REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: AG-E1-500 SC1

Product name(s): see Part A

Chemical active substance:

Ethofumesate, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Sponsor: Adama Agan Ltd.

Applicant: Country organisation / representative of ADAMA, as given in Part A

Submission date: March 2021

MS Finalisation date: January 2022 (initial Core Assessment)

June 2022 (final Core Assessment)

Version history

When	What
March 2021	dRR version 1 submitted by applicant
January 2022	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~~.
June 2022	Final report (Core Assessment updated following 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. Information no longer relevant ~~is struck through and shaded~~.

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

STATEMENT FOR OWNERSHIP

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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)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g 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	HU, SK	Sugar beet BEAVA Fodder beet BEAVC	F	annual dicot weeds and annual grass weeds	foliar spraying, overall	BBCH 10-18/ spring	a) 2 b) 2	a) 5 b) 5	a) 1 L/ha b) 2 L/ha	a) 500 b) 1000	100-400	n.a.	Max. rate of active must not exceed 1.0 kg/ha every 3 years (from all used products with ethofumesate).	A	A	R scenario R3 A scenarios D3, D4, R1	A	A	A	R
3	PL	Sugar beet BEAVA Fodder beet BEAVC	F	annual dicot weeds and annual grass weeds	foliar spraying, overall	BBCH 10-18/ spring	a) 3 b) 3	a) 5 b) 5	a) 0.6 L/ha b) 1.8 L/ha	a) 300 b) 900	100-400	n.a	Max. rate of active must not exceed 1.0 kg/ha every 3 years (from all used products with ethofumesate). At each time can be applied in tankmix: AG-E1-50 SC 0.5 L/ha + Goltix Titan 565 SC 1.5 L/ha ~~+ Atpolan BIO 80 EC 1.0 L/ha~~	A	A	R scenario R3 A scenarios D3, D4, R1	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

Explanation for column 15 – 21 "Conclusion"

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

Remarks table:

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

9.1.1 Overall conclusions

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)

The acute and chronic risks to ~~small omnivorous~~ birds from exposure to food stuffs contaminated with ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet are acceptable.

The risk to birds from exposure to ethofumesate in drinking water from puddles is acceptable.

The acute risks ~~to small herbivorous mammals~~ and chronic risks to ~~small insectivorous, large herbivorous and small omnivorous~~ mammals from exposure to food stuffs contaminated with ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet are acceptable.

The risk to birds and mammals from exposure to ethofumesate and metabolite NC 5493 in drinking water from puddles is acceptable.

Evaluation of the risk of secondary poisoning was triggered neither for ethofumesate nor its metabolites due to log Pow values being <3 for all compounds.

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.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risk to aquatic organisms from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet at 2x500 g a.s./ha with 5 d interval is acceptable in scenarios D3, D4 and R1 with no need for risk mitigation measures. In scenario R3 the risk is acceptable provided that 10 m vegetated filter strip to surface water bodies is respected.

For uses of AG-E1-500 SC1 to sugar beet at 3x300 g a.s./ha with 5 d interval the risk is acceptable in scenarios D3, D4 and R1 with no need for risk mitigation measures. In scenario R3 the risk is acceptable provided that 20 m vegetated filter strip to surface water bodies is respected.

The risk to aquatic organisms from exposure to the ethofumesate relevant metabolites, NC8493 and NC20645 is ~~are~~ acceptable without mitigation.

Since no studies on effects of AG-E1-500 SC1 used in a mixture with adjuvant Atpolan BIO 80 EC on rooted aquatic macrophytes (most sensitive aquatic species) were available, potentially increased toxicity of the herbicide due to the presence of adjuvant could not be addressed in the risk assessment and recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are made available.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to bees from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk to non-target arthropods from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable with no need for risk mitigation measures.

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

The risk to non-target soil organisms from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable.

The risk to soil microbial activity from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable.

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

The risk assessment for non-target terrestrial plants from AG-E1-500 SC1 was performed using two approaches:

- with consideration of the multiple applications (not fully in line with SANCO/10329/2002 rev. 2 final)
- with consideration of the single application (fully in line with SANCO/10329/2002 rev. 2 final).

Depending on the approach, acceptable risk could be concluded with various risk mitigation measures:

1. Approach accounting for multiple applications:
   - For applications at 2x1.0 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 75% using appropriate drift reducing techniques.
   - For applications at 3x0.6 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 50% using appropriate drift reducing techniques.
2. Approach performed with assumption of single application rate:
   - For applications at 2x1.0 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 50% using appropriate drift reducing techniques.
   - For applications at 3x0.6 L/ha: no risk mitigation measures necessary.

Concerned Member States must decide on applicability of the proposed risk mitigation measures and which option (with MAF or without MAF) is relevant in their countries.

Since no studies on effects of AG-E1-500 SC1 used in a mixture with adjuvant Atpolan BIO 80 EC on non-target terrestrial plants were performed, potentially increased toxicity of the herbicide due to the presence of adjuvant could not be addressed in the risk assessment and recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are made available.

~~The off-field risk to non-target plants from the use of AG-E1-500 SC1 on sugar beet/fodder beet is acceptable with a 5m buffer zone or 75% drift reduction nozzles.~~

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

No further data on effects of ethofumesate or formulation AG-E1-500 SC1 to other terrestrial organisms are available.

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 AG-E1-500 SC1

Grouping according to criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Effects on birds and mammals (Błąd! Nie można odnaleźć źródła odwołania. **and** Błąd! Nie można odnaleźć źródła odwołania.**)**			
Field crops	GAP uses 1 and 2: 2 x 1 L product/ha, BBCH 10-18	Crop group according to EFSA/2009/1438	Sugar beet, 2 × 1 L prod./ha based on (generic) focal species relevant at time of application
Effects on aquatic organisms (Błąd! Nie można odnaleźć źródła odwołania.**)**			
Field crops	GAP uses 1 and 2: 2 x 1 L product/ha, BBCH 10-18	Crop group according to FOCUS (2001 & 2015): sugar beet	Sugar beet, maximum annual application rate i.e. 2 L product/ha
Effects on bees (Błąd! Nie można odnaleźć źródła odwołania.**)**			
Field crops	GAP uses 1 and 2: 2 x 1 L product/ha, BBCH 10-18	Field crops according to ESCORT 2 (2000)	Field crops, maximum single application rate, i.e. 1× 2 L prod./ha
Effects on non-target arthropods (Błąd! Nie można odnaleźć źródła odwołania.**) and terrestrial non-target plants (**Błąd! Nie można odnaleźć źródła odwołania.**)**			
Field crops	GAP uses 1 and 2: 2 x 1 L product/ha, BBCH 10-18	Field crops according to ESCORT 2 (2000)	Sugar beet, maximum annual application rate i.e. 2 x 1 L product/ha
Effects on terrestrial soil meso- and macrofauna (Błąd! Nie można odnaleźć źródła odwołania.**) and soil microbial activity (**Błąd! Nie można odnaleźć źródła odwołania.**)**			
Field crops	GAP uses 1 and 2: 2 x 1 L product/ha, BBCH 10-18	Crop group according to FOCUS (1997 & 2014): beet crops	Sugar beet, maximum annual application rate i.e. 2 x 1 L product/ha

zRMS comments:

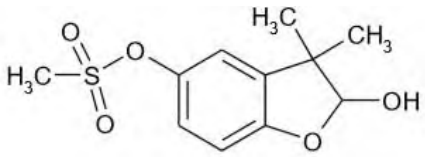
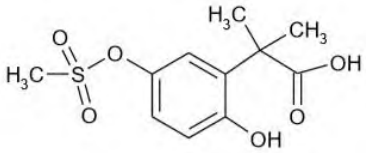
zRMS agrees with grouping of the intended uses of AG-E1-500 SC1 proposed in Table 9.1-2 above. In any case the exposure from twofold application at 1.0 L/ha (corresponding to 2x500 g a.s./ha) will be higher comparing to 3x0.6 L/ha (corresponding to 3x300 g a.s./ha) and will be thus protective for both intended uses of the product.

It should be, however, noted that although single use at 1000 g a.s./ha is not included in the GAP table, it was considered in the risk assessment for birds and mammals. Since acceptable risk was concluded with this worst case assumption, no further calculations were deemed necessary for the twofold application at 500 g a.s./ha, which would result with considerably lower exposure comparing to the cumulative rate.

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 AG-E1-500 SC1 is indicated in the table.

Table 9.1-3 Metabolites of Ethofumesate

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
NC 8493		258.3	Soil: 24.2% (photolysis)	For soil macro-, meso- and microorganisms as well as aquatic organisms
NC 20645		274.3	Water/Sediment: 18.8%	For aquatic organisms

zRMS comments:

Information regarding metabolites of ethofumesate provided in Table 9.1-3 above is in line with EU agreed data reported in EFSA Journal 2016:14(1):4374. Additional information has been added by the zRMS for completeness.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

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

Effects on birds of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. However, the provision of further data on the AG-E1-500 SC1 is not considered essential, because the risk assessment of the active substance indicates acceptable risk to birds from the use of ethofumesate in accordance with the proposed GAP.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Anas platyrhynchos	Ethofumesate	Oral Acute	LD_{50} = >2000 mg/kg bw	EFSA Conclusion 4374/2016
Anas platyrhynchos	Ethofumesate	Oral Acute	LD_{50} = >3552 mg/kg bw	EFSA Conclusion 4374/2016
Colinus virginianus	Ethofumesate	Oral Acute	LD_{50} = >2000 mg/kg bw	EFSA Conclusion 4374/2016
Colinus virginianus	Ethofumesate	Oral Acute	LD_{50} = >8743 mg/kg bw	EFSA Conclusion 4374/2016
Extrapolated endpoint	Ethofumesate	Acute	LD_{50} = 3776* mg/kg bw	EFSA Conclusion 4374/2016
Anas platyrhynchos	Ethofumesate	Dietary Reproductive toxicity	NOEL = 406.0 mg/kg bw/d (maximum test concentration, no effect on growth or reproduction)	EFSA Conclusion 4374/2016
Colinus virginianus	Ethofumesate	Dietary Reproductive toxicity	NOEL = 265.0 mg/kg bw/d (maximum test concentration, no effect on growth or reproduction)	EFSA Conclusion 4374/2016

* LD_{50} extrapolated according to the EFSA Guidance Document on Birds and Mammals (2009), based on the lowest endpoint for mallard duck and bobwhite quail of 2000 mg a.s./kg bw with an extrapolation factor of 1.888.

zRMS comments:

Avian toxicity data for ethofumesate are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

9.2.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to birds from use of AG-E1-500 SC1 in accordance with the proposed GAP.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

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

The results of the acute and reproductive first-tier risk assessments are summarised in the following table.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of AG-E1-500 SC1 in sugar beet/fodder beet

Intended use	**AG-E1- 500 SC in sugar beet/fodder beet, BBCH 10-18**				
Active substance/product	Ethofumesate				
Application rate (g/ha)	1 x 1000 g a.s./ha*				
Acute toxicity (mg/kg bw)	3776				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Sugar/fodder beet (root vegetables) BBCH 10-37	Small omnivorous bird	158.8	1	158.80	23.78
Reprod. toxicity (mg/kg bw/d)	265				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Sugar/fodder beet (root vegetables) BBCH 10-18	Small omnivorous bird	64.8	1	34.34	7.72

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
* This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application

zRMS comments:

The risk assessment presented in Table 9.2-2 above is agreed by the zRMS. Evaluation was performed with consideration of the single cumulative application rate of 1000 g a.s./ha, being protective for applications at 2x500 g a.s./ha and 3x300 g a.s./ha. On the basis of performed calculations acceptable acute and long-term dietary risk from exposure of birds to ethofumesate may be concluded.

9.2.2.2 Higher-tier risk assessment

A higher-tier risk assessment for the use of AG-E1-500 SC1 on sugar beet/fodder beet is not required as the first-tier risk assessment indicates acceptable risk for both acute and chronic exposure to birds.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario
Since AG-E1-500 SC1 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

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

With a K(f)oc of 118 mL/g, ethofumesate belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1000		
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.265
Reprod. toxicity (mg/kg bw/d)	=	265	quotient =	3.77

No specific calculations of exposure and TER are necessary as the ratios of effective application rate of ethofumesate to acute and reprotoxic endpoints for birds are less than 50.

zRMS comments:

As in case of the dietary exposure, the exposure of birds to ethofumesate via drinking water was also calculated with consideration of the cumulative application rate of 1000 g a.s./ha, protective for all intended uses of AG-E1-200 SC1. Calculations provided in table above are agreed by the zRMS. Acceptable risk from ethofumesate present in drinking water may be concluded.

It is noted that the drinking water risk assessment should include also exposure to pertinent soil metabolites of the active substance. Since no calculations were provided by the Applicant, respective evaluation for metabolite NC 8493 was performed by the zRMS and is presented below. The pseudo-application rate of the metabolite was calculated with consideration of the maximum cumulative rate of the parent (1000 g a.s./ha), molar ratio (0.902) and peak occurrence in soil (24.2%, soil photolysis study). In absence of the respective toxicity data, 10 times toxicity of the parent was assumed as representing worst case.

Effective application rate (g/ha)	=	218.3			Trigger
Acute toxicity (mg/kg bw)	=	377.6	quotient =	0.58	50 (Kfoc = 2.1 mL/g]
Reprod. toxicity (mg/kg bw/d)	=	26.5	quotient =	8.2	

Based on the above calculations, no unacceptable risk from exposure to metabolite NC 8493 in drinking water is expected following intended uses of AG-E1-200 SC1.

9.2.2.4 Effects of secondary poisoning

As stated in the EFSA Conclusion (2016), the log POW for ethofumesate was determined to be at 2.7 at 20 °C and 25 °C (pH 6.4). This value is below the relevant trigger of 3 and thus, a low potential for bioaccumulation is indicated and no deterministic risk assessments by calculating TER values have to be conducted. The logPOW for the three mentioned metabolites are below the trigger of 3 (i.e. log POW NC 8493 = 1.5; log POW NC 9607 = 2.2; log POW NC 20645 = -2.4 – 0.4).

In conclusion, a low potential for bioaccumulation is indicated for both the active substances and the metabolites of concern. So, no deterministic risk assessments by calculating TER values for fish- or earthworm-eating birds have to be conducted.

Risk assessment for earthworm-eating birds via secondary poisoning
Not required.

Risk assessment for fish-eating birds via secondary poisoning
Not required.

zRMS comments:

Evaluation of the risk of secondary poisoning for ethofumesate and its metabolites is not triggered due to log Pow values being <3 for all compounds.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The acute and chronic risks to small omnivorous birds from exposure to food stuffs contaminated with ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet are acceptable.

The risk to birds from exposure to ethofumesate and metabolite NC 5493 in drinking water from puddles is acceptable.

Evaluation of the risk of secondary poisoning was triggered neither for ethofumesate nor its metabolites due to log Pow values being <3 for all compounds.

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

9.3.1 Toxicity data

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

Effects on mammals of AG-E1-50 SC were not evaluated as part of the EU assessment of ethofumesate. However, the provision of further data on the AG-E1-50 SC is not considered essential, because the risk assessment of the active substance indicates acceptable risk to mammals from the use of ethofumesate in accordance with the proposed GAP.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	ethofumesate	Oral 1 d Acute	LD_{50} = >5000 mg/kg bw	EFSA Conclusion 4374/2016
Rat	ethofumesate	Dietary Reproductive toxicity Two-generation study	NOAEL = 60.9 mg/kg bw/d (offspring survival)	EFSA Conclusion 4374/2016

zRMS comments:

Mammalian toxicity data for ethofumesate are in line with EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

9.3.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to mammals from use of AG-E1-500 SC1 in accordance with the proposed GAP.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

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

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of AG-E1-500 SC1 in sugar beet/fodder beet

Intended use	**AG-E1-500 SC1 in sugar beet/fodder beet, BBCH 10-18**				
Active substance/product	Ethofumesate				
Application rate (g/ha)	1 x 1000 g a.s./ha*				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Sugar/fodder beet (root vegetables) BBCH 10-37	Small herbivorous mammal	118.4	1	118.4	42.23
Reprod. toxicity (mg/kg bw/d)	60.9				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Sugar/fodder beet (root vegetables) BBCH 10-18	Small herbivorous mammal (screening step)	48.3	0.53	25.60	**2.38**
Sugar beet BBCH 10-19	Small insectivorous mammal Common shrew *Sorex araneus*	4.2	0.53	2.23	27.36
Sugar beet BBCH 10-39	Large herbivorous mammal Rabbit *Oryctolagus cuniculus*	14.3	0.53	7.58	8.04
Sugar beet BBCH 10-39	Small omnivorous mammal Wood mouse *Apodemus sylvaticus*	7.8	0.53	4.13	14.73

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
* This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application

zRMS comments:

The risk assessment presented in Table 9.3-2 above is agreed by the zRMS. Evaluation was performed with consideration of the single cumulative application rate of 1000 g a.s./ha, being protective for applications at 2x500 g a.s./ha and 3x300 g a.s./ha. On the basis of performed calculations acceptable acute and long-term dietary risk from exposure of mammals to ethofumesate may be concluded.

9.3.2.2 Higher-tier risk assessment

A higher-tier risk assessment for the use of AG-E1-500 SC1 on sugar beet/fodder beet is not required as the first-tier risk assessment indicates acceptable risk for both acute and chronic exposure to mammals.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case

of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 118 mL/g, ethofumesate belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	1000		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.2
Reprod. toxicity (mg/kg bw/d)	=	60.9	quotient =	16.4

No specific calculations of exposure and TER are necessary as the ratios of effective application rate of ethofumesate to acute and reprotoxic endpoints for mammals are less than 50.

zRMS comments:

As in case of the dietary exposure, the exposure of mammals to ethofumesate via drinking water was also calculated with consideration of the cumulative application rate of 1000 g a.s./ha, protective for all intended uses of AG-E1-200 SC1. Calculations provided in table above are agreed by the zRMS. Acceptable risk from ethofumesate present in drinking water may be concluded.

It is noted that the drinking water risk assessment should include also exposure to pertinent soil metabolites of the active substance. Since no calculations were provided by the Applicant, respective evaluation for metabolite NC 8493 was performed by the zRMS and is presented below. The pseudo-application rate of the metabolite was calculated with consideration of the maximum cumulative rate of the parent (1000 g a.s./ha), molar ratio (0.902) and peak occurrence in soil (24.2%, soil photolysis study). In absence of the respective toxicity data, 10 times toxicity of the parent was assumed as representing worst case.

Effective application rate (g/ha)	=	218.3			Trigger
Acute toxicity (mg/kg bw)	=	500	quotient =	0.44	50 (Kfoc = 2.1 mL/g]
Reprod. toxicity (mg/kg bw/d)	=	6.09	quotient =	35.8	

Based on the above calculations, no unacceptable risk from exposure to metabolite NC 8493 in drinking water is expected following intended uses of AG-E1-200 SC1.

9.3.2.4 Effects of secondary poisoning

As stated in the EFSA Conclusion (2016), the log POW for ethofumesate was determined to be 2.7 at 20 °C and 25 °C (pH 6.4). This value is below the relevant trigger of 3 and thus, a low potential for bioaccumulation is indicated and no deterministic risk assessments by calculating TER values have to be conducted. The logPOW for the three mentioned metabolites are below the trigger of 3 (i.e. log POW NC 8493 = 1.5; log POW NC 9607 = 2.2; log POW NC 20645 = -2.4 – 0.4).

In conclusion, a low potential for bioaccumulation is indicated for both the active substances and the metabolites of concern. So, no deterministic risk assessments by calculating TER values for fish- or earthworm-eating mammals have to be conducted.

Risk assessment for earthworm-eating mammals via secondary poisoning
Not required.

Risk assessment for fish-eating mammals via secondary poisoning
Not required.

zRMS comments:

Evaluation of the risk of secondary poisoning for ethofumesate and its metabolites is not triggered due to log Pow values being <3 for all compounds.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The acute risks to small herbivorous mammals and chronic risks to small insectivorous, large herbivorous and small omnivorous mammals from exposure to food stuffs contaminated with ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet are acceptable.

The risk to mammals from exposure to ethofumesate in drinking water from puddles is acceptable.

Evaluation of the risk of secondary poisoning was triggered neither for ethofumesate nor its metabolites due to log Pow values being <3 for all compounds.

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

No additional data was submitted as part of the active substance renewal of ethofumesate. No further data is presented in this product submission.

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.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ethofumesate and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Cyprinus carpio	Ethofumesate	96h, ss	LC_{50} = 10.92 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Oncorhynchus mykiss	Ethofumesate	96h, ss	LC_{50} = 11.91 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Lepomis macrochirus	Ethofumesate	96h, ss	LC_{50} = 21.2 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Cyprinodon variegatus	Ethofumesate	96h, s	LC_{50} = 25.0 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Danio rerio	Ethofumesate	160 d (FLC), f	NOEC = 0.156 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Pimephales promelas	Ethofumesate	28 d (ELC), f	NOEC = 4.17 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Daphnia magna	Ethofumesate	48 h, s	EC_{50} = 13.52 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Americamysis bahia	Ethofumesate	96 h, s	EC_{50} = 5.4 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Crassostrea virginica	Ethofumesate	96 h, f	EC_{50} = 1.7 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Daphnia magna	Metabolite NC 8493	48 h, ss	EC_{50} = >10 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Daphnia magna	Metabolite NC 8493	48 h, s	EC_{50} = >100 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Daphnia magna	Metabolite NC 20645	48 h, ss	EC_{50} = >10 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Daphnia magna	Metabolite NC 20645	48 h, s	EC_{50} = >100 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Daphnia magna	Ethofumesate	21 d, ss	NOEC = 0.32 mg a.s./L $_{nom}$	EFSA Conclusion 4374/2016
Daphnia magna	Ethofumesate	21 d, ss	NOEC = 0.25 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Daphnia magna	Ethofumesate	21 d, ss	NOEC = 1.06 mg a.s./L $_{mm}$	EFSA Conclusion 4374/2016
Chironomus riparius	Ethofumesate	28 d, spiked water	NOEC = 3.82 mg a.s./L $_{im}$	EFSA Conclusion 4374/2016
Chironomus riparius	Ethofumesate	28 d, spiked water	NOEC = 5.33 mg a.s./L $_{im}$	EFSA Conclusion 4374/2016

Species	Substance	Exposure System	Results	Reference
Chironomus riparius	Ethofumesate	28 d, spiked water	NOEC = 14.05 mg a.s./L $_{im}$	EFSA Conclusion 4374/2016
Pseudokirchneriella subcapitata	Ethofumesate	72 h, s	E_rC_{50} = 16.3 mg a.s./L (NOEC = 5.91 mg a.s./L) $_{mm}$ E_yC_{50} = 9.68 mg a.s./L (NOEC = 5.91 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Anabaena flos-aquae	Ethofumesate	96 h, s	E_rC_{50} = >20 mg a.s./L (NOEC = 20 mg a.s./L) $_{nom}$ E_bC_{50} = >20 mg a.s./L (NOEC = 20 mg a.s./L) $_{nom}$	EFSA Conclusion 4374/2016
Skeletonema costatum	Ethofumesate	72 h, s	E_rC_{50} = >20 mg a.s./L (NOEC = 5 mg a.s./L) $_{nom}$ E_bC_{50} = 14.5 mg a.s./L (NOEC = 2.5 mg a.s./L) $_{nom}$	EFSA Conclusion 4374/2016
Pseudokirchneriella subcapitata	Metabolite NC 8493	72 h, s	E_rC_{50} = 20.7 mg a.s./L (NOEC = 0.367 mg a.s./L) $_{nom}$ E_yC_{50} = 0.865 mg a.s./L (NOEC = 0.367 mg a.s./L) $_{nom}$	EFSA Conclusion 4374/2016
Desmodesmus subspicatus	Metabolite NC 8493	72 h, s	E_rC_{50} = 4.83 mg a.s./L (NOEC = 1.33 mg a.s./L) $_{mm}$ E_yC_{50} = 1.87 mg a.s./L (NOEC = 1.33 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Desmodesmus subspicatus	Metabolite NC 20645	72 h, s	E_rC_{50} = 52.4 mg a.s./L (NOEC = 1.25 mg a.s./L) $_{mm}$ E_yC_{50} = 8.83 mg a.s./L (NOEC = 1.25 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Pseudokirchneriella subcapitata	Metabolite NC 20645	72 h, s	E_rC_{50} = >10 mg a.s./L (NOEC = 10 mg a.s./L) $_{nom}$ E_yC_{50} = >10 mg a.s./L (NOEC = 10 mg a.s./L) $_{nom}$	EFSA Conclusion 4374/2016
Lemna gibba	Ethofumesate	14 d, ss	E_rC_{50} = >52.8 mg a.s./L (NOEC = 4.3 mg a.s./L) $_{mm}$ E_bC_{50} = 50.4 mg a.s./L (NOEC = 4.3 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Lemna gibba	Ethofumesate	7 d, ss	E_rC_{50} = >42 mg a.s./L (NOEC = 26 mg a.s./L) $_{mm}$ E_bC_{50} = 35.0 mg a.s./L (NOEC = 17 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Myriophyllum spicatum	Ethofumesate	14 d, s	E_rC_{50} = 0.479 mg a.s./L (NOEC = 0.036 mg a.s./L) $_{mm}$ E_yC_{50} = 0.25 mg a.s./L (NOEC = 0.036 mg a.s./L) $_{mm}$	EFSA Conclusion 4374/2016
Higher-tier studies (micro- or mesocosm studies)				
None				

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

zRMS comments:

Endpoints presented in Table 9.5-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – AG-E1-500 SC1

Species	Substance	Exposure System	Results	Reference
Leuciscus idus	Ethosat 500 SC	96 h, ss	LC_{50} = 36.6 mg/L $_{nom}$ (15.1 mg a.s./L)	Scheerbaum D./ 2005/ FAG100321
Daphnia magna	AG-E1-500 SC1	48 h, s	EC_{50} = 46.33 mg/L $_{nom}$ (21.7 mg a.s./L)	Renner P./ 2020a/ 20 48 ADL 0001
Desmodesmus subspicatus	AG-E1-500 SC1	72 h, s	E_rC_{50} = 9.52 mg/L $_{nom}$ (4.5 mg a.s./L) E_yC_{50} = 7.97 mg/L $_{nom}$ (3.7 mg a.s./L) NOEC = 6.62 mg/L $_{nom}$ (3.1 mg a.s./L)	Renner P./ 2020b/ 20 48 AAL 0001
Myriophyllum spicatum	AG-E1-500 SC1	14 d, s	<u>Shoot fresh weight:</u> E_rC_{50} = 0.257 mg/L $_{nom}$ (0.12 mg a.s./L) E_yC_{50} = 0.183 mg/L $_{nom}$ (0.09 mg a.s./L) <u>Shoot dry weight:</u> E_rC_{50} >0.400 mg/L $_{nom}$ (0.19 mg a.s./L) E_yC_{50} >0.400 mg/L $_{nom}$ (0.19 mg a.s./L) <u>Main shoot length:</u> E_rC_{50} = 0.290 mg/L $_{nom}$ (0.14 mg a.s./L) E_yC_{50} = 0.129 mg/L $_{nom}$ (0.06 mg a.s./L) <u>Total shoot length:</u> E_rC_{50} > 0.400 mg/L $_{nom}$ (0.19 mg a.s./L) E_yC_{50} = 0.257 mg/L $_{nom}$ (0.12 mg a.s./L) overall NOE_rC = 0.0102 mg/L $_{nom}$ (0.005 mg a.s./L)	Renner P./ 2020c/ 20 48 AMS 0001
Higher-tier studies (micro- or mesocosm studies)				
None				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

zRMS comments:

Studies on toxicity of AG-E1-500 SC to aquatic organisms were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.5-2 are confirmed to be correct. Additional information has been added by the zRMS for completeness.

Study on acute toxicity of the product to fish has been performed with the previous variant of formulation AG-E1-500 SC1. According to information provided in Part C of the Core Assessment, introduced composition changes were minor (<1%) and are considered to have no impact on the ecotoxicological profile of the product. Hence, results of studies performed with the old version of the formulated product may be used in evaluation performed for the new version (AG-E1-500 SC).

No study on toxicity of the formulated product to *Lemna gibba* was performed, however studies performed with the active compound clearly demonstrated that *Myriophyllum spicatum* is much more sensitive comparing to *Lemna gibba*. Taking this into account, study on toxicity of the formulation to *M. spicatum* is deemed sufficient.

In order to compare the active substance and formulation toxicity, MDR values were calculated by the zRMS in the table below.

Species / time scale	Active substance endpoint [mg a.s./L]	Formulation endpoint [mg a.s./L]	MDR
Fish, acute	10.92	15.1	0.72
Daphnia magna, acute	13.52	21.7	0.62
Algae, chronic	16.3	4.5	3.62
Myriophyllum spicatum, chronic	0.479	0.12 ~~0.257~~	3.99 ~~1.86~~

All MDR values are between 0.2 and 5, indicating that active substance and formulation toxicity are in good agreement. Nevertheless, the endpoints for algae and *M. spicatum* derived in studies performed with the formulated product are slightly lower comparing to active substance endpoints and for this reason, in opinion of the zRMS, should be used in the risk assessment, especially calculation of the MDR values is performed in order to justify use of the measured formulation toxicity data in the risk assessment.

It was noted by the zRMS that according to the GAP in case of use No 3 application of AG-E1-500 SC as a tank mixture with another herbicide (Goltix Titan 565 SC) and adjuvant (Atpolan BIO 80 EC) is recommended. Goltix Titan 565 SC contains metamitron and quinmerac, which belong to different chemical groups than ethofumesate and for this reason, in line with current requirements, no specific risk assessment is deemed necessary for this mixture. It should be, however, pointed out that adjuvants are added in order to increase efficacy of the formulated product and it cannot be excluded that their addition to the mixture with AG-E1-500 SC1 would lead to more pronounced toxic effects on rooted aquatic macrophytes, being the most sensitive aquatic species. However, no study on effects of AG-E1-500 SC1 with adjuvant were performed by the Applicant and available data are not sufficient to conclude on the risk resulting from simultaneous exposure of aquatic macrophytes to AG-E1-500 SC1 and the adjuvant. Taking this into account, recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are provided by the Applicant.

9.5.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to aquatic organisms from use of AG-E1-500 SC1 in accordance with the proposed GAP.

9.5.2 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", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

9.5.2.1 Active substance

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Ethofumesate for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 2 x 500 g a.s./ha AG-E1-500 SC1 in sugar beet

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Crassostrea virginica***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50} ~~E_yC_{50}~~	NOEC	E_rC_{50} ~~E_yC_{50}~~
(µg/L)		10920	156	1700	250	4500 * ~~9680~~	3820	120 * ~~250~~
AF		100	10	100	10	10	10	10
RAC (µg/L)		109.2	15.6	17	25	450 * ~~968~~	382	12 * ~~25~~
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**							
Step 1								
	297.22	**2.7218**	**19.053**	**17.484**	**11.889**	0.66 ~~0.3071~~	0.7781	**24.8** ~~**11.889**~~
Step 2								
N-Europe	46.05	0.4217	**2.9519**	**2.7088**	**1.8420**	-	-	**3.8** ~~**1.8420**~~
S-Europe	84.94	0.7778	**5.4449**	**4.9965**	**3.3976**	-	-	**7.1** ~~**3.3976**~~
Step 3								
D3/ditch	2.276	-	0.1459	0.1339	0.0910	-	-	0.19 ~~0.0910~~
D4/pond	0.5254	-	0.0337	0.0309	0.0210	-	-	0.04 ~~0.0210~~
D4/stream	1.912	-	0.1226	0.1125	0.0765	-	-	0.16 ~~0.0765~~
R1/pond	0.3942	-	0.0253	0.0232	0.0158	-	-	0.03 ~~0.0158~~
R1/stream	5.612	-	0.3597	0.3301	0.2245	-	-	0.47 ~~0.2245~~
R3/stream	29.30	-	**1.8782**	**1.7235**	**1.1720**	-	-	**2.44** ~~**1.1720**~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Formulation endpoints expressed in terms of the active substance (used since lower than the active substance endpoints)

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Ethofumesate for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 3 x 330 g a.s./ha AG-E1-500 SC1 in sugar beet [1)]

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Crassostrea virginica***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50} ~~E_yC_{50}~~	NOEC	E_rC_{50} ~~E_yC_{50}~~
(µg/L)		10920	156	1700	250	4500 * ~~9680~~	3820	120 * ~~250~~
AF		100	10	100	10	10	10	10
RAC (µg/L)		109.2	15.6	17	25 ~~2.5~~	450 * ~~968~~	382	12 * ~~2.5~~
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	294.24	**2.6945**	**18.862**	**17.308**	**11.770**	0.65 ~~0.3040~~	0.7703	**24.5** ~~**11.770**~~
Step 2								
N-Europe	41.99	0.3845	**2.6917**	**2.4700**	**1.6796**	-	-	**3.5** ~~**1.6796**~~
S-Europe	78.16	0.7158	**5.0103**	**4.5976**	**3.1264**	-	-	**6.5** ~~**3.1264**~~
Step 3								
D3/ditch	1.259	-	0.0807	0.0741	0.0504	-	-	0.10 ~~0.0504~~
D4/pond	0.549	-	0.0352	0.0323	0.0220	-	-	0.05 ~~0.0220~~
D4/stream	1.119	-	0.0717	0.0658	0.0448	-	-	0.09 ~~0.0448~~
R1/pond	0.514	-	0.0330	0.0302	0.0206	-	-	0.04 ~~0.0206~~
R1/stream	8.677	-	0.5562	0.5104	0.3471	-	-	0.72 ~~0.3471~~
R3/stream	18.96	-	**1.2154**	**1.1153**	0.7584	-	-	**1.6** ~~0.7584~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* Formulation endpoints expressed in terms of the active substance (used since lower than the active substance endpoints)
[1)] Exposure calculated for application at 3x330 g a.s./ha, but in line with the GAP the intended rate is 3x300 g a.s./ha

For the intended use sugar beet, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic macrophytes ~~fish~~ as characterised by a E_rC_{50} for *Myriophyllum spicatum* of 120 µg a.s./L derived from formulation study ~~NOEC for *Danio rerio* of 156 µg/L~~ in connection with an assessment factor of 10) in one FOCUS Steps 3 scenario (R3). Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies. Performed calculations cover the risk to other aquatic species with PEC/RAC >1 in this scenario.

Table 9.5-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Ethofumesate based on FOCUS Step 4 calculations and toxicity data for most sensitive species (*M. spicatum*) ~~fish~~ with mitigation of spray drift and run-off for the use of 2 x 500 g a.s./ha AG-E1-500 SC1 in sugar beet

Intended use **Active substance** **Application rate (g/ha)**		Sugar beet Ethofumesate 2 x 500 (BBCH 10-18)				
Nozzle reduction	**No-spray buffer (m)**	1/3	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	R3 stream	29.3	29.3	29.3	13.25 ~~1.367~~	6.932 ~~0.272~~
90 %		29.3	n/c	n/c	n/c	n/c
RAC (µg/L) 12.0 ~~15.6~~		**PEC/RAC ratio**				
None	R3 stream	**2.4** ~~**1.878**~~	**2.4** ~~**1.878**~~	**2.4** ~~**1.878**~~	**1.10** ~~0.088~~	0.58 ~~0.017~~
90 %		**2.4** ~~**1.878**~~	n/c	n/c	n/c	n/c

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
n/c not calculated

A safe use is indicated with a vegetated filter strip of 20 m ~~10m~~ and a no-spray buffer of 10m.

Table 9.5-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Ethofumesate based on FOCUS Step 4 calculations and toxicity data for most sensitive species (*M. spicatum*) ~~fish~~ with mitigation of spray drift and run-off for the use of 3 x 330 g a.s./ha AG-E1-500 SC1 in sugar beet [1)]

Intended use **Active substance** **Application rate (g/ha)**		Sugar beet Ethofumesate 3 x 330 (BBCH 10-18)				
Nozzle reduction	**No-spray buffer (m)**	1/3	10	20	10	20
	Vegetated filter strip (m)	None	None	None	10	20
None	R3 stream	18.96	18.96	18.96	8.572 ~~2.070~~	4.486 ~~0.1585~~
90 %		18.96	n/c	n/c	n/c	n/c
RAC (µg/L) 12.0 ~~15.6~~		**PEC/RAC ratio**				
None	R3 stream	**1.58** ~~**1.2154**~~	**1.58** ~~**1.2154**~~	**1.58** ~~**1.2154**~~	0.71 ~~0.1327~~	0.37 ~~0.0102~~
90 %		**1.58** ~~**1.2154**~~	n/c	n/c	n/c	n/c

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
n/c not calculated
[1)] Exposure calculated for application at 3x330 g a.s./ha, but in line with the GAP the intended rate is 3x300 g a.s./ha

A safe use is indicated with a vegetated filter strip of 10m and a no-spray buffer of 10m.

zRMS comments:

The aquatic risk assessment performed by the Applicant is partially agreed by the zRMS.

As indicated in the commenting box in point 9.5.1 above, in opinion of the zRMS the lower of formulation and active substance endpoints should be considered in PEC/RAC calculations, which was the case for algae and *Myriophyllum spicatum*. The PEC/RAC values for these species were thus recalculated in Tables 9.5-3 to 9.5-6 above and the text amended accordingly. In addition to that considerably higher Step 4 PEC_{SW} values in scenario R3 were calculated by the zRMS efate expert and refined risk assessment in Tables 9.5-5 and 9.5-6 was amended accordingly.

Introduced corrections had no impact on the outcome of the evaluation performed for application at 3x300 g a.s./ha: acceptable risk could be concluded in scenarios D3, D4 and R1 with no need for risk mitigation measures, while in scenario R3 acceptable risk could be concluded with assumption of 10 m vegetated filter strip to surface water bodies.
In case of application at 500 g a.s./ha acceptable risk could be concluded in scenarios D3, D4 and R1 with no need for risk mitigation measures, but in scenario R3 acceptable risk could be concluded with assumption of 20 m vegetated filter strip to surface water bodies.

In line with information provided in the commenting box in point 9.5.1 above, formulation AG-E1-500 SC1 cannot be used as a tank mixture with adjuvant Atpolan BIO 80 EC until respective data addressing potentially higher toxicity of this mixture to rooted aquatic macrophytes are submitted by the Applicant. Recommendation on use of the product with adjuvant is thus deleted in the GAP table and the product label.

9.5.2.2 Relevant metabolites

The relevant global maximum FOCUS Step 1 and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios for ethofumesate metabolites NC8493 and NC20645 are presented in the tables below.

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for NC8493 for each organism group based on FOCUS Steps 1 and 3 calculations for the use of 2 x 500 g a.s./ha AG-E1-500 SC1 in sugar beet

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Algae**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} 1092[a]	NOEC 15.6[a]	EC_{50} >100000	E_rC_{50} ~~E_yC_{50}~~ 4830 ~~86.5~~	E_yC_{50} 47.9[a] ~~25[a]~~
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	1000 ~~10000~~	483 ~~86.5~~	4.79 ~~2.5~~
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	72.58	**6.6465**	**46.526**	0.073 ~~0.0073~~	0.15 ~~0.8391~~	**15.2** ~~**29.032**~~
Step 2						
N-Europe	<0.001	<0.0001	0.0006	-	-	<0.00021 ~~0.0004~~
S-Europe	<0.001	<0.0001	0.0006	-	-	<0.00021 ~~0.0004~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[a] Endpoint estimated by assuming 10 times greater toxicity than the parent

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for NC8493 for each organism group based on FOCUS Steps 1 and 3 calculations for the use of 3 x 330 g a.s./ha AG-E1-500 SC1 in sugar beet [1)]

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Algae**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} 1092[a]	NOEC 15.6[a]	EC_{50} >100000	E_rC_{50} ~~E_yC_{50}~~ 4830 ~~86.5~~	E_yC_{50} 47.9[a] ~~25[a]~~
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	1000 ~~10000~~	483 ~~86.5~~	4.79 ~~2.5~~
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	71.85	**6.5797**	**46.058**	0.073 ~~0.0072~~	0.15 ~~0.8306~~	**15.0** ~~**28.740**~~
Step 2						
N-Europe	<0.001	<0.0001	0.0006	-	-	<0.00021 ~~0.0004~~
S-Europe	<0.001	<0.0001	0.0006	-	-	<0.00021 ~~0.0004~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[a] Endpoint estimated by assuming 10 times greater toxicity than the parent
[1)] Exposure calculated for application at 3x330 g a.s./ha, but in line with the GAP the intended rate is 3x300 g a.s./ha

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for NC20645 for each organism group based on FOCUS Steps 1 and 3 calculations for the use of 2 x 500 g a.s./ha AG-E1-500 SC1 in sugar beet

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Algae**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} 1092[a]	NOEC 15.6[a]	EC_{50} >100000	E_rC_{50} ~~E_yC_{50}~~ >10000 ~~8830~~	E_yC_{50} 47.9[a] ~~25[a]~~
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	1000 ~~10000~~	>1000 ~~883~~	4.79 ~~2.5~~
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	67.06	**6.1410**	**42.987**	0.067 ~~0.0067~~	<0.07 ~~0.7753~~	**14.0** ~~**26.824**~~
Step 2						
N-Europe	9.48	0.8681	**6.0769**	-	-	**2.0** ~~**3.7920**~~
S-Europe	17.53	**1.6053**	**11.237**	-	-	**3.7** ~~**7.0120**~~
Step 3						
D3/ditch	0.0003	<0.0001	0.0002	-	-	0.00006 ~~0.0001~~
D4/pond	0.0075	0.0007	0.0048	-	-	0.002 ~~0.0030~~
D4/stream	0.0007	0.0001	0.0005	-	-	0.0001 ~~0.0003~~
R1/pond	0.0085	0.0008	0.0055	-	-	0.002 ~~0.0034~~
R1/stream	0.0077	0.0007	0.0047	-	-	0.002 ~~0.0029~~
R3/stream	0.1108	0.0101	0.0710	-	-	0.023 ~~0.0443~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[a] Endpoint estimated by assuming 10 times greater toxicity than the parent

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for NC20645 for each organism group based on FOCUS Steps 1 and 3 calculations for the use of 3 x 300 g a.s./ha AG-E1-500 SC1 in sugar beet

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Algae**	**Aquatic macrophytes** ~~**Higher-tier information**~~
Test species		***Cyprinus carpio***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} 1092[a]	NOEC 15.6[a]	EC_{50} >100000	E_rC_{50} ~~E_yC_{50}~~ >10000 ~~8830~~	E_yC_{50} 47.9[a] ~~25[a]~~
AF		100	10	100	10	10
RAC (µg/L)		10.92	1.56	1000 ~~10000~~	>1000 ~~883~~	4.79 ~~2.5~~
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**					
Step 1						
	66.39	**6.0797**	**42.558**	0.066 ~~0.0066~~	<0.07 0.0752	**13.9** ~~**26.556**~~
Step 2						
N-Europe	10.52	0.9634	**6.7436**	-	-	**2.2** ~~**4.2080**~~
S-Europe	19.88	**1.8205**	**12.744**	-	-	**4.2** ~~**7.9520**~~
Step 3						
D3/ditch	0.0004	<0.0001	0.0002	-	-	0.000008 ~~0.0001~~
D4/pond	0.0079	0.0007	0.0051	-	-	0.002 ~~0.0032~~
D4/stream	0.0008	0.0001	0.0005	-	-	0.0002 ~~0.0003~~
R1/pond	0.0112	0.0010	0.0072	-	-	0.002 ~~0.0045~~
R1/stream	0.0120	0.0011	0.0077	-	-	0.003 ~~0.0048~~
R3/stream	0.0717	0.0066	0.0460	-	-	0.015 ~~0.0287~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
[a] Endpoint estimated by assuming 10 times greater toxicity than the parent

zRMS comments:

The aquatic risk assessment for ethofumesate metabolites provided above is in general agreed by the zRMS. For species for which no toxicity data were available for the metabolite, 10 times toxicity of the parent has been assumed as a worst case. No separate chronic risk assessment for *Daphnia magna* was performed, however long-term RAC for fish (1.56 µg/L) covered the long-term RAC for *Daphnia magna* (2.5 µg/L with assumption of 10 times toxicity of the parent).

It was noted that the risk assessment for algae and aquatic macrophytes was performed with consideration of E_yC_{50} values although in line with EFSA aquatic guidance (2013) ErC50 value should be considered. Respective corrections were thus introduced by the zRMS in tables above, but they had no impact on the outcome of the performed calculations.

Overall, acceptable risk from ethofumesate metabolites could be concluded following all intended uses of AG-E1-500 SC1 with no need for risk mitigation measures.

During the commenting period it was noted that the RAC values for aquatic invertebrates were not correctly calculated in the risk assessment for metabolites in Tables 9.5- to 9.5-10 (1000 should have been used instead of 10000). Respective corrections were made in tables mentioned, but they had no impact on the outcome of the aquatic risk assessment performed for metabolites.

9.5.3 Overall conclusions

The risk to aquatic organisms from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet at 2x500 g a.s./ha with 5 d interval is acceptable in scenarios D3, D4 and R1 with no need for risk mitigation measures. In scenario R3 the risk is acceptable provided that 10 m vegetated filter strip to surface water bodies is respected.

For uses of AG-E1-500 SC1 to sugar beet at 3x300 g a.s./ha with 5 d interval the risk is acceptable in scenarios D3, D4 and R1 with no need for risk mitigation measures. In scenario R3 the risk is acceptable provided that 20 m vegetated filter strip to surface water bodies is respected.

The risk to aquatic organisms from exposure to the ethofumesate relevant metabolites, NC8493 and NC20645 is ~~are~~ acceptable without mitigation.

Since no studies on effects of AG-E1-500 SC1 used in a mixture with adjuvant Atpolan BIO 80 EC on rooted aquatic macrophytes (most sensitive aquatic species) were available, potentially increased toxicity of the herbicide due to the presence of adjuvant could not be addressed in the risk assessment and recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are made available.

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 AG-E1-500 SC1, 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."

~~with a 10m vegetative strip and a 10m no-spray buffer zone. This is based on assessment of the most sensitive ecotoxicity endpoint, the chronic toxicity to the fish *Danio rerio* and the most vulnerable scenario, R3 stream.~~

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with ethofumesate. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.**Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Ethofumesate	Oral	LD_{50} = >106.3 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Oral	LD_{50} = >50 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Oral	LD_{50} = >100 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Contact	LD_{50} = >100 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Contact	LD_{50} = >50 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Contact	LD_{50} = >100 µg a.s./bee	EFSA Conclusion 4374/2016
Apis mellifera	Ethofumesate	Chronic, 10d	LC_{50} = >120 mg a.s./kg LDD_{50} = >4.4 µg a.s./bee/d	EFSA Conclusion 4374/2016
Apis mellifera	AG-E1-500 SC1	Oral	LD_{50} = >500 µg product/bee (> 234.1 µg a.s./bee)	Franke M./ 2020/ 20 48 BAA 0004
Apis mellifera	AG-E1-500 SC1	Contact	LD_{50} = >500 µg product/bee (> 234.1 µg a.s./bee)	Franke M./ 2020/ 20 48 BAA 0004
Apis mellifera	AG-E1-500 SC1	Chronic, 10d	LDD_{50} > 115.77 µg a.s./bee/day NOEDD = 115.77 µg a.s./bee/day	Ansaloni T./ 2020a/ S19-20080
Apis mellifera	AG-E1-500 SC1	Larval toxicity, 22d	ED_{50} = 164.11 µg a.s./larva/developmental period NOED = 140.00 µg a.s./larva/developmental period	Ansaloni T./ 2020b/ S19-20081
Higher-tier studies (tunnel test, field studies)				
None				

zRMS comments:

The bee toxicity data for ethofumesate presented in Table 9.6-1 are in line with EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

The studies performed with AG-E1-500 SC1 were evaluated and agreed by the zRMS (for details, please refer to

respective points in Appendix 2). Endpoints for the formulated product reported in Table 9.6-1 are confirmed to be correct.

9.6.1.1 Justification for new endpoints

No new endpoints were used in the assessment of risk to aquatic organisms from use of AG-E1-500 SC1 in accordance with the proposed GAP.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The EFSA Guidance on Risk Assessment on Bees, EFSA Journal 2013; 11(7): 3295, is not yet noted in the Standing Committee SCoPAFF. According to the EFSA document "Outline of the revision of the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp.* And solitary bees) (EFSA,2013)" dated July 2019, EFSA Guidance 3295, 2013 continues to be reviewed and revised in a programme of work which will continue throughout 2020. It is anticipated that the finalised guidance document will be published in March 2021.

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of AG-E1-500 SC1 in sugar beet/fodder beet in accordance with SANCO/10329/2002 rev. 2

Intended use	**AG-E1-500 SC1 in sugar beet/fodder beet**		
Active substance	Ethofumesate		
Application rate (g/ha)	1 x 1000 g/ha*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>50 ~~>100~~	1000	20 ~~10~~
Contact toxicity	>50 ~~>100~~		20 ~~10~~
Product	AG-E1-500 SC1		
Application rate (g product/ha)	1 x 2000 * (equivalent to 1000 g a.s./ha)		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>234.1	1000	4.27
Contact toxicity	>234.1		4.27

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application

Both the Hazard Quotients for the oral (QHO) and the contact exposure (QHC) are below the relevant trigger of 50. Thus, according to SANCO/10329/2002 rev.2, an overall acceptable risk to honeybees can be concluded from the application of AG-E1-500 SC1.

The chronic effects were evaluated by feeding tests with adult and juvenile honeybees. The results show low toxicity of AG-E1-500 SC1 for adult honeybees (LLD50 = >115.77 µg a.s./bee/day) and for juvenile bees (ED50 = 164.11 µg a.s./larva/developmental period). For the details of the studies, please refer to data point KCP 10.3.1.2 and KCP 10.3.1.3 in Appendix 2.

As stated in the RAR (2015) of ethofumesate, the risk for honeybees to get in contact with contaminated nectar and pollen is negligible as sugar beets do not build flowers within the first year. Sugar beets are harvested by the end of the first year at BBCH 49 which is before flowering. In the rare case that shoots with flowers are produced in the first year or beets are flowering in the second year (if beets are grown for seed production) no risk for honeybees is expected as beet flowers are wind pollinated.
Sugar beet flowers are not mentioned in any standard or handbook on honeybee foraging plants (e.g. Maurizio & Schaper, 1994[1] ; Pritsch, 2007[2]).

Moreover, bees are not likely to be exposed to the whole formulation for the same reason, particularly as application is distinctly before flowering.
In conclusion, it can be concluded that the acute ~~and chronic~~ risk for bees can be considered as acceptable, both from the toxicity and the exposure point of view.

zRMS comments:

The risk assessment for bees presented in Table 9.6-2 above is agreed by the zRMS.

It is noted that several toxicity values are reported in EFSA Journal 2016;14(1):4374 (>50 to >106.3 µg a.s./bee for oral toxicity and >50 to >100 µg a.s./bee for contact toxicity). Since all endpoints are "greater than" values, it is justified to consider the maximum reported endpoints in the risk assessment and for this reason for oral exposure the LD_{50} of >106.3 µg a.s./bee could be considered. Nevertheless, as acceptable risk could be concluded for lower endpoint of >100 µg a.s./bee, the HQ values were not corrected by the zRMS.

In case of the risk assessment for the formulated product additional information has been added by the zRMS in Table 9.6-2 in order to clarify that the rate of the product as well as endpoints were expressed in terms of the active substance.

Since evaluation was based on the maximum cumulative application rate of the product and active substance, calculated HQ values are protective also for split applications.

Overall, acceptable risk to bees may be concluded from all intended uses of AG-E1-500 SC1 in the Central Zone.

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.

Although the chronic and larvae risk assessment is currently not required at the Central Zone level, one of the commenting Member States asked to perform the risk assessment in line with EFSA (2013) in order to facilitate national authorization of the product. The zRMS decided to provide additional calculations which were performed using EFSA Bee-Tool v. 3 for the cumulative application rate of 1000 g a.s./ha, representing worst case.

Screening step risk assessment (sugar beet, BBCH 10-18, 1x1.0 kg product/ha)

Contact route of exposure				
	"calculation factor" (linked with dust)	**HQ**	Trigger	Risk indicator
HB	1	4.3	42	OK
Oral route of exposure (pollen and nectar)				
	"calculation factor" (Ef x SV)	**ETR**	Trigger	Risk indicator
HB - acute	7.6	0.03	0.2	OK
HB - chronic	7.6	0.066	0.03	!

[1] Maurizio, A., Schaper, F., 1994, Das Trachtpflanzenbuch
[2] Pritsch, G., 2007, Bienenweide

HB - larvae	4.4	0.03	0.2	OK

Tier 1 chronic risk assessment

Crop	Category	Scenario	Ef	SV HB	TWA HB	ETR HB	Trigger	Risk indicator
Sugar beet BBCH 10-18	chronic	treated crop	1	0.92	0.72	0.036	0.03	!
	chronic	weeds	1	2.9	0.72	0.018	0.03	OK
	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	OK
	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	OK
	chronic	next crop	1	0.54	0.72	0.003	0.03	OK

Based on calculations performed in line with indications of EFSA (2013), acceptable acute oral and contact risk to adult bees as well as chronic risk to larvae may be concluded from the intended uses of AG-E1-5200 SC1 already at the screening step. The chronic risk to bees is acceptable in weeds, field margin, adjacent crop and next crop scenarios. However, chronic risk to bees in the treated crop scenario is potentially unacceptable and should be further resolved at the product authorisation in Member States considering indications of the not yet noted EFSA guidance in their national assessments. Risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

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

Not relevant.

9.6.3 Effects on bumble bees

The EFSA guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* sp. and solitary bees), EFSA Journal 2013; 11(7):3295, has not yet entered into force at the time of preparing this dossier. No studies on bumble bees are available in the active substance dossier and no studies on bumble bees have been carried out on the product AG-E1-500 SC1.

9.6.4 Effects on solitary bees

The EFSA guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* sp. and solitary bees), EFSA Journal 2013; 11(7):3295, has not yet entered into force at the time of preparing this dossier. No studies on solitary bees are available in the active substance dossier and no studies on solitary bees have been carried out on the product AG-E1-500 SC1.

9.6.5 Overall conclusions

The risk to bees from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet was assessed in line with indications of the current guidance document (SANCO/10329/2002 rev 2 final) and is acceptable. The Hazard Quotients for oral (Q_{HO}) and contact exposure (Q_{HC}) are below the trigger of 50. ~~Also, the~~ Results of chronic feeding studies show low toxicity to larvae and adults.

In addition, formulation is applied before flowering period, and sugar beet can be considered as no attractive to bees (harvest before flowering period). In consequence, according to EPPO guideline (EPPO 3/10 (3);09-2010), there is no exposure to bees and so acceptable risk to bees. Therefore, an acceptable risk to bees is expected from the application of AG-E1-500 SC1.

Risk assessment for bumblebees and solitary bees was not performed as being not yet a data requirement.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been not carried out with ethofumesate.

Effects on non-target arthropods of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri	Ethofumesate 500 SC	Laboratory test Glass plates (2D)	LR_{50} = >1000 g a.s./ha ER_{50} = >1000 g a.s./ha	EFSA Conclusion 4374/2016
~~*Typhlodromus pyri*~~	~~Ethofol 500 SC~~	~~Laboratory test~~ ~~Glass plates (2D)~~	~~LR_{50} = >1000 g a.s./ha~~ ~~ER_{50} = >1000 g a.s./ha~~	~~EFSA Conclusion 4374/2016~~
Aphidius rhopalosiphi	Ethofumesate 500 SC	Laboratory test Glass plates (2D)	LR_{50} = >1000 g a.s./ha ER_{50} = >1000 g a.s./ha	EFSA Conclusion 4374/2016
~~*Aphidius rhopalosiphi*~~	~~Ethofol 500 SC~~	~~Laboratory test~~ ~~Glass plates (2D)~~	~~LR_{50} = >1000 g a.s./ha~~ ~~ER_{50} = >1000 g a.s./ha~~	~~EFSA Conclusion 4374/2016~~
Aleochara bilneata	Tramat 500	Laboratory test Quartz sand (2D)	LR_{50} = >1252.5 g a.s./ha ER_{50} = >1252.5 g a.s./ha	EFSA Conclusion 4374/2016
Chrysoperla carnea	Tramat 500	Laboratory test Glass plates (2D)	LR_{50} = >2000 g a.s./ha ER_{50} = >2000 g a.s./ha	EFSA Conclusion 4374/2016
Poecilus cupreus	Tramat 500	Laboratory test Quartz sand (2D)	LR_{50} = >2000 g a.s./ha	EFSA Conclusion 4374/2016
Typhlodromus pyri (protonymphs)	AG-E1-500 SC1	Laboratory test Glass plates (2D)	LR_{50} = >5000 g a.s./ha ER_{50} = >50000 g a.s./ha	Röhlig U./ 2020a/ 20 48 NTL 0001
Aphidius rhopalosiphi	AG-E1-500 SC1	Extended laboratory test wheat plants (3D)	ER_{50} = >50000 g a.s./ha NOER = 50000 g a.s./ha Red. of reproduction: 1.0 % at 5000 g a.s./ha 4.3 % at 2810 g a.s./ha -1.9 % at 1580 g a.s./ha -4.3 % at 890 g a.s./ha 2.4 % at 500 g a.s./ha	Röhlig U./ 2020b/ 20 48 NAL 0001
Field or semi-field tests				
None				

zRMS comments:

The toxicity endpoints for the representative formulations presented in Table 9.7-1 are in line with the EU agreed data reported in EFSA Journal 2016;14(1):4374. Formulation Ethofumesate 500 SC (Tramat 500) was a representative formulation of the ethofumesate Task Force comprising of ADAMA (Applicant for AG-E1-500 SC1) and Bayer CropScience AG. Analysis of the information available in Volume 4 (2015) and Part C of the

Core Assessment for AG-E1-500 SC1 indicates some differences in compositions of both products, but in opinion of the zRMS with no impact on the ecotoxicological profile. Taking this into account, results for Ethofumesate 500 SC (Tramat 500) may be used in the risk assessment performed for AG-E1-500 SC. Formulation Ethofol 500 SC belongs to another Applicant not being a part of the ethofumesate EU Task Force and results of studies performed with this product will be thus not used in the risk assessment.

Studies on toxicity of AG-E1-500 SC to non-target arthropods were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.7-1 are confirmed to be correct.

9.7.1.1 Justification for new endpoints

The non-target arthropod endpoints presented in EFSA Conclusion 4374/2016 are derived from studies carried out with the representative formulations used in the active substance renewal. AG-E1-500-SC1 was not a representative formulation. Non-target arthropod risk assessments in this dRR were carried out using the endpoints from studies on AG-E1-500 SC1, the results of which are comparable to the EU agreed endpoints.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods 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 in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of AG-E1-500 SC1 in sugar beet/fodder beet

Intended use	**AG-E1-500-SC1 in sugar beet/fodder beet**		
Active substance/product	Ethofumesate		
Application rate (g/ha)	1 x 1000 g/ha*		
MAF	1.0		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri (Tier I, 2D)	>5000	1000	<0.2
Aphidius rhopalosiphi (Tier II, 3D)	>5000		<0.2

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in bold breach the relevant trigger. * This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application

As outlined in the table above, an acceptable in-field risk can be concluded in Tier 1 for the indicator species *T. pyri* and *A. rhopalosiphi* for intended uses.

zRMS comments:

The in-field risk assessment presented in Table 9.7-2 above is in general agreed by the zRMS. It is, however, noted that LR_{50} value for *Aphidius rhopalosiphi* originates from Tier II study with AG-E1-500 SC1 and no Tier I study was performed with this species. Taking this into account, risk posed to additional species should be also taken into account in order to conclude acceptable in-field risk. Respective information has been added in Table 9.7-2 for clarity.

No Tier I or Tier II studies on toxicity to additional arthropod species were performed with AG-E1-500 SC1. However, in the course of the EU review laboratory studies with *Aleochara bilineata*, *Chrysoperla carnea* and

Poecilus cupreus were performed with similar formulation Ethofumesate 500 SC (aka Tramat 500) and their results may be used in the risk assessment performed for AG-E1-500 SC1, since based on analysis of information available in Volume 4 and Part C differences in composition of both formulations are not expected to have any impact on the ecotoxicological profile.

LR_{50} values for three additional species tested ranged from >1252.5 to >2000 g a.s./ha and were higher than the maximum cumulative application rate of AG-E1-500 SC1 (1000 g a.s./ha). On this basis acceptable in-field risk may be concluded from all intended uses of AG-E1-500 SC1 in the Central Zone (including split applications).

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of AG-E1-500 SC1 in sugar beet/fodder beet

Intended use	**AG-E1-500-SC1 in sugar beet/fodder beet**				
Active substance/product	Ethofumesate				
Application rate (g/ha)	1 x 1000 g/ha*				
MAF	1.0				
vdf	10 / 5 (Tier 1, 2D)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri (Tier I, 2D, vdf = 10)	>5000	2.77	2.77	10	<0.00554
Typhlodromus pyri (Tier I, 2D, vdf = 5)	>5000		5.54		<0.001
Aphidius rhopalosiphi (Tier II, 3D)	>5000		27.7		<0.0554 ~~0.00554~~

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger. * This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application.

As outlined in the table above, an acceptable off-field risk can be concluded in Tier 1 for the indicator species *T. pyri* and *A. rhopalosiphi* for intended uses.

zRMS comments:

The off-field risk assessment for *Typhlodromus pyri* presented in Table 9.7-3 above is agreed by the zRMS. However, the risk assessment for Aphidius rhopalosiphi was originally based on the same exposure estimates, although the LR_{50} value originates from Tier II study performed in 3D system (wheat plants) and for this reason no VDF should be considered. Respective calculations were thus introduced by the zRMS in Table 9.7-3.

It is also noted that currently at the EU level there is discussion on the VDF to be used in calculation of the off-field exposure since available data indicate that VDF of 10 recommended by the ESCORT 2 guidance is underprotective. According to indications of EFSA supporting publication 2019:EN-1673, VDF of 5 may be considered as sufficient interim solution. However, it is also indicated that such an interim solution should be reflected in the current guidance document for terrestrial ecotoxicology (SANCO/10329/2002 rev 2 final) and its implementation should be further discusses. Since publication of the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology, the terrestrial guidance was not updated and no further discussion took place regarding implementation of VDF of 5 into the risk assessment scheme in line with indications of EFSA (2019). Taking this into account, consideration of VDF of 5 is not yet mandatory. Nevertheless, for convenience of the cMS that do prefer VDF of 5, additional calculations were performed by the zRMS and included in Table 9.7-3. Since VDF is applicable only for 2D study, calculations were performed only for *T. pyri* since for *A. rhopalisphi* only 3D study was available.

Overall, acceptable risk could be concluded for both species with no need for risk mitigation measures, regardless of the VDF.

Additionally it is noted by the zRMS that LR_{50} value for *A.rhopalosiphi* originates from Tier II study with AG-

E1-500 SC1 and no Tier I study was performed with this species. Taking this into account, risk posed to additional species should be also taken into account in order to conclude acceptable off-field risk. Respective information has been added in Table 9.7-3 for clarity.

No Tier I or Tier II studies on toxicity to additional arthropod species were performed with AG-E1-500 SC1. However, in the course of the EU review laboratory studies with *Aleochara bilineata*, *Chrysoperla carnea* and *Poecilus cupreus* were performed with similar formulation Ethofumesate 500 SC (aka Tramat 500) and their results may be used in the risk assessment performed for AG-E1-500 SC1, since based on analysis of information available in Volume 4 and Part C differences in composition of both formulations are not expected to have any impact on the ecotoxicological profile.

LR_{50} values for three additional species tested ranged from >1252.5 to >2000 g a.s./ha and were considerably higher than the maximum off-field rate of AG-E1-500 SC1 (277 g a.s./ha, including correction factor of 10). On this basis acceptable off-field risk may be concluded from all intended uses of AG-E1-500 SC in the Central Zone (including split applications) with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk to non-target arthropods from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable. Risk mitigation measure is not required.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Ethofumesate 500 SC~~	~~Overspray 56 d, chronic 10 % peat content~~	~~NOEC = 25 mg a.s./kg dw NOEC$_{corr}$ = 12.5 mg a.s./kg dw*~~	~~EFSA Conclusion 4374/2016~~
Eisenia andrei	AG-E1-500 SC1	Mixed into substrate 56 d, chronic, 10 % peat content	NOEC = 120.5 mg product/kg dw (53.3 mg a.s./kg dw) EC_{10} = 137.4 mg product/kg dw (60.8 mg a.s./kg dw) **$NOEC_{corr}$ = 26.7 mg a.s./kg dws**	Friedrich S./ 2020a/ 20 48 TEC 0002
Eisenia fetida	Metabolite NC 8493	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 16 mg/kg dw (max. concentration tested)	EFSA Conclusion 4374/2016
Eisenia fetida	Metabolite NC 8493	Mixed into substrate 56 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw** (limit test)	EFSA Conclusion 4374/2016
Eisenia fetida	Metabolite NC 20645	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100 mg/kg dw	EFSA Conclusion 4374/2016
~~*Folsomia candida*~~	~~Ethofol 500 SC~~	~~Mixed into substrate, 28 d, chronic, 5 % peat content~~	~~NOEC = 26.7 mg a.s./kg dw NOEC$_{corr}$ = 13.35 mg a.s./kg dw*~~	~~EFSA Conclusion 4374/2016~~
Folsomia candida	AG-E1-500 SC1	Mixed into substrate, 28 d, chronic, 5 % peat content	NOEC = 349 mg product/kg dw (154 mg a.s./kg dw) EC_{10} = 378 mg product/kg dw (167 mg a.s./kg dw) **$NOEC_{corr}$ = 77.0 mg a.s./kg dws**	Friedrich S./ 2020b/ 20 48 TCC 0003
Folsomia candida	Metabolite NC 8493	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC =556 mg/kg dw**	EFSA Conclusion 4374/2016
Folsomia candida	Metabolite NC 20645	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 100 mg/kg dw (max. dose tested)	EFSA Conclusion 4374/2016
~~*Hypoaspis aculeifer*~~	~~Ethofumesate 500 SC~~	~~Mixed into substrate, 14 d, chronic, 5 % peat content~~	~~NOEC = 44.2 mg a.s./kg dw NOEC$_{corr}$ = 22.1 mg a.s./kg dw*~~	~~EFSA Conclusion 4374/2016~~

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	AG-E1-500 SC1	Mixed into substrate, 14 d, chronic, 5 % peat content	NOEC = 1130 mg product/kg dw (500 mg a.s./kg dw, max. dose tested) EC_{10} >1130 mg product/kg dw (>500 mg a.s./kg dw) **$NOEC_{corr}$ = 250 mg a.s./kg dws**	Friedrich S./ 2020c/ 20 48 THC 0002
Hypoaspis aculeifer	Metabolite NC 8493	Mixed into substrate 14 d, chronic 5 % peat content	**NOEC = 309 mg/kg dw**	EFSA Conclusion 4374/2016
Field studies				
None				
Litter bag test				
None				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Values highlighted in **bold** were used in the risk assessment

zRMS comments:

The toxicity data for the representative formulations and ethofumesate metabolites given in Table 9.8-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374. Additional information on chronic toxicity of metabolite NC 8493 has been added by the zRMS to Table 9.8-1 as being initially not presented by the Applicant.

Analysis of the information available in Volume 4 (2015) for Ethofumesate 500 SC (being the representative formulation of the ethofumesate Task Force comprising of ADAMA and Bayer CropScience AG) and Part C of the Core Assessment for AG-E1-500 SC1 indicates some differences in compositions of both products, but in opinion of the zRMS they have no significant impact on the ecotoxicological profile. Nevertheless, as toxicity to all relevant soil species was investigated in studies performed with the formulation being a subject of this zonal assessment, in opinion of the zRMS the risk assessment should be based on these data and not results of studies performed with another formulation, even if comparable with AG-E1-500 SC1.
Formulation Ethofol 500 SC belongs to the EU Applicant not being part of the Task Force and was also not considered in the risk assessment for AG-E1-500 SC1.

Endpoints for metabolite NC 20645 are retained in Table 9.8-1 as being reported in the LoEP, however no risk assessment is required for this compound which was formed in soil at max 4.8% and was not included in soil residue definition for the risk assessment provided in EFSA Journal 2016;14(1):4374.

Studies on toxicity of AG-E1-500 SC to earthworms, *Folsomia candida* and *Hypoaspis aculeifer* were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.8-1 are confirmed to be correct with some additional information added by the zRMS.

All endpoints that were considered not relevant for the risk assessment for AG-E1-500 SC1 were struck through in Table 9.8-1 above.

9.8.1.1 Justification for new endpoints

New studies and endpoints are provided for the formulated product AG-E1-500 SC1 to address new data requirements.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for ethofumesate.

Although tests using earthworm and the non-target soil organism *F. candida* have been conducted with the metabolite NC 20645 (see Table 9.8-1), this metabolite will not be included in the following risk assessment, since it is not a major metabolite in soil (water/sediment metabolite).

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of AG-E1-500 SC1 in sugar beet/fodder beet

Intended use	Sugar beet (2 x 1 L product/ha, BBCH 10-18)		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg a.s./kg dw)**	**PEC_{soil} (mg a.s./kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
~~Ethofumesate~~	~~12.5~~	~~1.122[a]~~	~~11.14~~
AG-E1 500 SC1	26.7 * ~~120.5~~	1.1332[a] ~~1.195[b]~~	23.6 ~~100.8~~
NC8493	100	0.2325[b] ~~0.116[b]~~	430.1 ~~862.1~~
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
F. candida			
~~Ethofumesate~~	~~13.35~~	~~1.122[a]~~	~~11.90~~
AG-E1-500 SC1	77.0 * ~~349~~	1.1332[a] ~~1.195[b]~~	67.9 ~~292.1~~
NC8493	556	0.2325[b] ~~0.116[b]~~	2391 ~~4793~~
H. aculeifer			
~~Ethofumesate~~	~~22.1~~	~~1.122[a]~~	~~19.70~~
AG-E1-500 SC1	250 * ~~1130~~	1.1332[a] ~~1.195[b]~~	220.6 ~~945.6~~
NC8493	309	0.2325[b] ~~0.116[b]~~	1329 ~~2664~~

TER values shown in bold fall below the relevant trigger.
[a] $PEC_{accumulation}$, [b] $PEC_{soil\ initial}$
* endpoint corrected due to log Pow >2

The acute and chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to ethofumesate and its relevant metabolite NC 8493 are greater than the triggers of 10 and 5, respectively. The same results were gained for the formulation AG-E1-500 SC1,

indicating that the acute and chronic risk to earthworms and other non-target soil organisms is acceptable following application according to the intended uses.

zRMS comments:

No toxicity data for ethofumesate were available from the EU review and all endpoints for soil macro- and meso-fauna reported in the LoEP were derived from the studies performed with the representative formulations. In opinion of the zRMS in absence of the active substance endpoints the risk assessment should be performed with consideration of endpoints derived from studies performed with formulation under evaluation (here: AG-E1-500 SC1) and the risk assessment based on endpoints for representative formulations considered to be active substance data is not relevant, since these are not active substance data. It should be also noted that in line with indications of the Commission Regulation (EU) No 283/2013:

In the case of certain study types, the use of a representative plant protection product instead of the active substance as manufactured may be more appropriate, for example testing of non-target arthropods, bees, earthworm reproduction, soil micro-flora and non-target terrestrial plants [...]

Taking this into account, the risk assessment based on results of the formulation under evaluation is sufficient and no additional calculations for the active substance are deemed necessary, provided that all respective toxicity data for the evaluated formulation are available.

Taking this into account the risk assessment presented in Table 9.8-2 was amended accordingly:

- calculations based on the representative formulations endpoints were struck through,
- the corrected endpoints for AG-E1-500 SC1 were expressed in terms of the active substance and compared with $PEC_{SOIL,ACCU}$ agreed in area of Section 8.

The risk assessment for metabolite NC 8493 presented in Table 9.8-2 was corrected by the zRMS with consideration of PEC_{SOIL} agreed in area of Section 8. It was noted that two toxicity endpoints are available for earthworms (NOEC of 16 and 100 mg pm/kg dws). Nevertheless, consideration of the higher value is justified, since both endpoints were set to the maximum concentration tested.

All calculations were based on PEC_{SOIL} values estimated from application of the maximum cumulative application rate of AG-E1-500 SC1 (1000 g a.s./ha), being protective also for the split applications.

The zRMS agrees with the Applicant to exclude metabolite NC 20645 from the risk assessment, since this compound was formed in soil at max 4.8% and was not included in the soil residue definition for the risk assessment provided in EFSA Journal 2016;14(1):4374.

Overall, based on the updated calculations, acceptable risk to earthworms and other relevant soil macro-organisms may be concluded from all intended uses of AG-E1-500 SC.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risk to non-target soil organisms from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of AG-E1-500 SC1 were not evaluated as part of the EU assessment of active substance 1. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
~~N-mineralisation~~	~~Ethofol 500 SC~~	~~28 d, aerobic soil type~~	~~-4.38% effect at 1.29 mg a.s./kg soil dw~~ ~~-18.87% effect at 6.47 mg a.s./kg soil dw~~	~~EFSA Conclusion 4374/2016~~
~~N-mineralisation~~	~~Ethofumesate 500 SC~~	~~42 d, aerobic soil type~~	~~-16.0% effect at 1.3 mg a.s./kg soil dw~~ ~~-14.3% effect at 13.0 mg a.s./kg soil dw~~	~~EFSA Conclusion 4374/2016~~
N-mineralisation	AG-E1-500 SC1	28 d, aerobic soil type	+6.5% effect at 1.51 mg product/kg soil dw (0.71 mg a.s./kg soil dw) -6.5% effect at 15.07 mg product/kg soil dw (7.05 mg a.s./kg soil dw)	Persdorf U./ 2020/ 20 48 SMN 0003
N-mineralisation	Metabolite NC 8493	28 d, aerobic loamy sand soil	-1.4% effect at 1.20 mg/kg soil dw -15.2% effect at 12 mg/kg soil dw	EFSA Conclusion 4374/2016
N-mineralisation	Metabolite NC 20645	28 d, aerobic loamy sand soil	+6.9% effect at 1.38 mg/kg soil dw +6.7% effect at 13.8 mg/kg soil dw	EFSA Conclusion 4374/2016

zRMS comments:

The toxicity data for the representative formulations and ethofumesate metabolites given in Table 9.9-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

Analysis of the information available in Volume 4 (2015) for Ethofumesate 500 SC (being the representative formulation of the ethofumesate Task Force comprising of ADAMA and Bayer CropScience AG) and Part C of the Core Assessment for AG-E1-500 SC1 indicates some differences in compositions of both products, but in opinion of the zRMS they have no significant impact on the ecotoxicological profile. Nevertheless, as effect on soil nitrogen transformation were investigated in study performed with the formulation being a subject of this zonal assessment, in opinion of the zRMS the risk assessment should be based on these data and not results of studies performed with another formulation, even if comparable with AG-E1-500 SC1.
Formulation Ethofol 500 SC belongs to the EU Applicant not being part of the Task Force and was also not considered in the risk assessment for AG-E1-500 SC1.

Endpoints for metabolite NC 20645 are retained in Table 9.8-1 as being reported in the LoEP, however no risk

assessment is required for this compound which was formed in soil at max 4.8% and was not included in soil residue definition for the risk assessment provided in EFSA Journal 2016;14(1):4374.

Study on effects of AG-E1-500 SC on soil nitrogen transformation was evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.9-1 are confirmed to be correct with some additional information added by the zRMS.

All endpoints that were considered not relevant for the risk assessment for AG-E1-500 SC1 were struck through in Table 9.9-1 above.

9.9.1.1 Justification for new endpoints

New studies and endpoints are provided for the formulated product AG-E1-500 SC1 to address new data requirements.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Although tests on the effects on soil nitrogen mineralisation have been conducted with the metabolite NC 20645 (see Table 9.8 1), this metabolite will not be included in the following risk assessment, since it is not a major metabolite in soil (water/sediment metabolite).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of AG-E1-500 SC1 on sugar beet

Intended use	Sugar beet (2 x 1 L product/ha, BBCH 10-18)		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
~~Ethofumesate~~	~~13.0 (at 42 d)~~	~~1.122[a]~~	~~yes~~
AG-E1-500 SC1	7.05 ~~15.07~~ (at 28 d)	1.1332[a] ~~1.195[b]~~	yes
NC8493	12 (at 28 d)	0.2325[b] ~~0.116[b]~~	yes

[a] $PEC_{accumulation}$, [b] $PEC_{soil\ initial}$

zRMS comments:

No toxicity data for ethofumesate were available from the EU review and all endpoints related to effects on soil nitrogen transformation reported in the LoEP were derived from the studies performed with the representative formulations. In opinion of the zRMS in absence of the active substance endpoints the risk assessment should be performed with consideration of endpoints derived from studies performed with formulation under evaluation (here: AG-E1-500 SC1) and the risk assessment based on endpoints for representative formulations considered to be active substance data is not relevant, since these are not active substance data. It should be also noted that in line with indications of the Commission Regulation (EU) No 283/2013:

In the case of certain study types, the use of a representative plant protection product instead of the active substance as manufactured may be more appropriate, for example testing of non-target arthropods, bees, earthworm reproduction, soil micro-flora and non-target terrestrial plants [...]

Taking this into account, the risk assessment based on results of the formulation under evaluation is sufficient and no additional calculations for the active substance are deemed necessary, provided that all respective toxicity data for the evaluated formulation are available.

Taking this into account the risk assessment presented in Table 9.9-2 was amended accordingly:

- risk assessment based on the representative formulation endpoints was struck through,
- the endpoint for AG-E1-500 SC1 was expressed in terms of the active substance and compared with $PEC_{SOIL,ACCU}$ agreed in area of Section 8.

The risk assessment for metabolite NC 8493 presented in Table 9.9-2 was corrected by the zRMS with consideration of PEC_{SOIL} agreed in area of Section 8.

The risk assessment was based on PEC_{SOIL} values estimated from application of the maximum cumulative application rate of AG-E1-500 SC1 (1000 g a.s./ha), being protective also for the split applications.

The zRMS agrees with the Applicant to exclude metabolite NC 20645 from the risk assessment, since this compound was formed in soil at max 4.8% and was not included in the soil residue definition for the risk assessment provided in EFSA Journal 2016;14(1):4374.

Overall, based on the updated evaluation, no unacceptable effects on soil microbial activity are expected from all intended uses of AG-E1-500 SC in the Central Zone (including split applications).

9.9.3 Overall conclusions

No effects outside a range of ± 25 % compared to the control were observed at exposure levels which clearly exceed the maximum exposure levels calculated in consideration of the worst-case application scenario for AG-E1-500 SC1. The risk to soil microbial activity from the use of ethofumesate applied as the formulation AG-E1-500 SC1 to sugar beet is acceptable.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of AG-E1-500 SC1 were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
~~*Avena sativa*~~	~~Ethefol 500 SC~~	~~21 d Seedling emergence~~	~~ER_{50} = 0.328 L prod/ha~~	~~EFSA Conclusion 4374/2016~~
~~*Triticum aestivum*~~	~~Ethofumesate 500 SC~~	~~21 d Seedling emergence~~	~~ER_{50} = 0.101 L prod/ha~~	~~EFSA Conclusion 4374/2016~~
~~Species Sensitivity Distribution (SSD)~~	~~Ethofumesate 500 SC~~	~~21 d Seedling emergence~~	~~HR_5 = 0.191 L prod/ha (mean)~~	~~EFSA Conclusion 4374/2016~~
~~*Avena sativa*~~	~~Ethefol 500 SC~~	~~21 d Vegetative vigour~~	~~ER_{50} > 2 L prod/ha~~	~~EFSA Conclusion 4374/2016~~
~~*Triticum aestivum*~~	~~Ethofumesate 500 SC~~	~~21 d Vegetative vigour~~	~~ER_{50} = 1.24 L prod/ha~~	~~EFSA Conclusion 4374/2016~~
Fagopyrum esculentum[1] d *Glycine max*[2] d *Helianthus annuus*[3] d *Lepidium sativum*[4] d *Linum usitatissimum*[5] d *Medicago sativa*[6] d *Solanum lycopersicum*[7] d *Vigna radiata*[8] d *Hordeum vulgare*[9] m *Triticum aestivum*[10] m	AG-E1-500 SC1	21 d Seedling emergence	ER_{50} shoot dry weight = 0.098 L prod/ha *Triticum aestivum*[10] m	Duffner A./ 2020a/ S19-22437
Fagopyrum esculentum[1] d *Glycine max*[2] d *Helianthus annuus*[3] d *Lepidium sativum*[4] d *Linum usitatissimum*[5] d *Medicago sativa*[6] d *Solanum lycopersicum*[7] d *Vigna radiata*[8] d *Hordeum vulgare*[9] m *Triticum aestivum*[10] m	AG-E1-500 SC1	21 d Vegetative vigour	ER_{50} shoot dry weight = 0.37 L prod/ha *Medicago sativa*[6] d	Duffner A./ 2020b/ S19-22438

m: monocotyledonous; d: dicotyledonous

zRMS comments:

The toxicity data for the representative formulations and ethofumesate metabolites given in Table 9.10-1 are in line with the EU agreed endpoints reported in EFSA Journal 2016;14(1):4374.

Analysis of the information available in Volume 4 (2015) for Ethofumesate 500 SC (being the representative formulation of the ethofumesate Task Force comprising of ADAMA and Bayer CropScience AG) and Part C of the Core Assessment for AG-E1-500 SC1 indicates some differences in compositions of both products, but in opinion of the zRMS they have no significant impact on the ecotoxicological profile. Nevertheless, as effect on seedling emergence and vegetative vigour were investigated in studies performed with the formulation being a subject of this zonal assessment, in opinion of the zRMS the risk assessment should be based on these data and not results of studies performed with another formulation, even if comparable with AG-E1-500 SC1.
Formulation Ethofol 500 SC belongs to the EU Applicant not being part of the Task Force and was also not considered in the risk assessment for AG-E1-500 SC1.

Studies on toxicity of AG-E1-500 SC to non-target terrestrial plants were evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.10-1 are confirmed to be correct.

It was noted by the zRMS that according to the GAP in case of use No 3 application of AG-E1-500 SC as a tank mixture with another herbicide (Goltix Titan 565 SC) and adjuvant (Atpolan BIO 80 EC) is recommended. Goltix Titan 565 SC contains metamitron and quinmerac, which belong to different chemical groups than ethofumesate and for this reason no specific risk assessment is deemed necessary for this mixture, in line with current requirements. It should be, however, pointed out that adjuvants are added in order to increase efficacy of the formulated product and it cannot be excluded that their addition to the mixture with AG-E1-500 SC1 would lead to more pronounced toxic effects on non-target terrestrial plants. However, no study on effects of AG-E1-500 SC1 with adjuvant were performed by the Applicant and available data are not sufficient to conclude on the risk resulting from simultaneous exposure of NTTPs to AG-E1-500 SC1 and the adjuvant. Taking this into account, recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are provided by the Applicant.

All endpoints that were considered not relevant for the risk assessment for AG-E1-500 SC1 were struck through in Table 9.10-1 above.

9.10.1.1 Justification for new endpoints

New studies and endpoints are provided for the formulated product AG-E1-500 SC1 to address new data requirements.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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

Table 9.10-2: Assessment of the risk for non-target plants due to the use of 1 x 2 L AG-E1-500 SC1/ha in sugar beet/fodder beet

Intended use	AG-E1-500 SC1 in sugar beet/fodder beet			
Active substance/product	Ethofumesate			
Application rate (g/ha)	1 x 2 L product/ha*			
MAF	1			
Test species	**ER_{50} (L product/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Triticum aestivum (seedling emergence)	0.098	0.0277	0.0554	**1.769**

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger. * This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application.

The calculation in the table above is carried out with the worst-case scenario of one application of 2L product/ha. This use is not proposed in the GAP table provided in section 9.1., therefore further risk calculations are presented in the tables below using the details from the GAP.

Table 9.10-3: Assessment of the risk for non-target plants due to the use of 2 x 1 L AG-E1-500 SC1/ha in sugar beet/fodder beet

Intended use	AG-E1-500 SC1 in sugar beet/fodder beet			
Active substance/product	Ethofumesate			
Application rate (g/ha)	2 x 1 L product/ha			
MAF	1.7			
Test species	**ER_{50} (L product/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Triticum aestivum (seedling emergence)	0.098	0.0238 ~~0.0277~~	0.040 ~~0.047~~	**2.45** ~~**2.081**~~

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.10-4: Assessment of the risk for non-target plants due to the use of 3 x 0.66 L AG-E1-500 SC1/ha in sugar beet/fodder beet

Intended use	AG-E1-500 SC1 in sugar beet/fodder beet			
Active substance/product	Ethofumesate			
Application rate (g/ha)	3 x 0.6 ~~0.66~~ L product/ha			
MAF	2.3			
Test species	**ER_{50} (L product/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Triticum aestivum	0.098	0.0201 ~~0.0277~~	0.028 ~~0.0420~~	**3.5** ~~**2.331**~~

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

zRMS comments:

The worst case risk assessment performed in Table 9.10-2 with consideration of the cumulative application rate is agreed by the zRMS. No acceptable risk could be concluded and for this reason the Applicant performed TER calculations based on the detailed GAP.

Calculations presented in Tables 9.10-3 and 9.10-4 were performed with consideration of MAF in order to account for multiple applications, but the drift rate relevant for single application was assumed, although drift rates for multiple applications are considerably lower (2.38% and 2.01% for two and three applications, respectively).
It should be also noted that in line with indications of SANCO/10329/2002 rev. 2 final, single application rate should be considered in the risk assessment for non-target terrestrial plants and for this reason the correct exposure estimates should be calculated for single application rate, drift rate relevant for single application and MAF of 1.

Nevertheless, as some Member States do require consideration of multiple applications, calculations presented in Tables 9.10-3 and 9.10-4 were retained for their convenience, but with off-field rates corrected for the respective drift values.

It was also noted that for none of uses indicated in GAP table application at 3x0.66 L/ha is being proposed, so the rate in Table 9.10-4 was corrected to 3x0.6 L/ha, in line with the Central Zone GAP.
The corrected calculations also resulted with TER values below the trigger of 5 and refined risk assessment performed with consideration of the risk mitigation measures is presented in point 9.10.2.4 below.

For Member States that entirely follow indications of the SANCO/10329/2002 rev. 2 final (like e.g. Poland) the risk assessment based on the single application rate is presented below.

Intended use	AG-E1-500 SC1 in sugar beet/fodder beet			
Active substance/product	Ethofumesate			
Application rate (g/ha)	2 x 1 L product/ha			
Test species	**ER_{50} (L product/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Triticum aestivum (seedling emergence)	0.098	0.0277	0.0277	**3.5**

Intended use	AG-E1-500 SC1 in sugar beet/fodder beet			
Active substance/product	Ethofumesate			
Application rate (g/ha)	3 x 0.6 L product/ha			
Test species	**ER_{50} (L product/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Triticum aestivum (seedling emergence)	0.098	0.0277	0.017	5.8

When indications of the terrestrial guidance are followed, no acceptable risk from application of AG-E1-500 SC1 may be concluded for application ad 2x1.0 L/ha and further assessment is performed in point 9.10.2.4 below.

For split application at 3x0.6 L/ha acceptable risk to non-target plants may be concluded with no need for risk mitigation measures.

All calculations were performed using the lowest available endpoint among all species tested in both studies (seedling emergence and vegetative vigour).

Concerned Member States must decide which option (with MAF or without MAF) is relevant in their countries.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-5: Risk assessment for non-target terrestrial plants due to the use 1 x 2 L AG-E1-500 SC1/ha in sugar beet/fodder beet) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		AG-E1-500 SC1 in sugar beet/fodder beet		
Active substance/product		Ethofumesate		
Application rate (g/ha)		1 x 2 L product/ha*		
MAF		1		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**
1 m (default for field crops) ~~1/3~~	0.0277	0.0554	0.0277	0.0139
5	0.0057	0.0114	n/c	n/c
Toxicity value		**TER**		
ER_{50} = 0.098 L product/ha		**criterion: TER ≥ 5**		
1 m (default for field crops) ~~1/3~~		**1.769**	**3.538**	7.05 ~~7.076~~
5		8.597	n/c	n/c

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger. * This is in accordance with EFSA Journal 2016;14(1):4374 which states that split applications are covered by the risk assessment for the single application. n/c not calculated.

The calculation in the table above is carried out with the worst-case scenario of one application of 2L product/ha. This use is not proposed in the GAP table provided in section 9.1., therefore further risk calculations are presented in the tables below using the details from the GAP.

Table 9.10-6: Risk assessment for non-target terrestrial plants due to the use 2 x 1 L AG-E1-500 SC1/ha in sugar beet/fodder beet) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		AG-E1-500 SC1 in sugar beet/fodder beet		
Active substance/product		Ethofumesate		
Application rate (g/ha)		2 x 1 L product/ha		
MAF		1.7		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**
1 m (default for field crops) ~~1/3~~	0.0238 ~~0.0277~~	0.040 ~~0.047~~	0.020 ~~0.024~~	0.010 ~~0.012~~
5	0.0047 ~~0.0057~~	0.008 ~~0.010~~	n/c	n/c
Toxicity value		**TER**		
ER_{50} = 0.098 L product/ha		**criterion: TER ≥ 5**		
1 m (default for field crops) ~~1/3~~		**2.42** ~~**2.081**~~	**4.84** ~~**4.162**~~	9.69 ~~8.325~~
5		12.3 ~~10.114~~	n/c	n/c

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger. n/c not calculated.

Table 9.10-7: Risk assessment for non-target terrestrial plants due to the use 3 x 0.66 L AG-E1-500 SC1/ha in sugar beet/fodder beet) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use **Active substance/product** **Application rate (g/ha)** **MAF**		AG-E1-500 SC1 in sugar beet/fodder beet Ethofumesate 3 x 0.6 ~~0.66~~ L product/ha 2.3		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**
1 m (default for field crops) ~~1/3~~	0.00201 ~~0.0277~~	0.028 ~~0.042~~	0.014 ~~0.021~~	n/c ~~0.011~~
5	0.0041 ~~0.0057~~	0.006 ~~0.009~~	n/c	n/c
Toxicity value ER_{50} = 0.098 L product/ha		**TER** **criterion: TER ≥ 5**		
1 m (default for field crops) ~~1/3~~		**3.5** ~~**2.331**~~	7.1 ~~**4.661**~~	n/c ~~9.323~~
5		17.3 ~~11.33~~	n/c	n/c

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger. n/c not calculated.

zRMS comments:

The worst case risk assessment performed in Table 9.10-5 with consideration of the cumulative application rate is agreed by the zRMS. Acceptable risk to non-target terrestrial plants could be concluded provided that 5 m unsprayed buffer zone to non-agricultural land is respected or the spray drift is reduced by 75% using appropriate drift reducing techniques.

In calculations performed for the detailed GAP the same mistakes as in point 9.10.2.2 were made, i.e. multiple applications were considered together with spray drift relevant for the single application.
When respective corrections were made by the zRMS in Tables 9.10-6 and 9.10-7, acceptable risk to non-target terrestrial plants could be concluded provided that following risk mitigation measures are respected:

- For applications at 2x1.0 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 75% using appropriate drift reducing techniques.
- For applications at 3x0.6 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 50% using appropriate drift reducing techniques.

It should be also noted that above risk mitigation measures would be relevant in Member States that require consideration of multiple applications in the risk assessment for non-target terrestrial plants. However, in line with indications of SANCO/10329/2002 rev. 2 final, single application rate should be considered in the risk assessment for non-target terrestrial plants and for this reason the correct exposure estimates should be calculated for single application rate, drift rate relevant for single application and MAF of 1. Therefore, for Member States that entirely follow indications of the SANCO/10329/2002 rev. 2 final (like e.g. Poland) the risk mitigation measures relevant for the single rate at 1.0 L/ha were identified and are presented below. For application at 3x0.6 L/ha no risk mitigation measures are deemed necessary when approach from the terrestrial guidance is taken into account (for details of calculation, please refer to commenting box in point 9.10.2.2 above).

Intended use **Active substance/product** **Application rate (g/ha)**		AG-E1-500 SC1 in sugar beet/fodder beet Ethofumesate 2 x 1.0 L product/ha		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**
1 m (default for field crops)	0.0277	0.028	0.014	n/c
5	0.0057	0.006	n/c	n/c
Toxicity value ER_{50} = 0.098 L product/ha		**TER** **criterion: TER ≥ 5**		
1 m (default for field crops)		**3.5**	7.1	n/c
5		17.2	n/c	n/c

When indications of the terrestrial guidance are followed, acceptable risk from application of AG-E1-500 SC1 at 2x1.0 L/ha may be concluded provided that 5 m unsprayed buffer zone to non-agricultural land is respected or the spray drift is reduced by 50% using appropriate drift reducing techniques.

All calculations were performed using the lowest available endpoint among all species tested in both studies (seedling emergence and vegetative vigour).

In line with information provided in the commenting box in point 9.10.1 above, formulation AG-E1-500 SC1 cannot be used as tank mixture with adjuvant Atpolan BIO 80 EC until respective data addressing potentially higher toxicity of this mixture to NTTPs are submitted by the Applicant. Recommendation on use of the product with adjuvant is thus deleted in the GAP table and the product label.

Concerned Member States must decide on applicability of the proposed risk mitigation measures and which option (with MAF or without MAF) is relevant in their countries.

9.10.3 Overall conclusions

The risk assessment for non-target terrestrial plants from AG-E1-500 SC1 was performed using two approaches:

- with consideration of the multiple applications (not fully in line with SANCO/10329/2002 rev. 2 final)
- with consideration of the single application (fully in line with SANCO/10329/2002 rev. 2 final).

Depending on the approach, acceptable risk could be concluded with various risk mitigation measures:

3. Approach accounting for multiple applications:
   - For applications at 2x1.0 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 75% using appropriate drift reducing techniques.
   - For applications at 3x0.6 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 50% using appropriate drift reducing techniques.
4. Approach performed with assumption of single application rate:
   - For applications at 2x1.0 L/ha: 5 m unsprayed buffer zone to non-agricultural land or reduction of the spray drift by 50% using appropriate drift reducing techniques.
   - For applications at 3x0.6 L/ha: no risk mitigation measures necessary.

Concerned Member States must decide on applicability of the proposed risk mitigation measures and which option (with MAF or without MAF) is relevant in their countries.

Since no studies on effects of AG-E1-500 SC1 used in a mixture with adjuvant Atpolan BIO 80 EC on non-target terrestrial plants were performed, potentially increased toxicity of the herbicide due to the presence of adjuvant could not be addressed in the risk assessment and recommendation on use of the product with adjuvant is deleted in the GAP table and the product label until respective data are made available.

~~The off-field risk to non-target plants from the use of AG-E1-500 SC1 on sugar beet/fodder beet is acceptable with a 5m buffer zone or 75% drift reduction nozzles.~~

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

No further data on effects of ethofumesate or formulation AG-E1-500 SC1 to other terrestrial organisms are available.

9.12 Monitoring data (KCP 10.8)

No further monitoring data on ethofumesate or formulation AG-E1-500 SC1 re available.

9.13 Classification and Labelling

Formulation AG-E1-500 SC1 is classified as H410 Very toxic to aquatic life with long lasting effects.

In accordance with ECHA Guidance on the Application of the CLP Criteria v. 5.0, July 2017, AG-E1-500 SC1 is classified as aquatic environment hazard category chronic 1 because:

Short-term (acute) aquatic hazard

- 96h LC_{50} (for fish) ≤1 mg/L – *Leuciscus idus* 96h LC_{50} = 36.6 mg product/L
- 48h EC_{50} (for crustacea) ≤1 mg/L - *Daphnia magna* 48h EC_{50} = 46.3 mg product/L
- 72h or 96h E_rC_{50} (for algae or other aquatic plants) ≤1 mg/L
  - *Desmodesmus subspicatus* 72h E_rC_{50} = 9.52 mg product/L
  - *Myriophyllum spicatum* 14d E_rC_{50} = 0.29 mg product/L

→ Acute 1

Long-term (chronic) aquatic hazard (non-rapidly degradable substances)

- Chronic NOEC or EC_x (for algae or other aquatic plants) ≤0.1 mg/L for category chronic 1
  Myriophyllum spicatum 14d NOE_rC = 0.0102 mg product/L (AG-E1-500 SC1)

→ Chronic 1

The signal word WARNING is associated with hazard statement H410.

The recommended precautionary statements are:
P501 Dispose of contents/container in accordance with local regulations
P391 Collect spillage

zRMS comments:

CLP classification of AG-E1-500 SC provided by the Applicant above is agreed by the zRMS.
Following classification and labelling are considered relevant:

Hazard pictograms:	GHS09 
Signal word:	Warning
Hazard statement(s):	H410 - Very 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
KCP 10.2/01	xxxxxxxxxxxxx	2005	Ethosat 500 Fish (Golden Orfe), Acute Toxicity Test, Semi-Static, 96 h xxxxxxxxxxxxxxxxxxxxxxxxxxx GLP Unpublished	Y	Adama
KCP 10.2/02	Renner P.	2020a	Acute toxicity of AG-E1-500 SC1 to Daphnia magna in a 48-hour static test 20 48 ADL 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.2/03	Renner P.	2020b	Effects of AG-E1-500 SC1 on *Desmodesmus subspicatus* in an algal growth inhibition test 20 48 AAL 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.2/04	Renner P.	2020c	Effects of AG-E1-500 SC1 on *Myriophyllum spicatum* in a static water-sediment system 20 48 AMS 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.1.1.1/01	Franke M.	2020	Acute toxicity of AG-E1-500 SC1 to the honeybee *Apis mellifera* L. under laboratory conditions 20 48 BAA 0004 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.1.1.2/01	Franke M.	2020	Please refer to KCP 10.3.1.1.1/01	N	Adama
KCP 10.3.1.2/01	Ansaloni T.	2020a	AG-E1-500 SC 1: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee, *Apis mellifera* L. under Laboratory Conditions S19-20080 Trialcamp S.L.U., Alcàsser, Spain GLP Unpublished	N	Adama

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 10.3.1.3/01	Ansaloni T.	2020b	AG-E1-500 SC 1: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions S19-20081 Trialcamp S.L.U., Alcàsser, Spain GLP Unpublished	N	Adama
KCP 10.3.2/01	Röhlig U	2020a	Effects of AG-E1-500-SC1 on the predatory mite *Typhlodromus pyri* Scheuten in a laboratory test 20 48 NTL 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.3.2/02	Röhlig U.	2020b	Effects of AG-E1-500-SC1 on the parasitic wasp *Aphidius rhopalosiphi* (De Stefani-Perez) in a laboratory test 20 48 NAL 0001 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.1.1/01	Friedrich S.	2020a	Effects of AG-E1-500 SC1 on the reproduction of the earthworm *Eisenia andrei* in artificial soil 20 48 TEC 0002 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.2.1/01	Friedrich S.	2020b	Effects of AG-E1-500 SC1 on the reproduction of the collembolan *Folsomia candida* 20 48 TCC 0003 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.4.2.1/02	Friedrich S.	2020c	Effects of AG-E1-500 SC1 on the reproduction of the predatory mite *Hypoaspis aculeifer* 20 48 THC 0002 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama
KCP 10.5/01	Persdorf U.	2020	Effects of AG-E1-500-SC1 on the activity of soil microflora (Nitrogen transformation test) 20 48 SMN 0003 BioChem agrar, Machern OT Gerichshain, Germany GLP Unpublished	N	Adama

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.6.2/01	Duffner A.	2020a	AG-E1-500 SC1: Effects on the Seedling Emergence and Seedling Growth of Non-Target Terrestrial Plant Specied under Greenhouse Conditions S19-22437 Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	Adama
KCP 10.6.2/02	Duffner A.	2020b	AG-E1-500 SC1: Effects on the Vegetative Vigour of Non-Target Terrestrial Plant Specied under Greenhouse Conditions S19-22438 Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP Unpublished	N	Adama

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 ethofumesate and its relevant metabolites was taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for ethofumesate.

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

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
There were no data 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

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

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

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

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

Acute toxicity to fish

Comments of zRMS:	The study was conducted in line with OECD 203 (1992) guideline with a minor deviation. It was reported in the study that the holding and testing temperature was 16±2°C (instead of 20-24°C) because the fish originated from outdoor cultures and due to wintertime the higher temperatures were not tolerable by the fish. This deviation is considered to have no impact on the outcome of the study since all the validity criteria were met. The mean measured concentrations of the active substance were maintained within 80-120% of nominal. Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: LC_{50} = 36.6 mg product/L (based on nominal concentration)

Reference:	**KCP 10.2/01**
Report	Ethosat 500 Fish (Golden Orfe), Acute Toxicity Test, Semi-Static, 96 h, Scheerbaum D., 2005, FAG100321
Guideline(s):	Yes, OECD 203 (1992) and EC Directive 92/69 Method C1 (1992)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	Ethosat 500
Batch No.	00401205
Active ingredient content	508 g ethofumesate/L
Appearance	Slightly beige liquid
pH	7.81 (1%)
Water solubility	39-44 mg/L at pH 3-11 and 20ºC
Hydrolytic stability	Stable at pH 5, 7, 9
Photostability in water	8-13 d at 12 h/d sunlight
Density	1.230 g/mL at 20ºC
Partition coefficient	269

Test organism:

Test species	Golden Orfe, *Leuciscus idus*
Origin	Fischzucht Hausschild, Germany
Acclimation time	12 days
Mean body weight at test start	4.25 g
Mean body length at test start	7.43 cm
No. fish per test substance concentration	7

Test conditions:

Test substance concentrations	0, 2.5, 5.0, 10, 20, 40 mg/L
Test duration	96 h
Test water renewal	Daily
Water pH	6.0-8.5
Hardness	10-250 mg $CaCO_3$/L
Water temperature	16 ± 2ºC
Dissolved oxygen	Minimum 60% saturation
Test vessel	Glass aquaria with loose glass tops, 40L
Light intensity	0.1 – 10 µmol photons/m^2/s
Photoperiod	12 h light

Observations:

Water quality	pH, temperature, oxygen saturation – daily hardness – at test start and any renewal of control replicate
Temperature	Room temperature – continuous
Fish observations	Mortality and visible abnormalities - 2, 24, 48, 72, 96 h Body weight and length – test start
Test substance concentration	Fresh media – 0, 72h Old media – 24, 96h

Analytical method:

Method type	HPLC
Equipment	Waters 712 WISP autosampler, Waters Tunable Absorbance UN detector, Waters 510 pump, Waters Empower Pro software
Column	CC125/3 Nucleosil 100-5 C18 with CC8/3 Nucleosil 100-5 C18 pre-column
Temperature	Ambient
Mobile phase	50% acetonitrile, 50% o-phosphoric acid (0.1%)
Flow rate	0.7 mL/min
Wavelength	210 nm
Run time	10 min

Statistics

LC_{50} values were calculated by probit analysis according to Weber (1986). Confidence intervals for LC_{50} were calculated according to Breitig & Tumpling (1982).

Results and discussions

Validity criteria:

- Oxygen saturation must be above 60%
- Mortality in control fish must be 1 or less out of 7
- Recovery rates of the active ingredient must be minimum 80% of the nominal concentration, otherwise mean measured concentrations must be determined

The study was considered to be valid as all validity criteria were met. The oxygen concentration remained above 60% saturation and there were no mortalities in the control group. The recovery rates of the test substance were above 80% throughout the test, therefore the NOEC and LC50 values can be determined based on nominal test substance concentrations.

Test substance concentrations are presented in the table below.

Table A2.2.1.1.1-1. Concentrations and % recovery rates of ethofumesate in test waters

Product (mg/L)	Active substance (mg/L)	0h (fresh media)		24h (old media)		72h (fresh media)		96h (old media)	
		Mean (mg/L)	% recovery	Mean (mg/L)	% recovery	Mean (mg/L)	% recovery	Mean (mg/L)	% recovery
40	18.1	18.2	101	17.2	95	19.1	106	19.1	106
20	9.04	9.07	100	8.48	94	9.61	106	9.41	104
10	4.52	4.77	106	4.39	97	4.86	108	4.78	106
5	2.26	2.28	101	2.09	92	2.41	107	2.35	104
2.5	1.13	1.05	93	0.92	81	1.17	104	1.08	96
Control	Control	<LOD	-	<LOD	-	<LOD	-	<LOD	-

LOD – 0.01 mg/L

Observations of mortality and visible abnormalities are presented in the table below.

Table A2.2.1.1.1-2. Observations of mortality and visible abnormalities in acute fish toxicity test of Ethosat 500 SC.

Conc. (mg/L)	Visible abnormalities	Test duration (h)				
		2	**24**	**48**	**72**	**96**
40	Lethargy	-	2/7	2/5	2/2	2/2
	Lying on its side	1/7	1/7	-	-	-
	Slow escape reflex	6/7	-	-	-	-
	Missing escape reflex	-	2/7	-	-	-
	Dead	-	2/7	3/5	-	-
20	Normal	7/7	-	-	-	-
	Slow escape reflex	-	7/7	2/7	5/7	3/7
	Missing escape reflex	-	-	5/7	2/7	4/7
10	Normal	7/7	7/7	7/7	7/7	7/7
5	Normal	7/7	7/7	7/7	7/7	7/7
2.5	Normal	7/7	7/7	7/7	7/7	7/7
Control	Normal	7/7	7/7	7/7	7/7	7/7

Cumulative mortality data are presented in the table below.

Table A2.2.1.1.1-3. Percent cumulative mortality in acute fish toxicity test of Ethosat 500 SC.

Conc (mg/L)	Test duration (h)				
	2	**24**	**48**	**72**	**96**
40	0	29	71	71	71
20	0	0	0	0	0
10	0	0	0	0	0
5	0	0	0	0	0
2.5	0	0	0	0	0
Control	0	0	0	0	0

The results above indicate that the nominal test item concentration of 10 mg/L caused no mortality or non-lethal effects, therefore the 96h NOEC is 10 mg/L.

The 96h LC_{50} was calculated as 36.6 mg/L (95% confidence intervals 32.4 – 40 mg/L).

Conclusion

In a 96 hour test to determine the acute toxicity of Ethosat 500 SC to the Golden Orfe, *Leuciscus idus*, the LC_{50} was determined to be 36.6 mg/L and the NOEC was 10 mg/L, based on nominal test substance concentrations.

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Acute toxicity to Daphnia

Comments of zRMS:	The study was conducted in line with OECD 202 with a minor deviation. It was noted that the breeding medium (M4) was different from the test medium (reconstituted water) and it was not stated if a pre-test acclimation period was performed to compensate for the change in the medium. However, this deviation is considered to have no effect on the outcome of the study since all the validity criteria were met. The mean measured concentrations of the active substance were maintained within 80-120% of nominal. Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: EC_{50} = 46.33 mg product/L (based on nominal concentration)

Reference:	**KCP 10.2/02**
Report	Acute toxicity of AG-E1-500 SC1 to Daphnia magna in a 48-hour static test, Renner P., 2020a, 20 48 ADL 0001
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021

Test organism:

Test species	*Daphnia magna* Straus
Origin	In-house culture, originally obtained from RWTH Aachen University, Institute for Environmental Research (Biology V), Worringerweg 1, 52074 Aachen, Germany
Age at test start	< 24 h
Acclimation	The test organisms were bred in M4 medium under the same conditions as in the test.

Test conditions:

Test substance concentrations	6.29, 12.46, 25.03, 50.06, 100.00 mg product/L (nominal) The test concentrations were chosen based on a non-GLP range finding test. A stock solution was prepared by dissolving AG-E1-500 SC1 in test medium. Dilutions of this stock solution were performed with test medium to obtain the selected test concentrations.
Control	Untreated test medium
Test duration	48 h
Test medium	Reconstituted water, prepared 1 day in advance $CaCl_2$ x 2 H_2O 294 mg/L $MgSO_4$ x 7 H_2O 123 mg/L $NaHCO_3$ 65 mg/L KCl 5.8 mg/L Ca:Mg 4:1 (based on molarity) Na:K 10:1 (based on molarity)
Test type	Static
Test water renewal	None
Test medium pH	7.40 at test start 7.45-7.56 measured at 0 h 7.81-7.92 measured at 48 h
Hardness	231 mg $CaCO_3$/L
Water temperature	19.7-20.3°C
Dissolved oxygen	8.54 mg O_2/L at test start 8.40-8.88 mg O_2/L measured at 0 h

	8.17-8.37 mg O_2/L measured at 48 h
Test vessels	Glass beakers covered by plastic lids (25 mL)
Test volume	10 mL
No. daphnia per test vessel	5 (loading: 2 mL test solution per *Daphnia*)
No. daphnia per test substance concentration	20
No. test vessels per test substance concentration	4 + 3 additional vessels (1 for measuring, 1 for analysis, 1 retain specimen)
Light intensity	20 $\mu Em^{-2}/s^{-1}$
Photoperiod	16 h light : 8 h dark, daily

Observations:

Water quality	pH – 0, 48 h oxygen saturation – 0, 48 h
Water temperature	Continuous
Daphnia observations	Immobility (including sub-lethal effects) – 3, 24 and 48 h
Test substance concentration	0, 48 h

Analytical method:

Method type	HPLC-MS
Equipment	Shimadzu LC-20ADXR pumps, Shimadzu DGU-20A3R degasser, Shimadzu SIL-20ACXR autosampler, Shimadzu CTO-20A column oven, Shimadzu CBM-20A controller, Shimadzu LabSolutions Version 5.86 data system
Column	ACE Excel 3 SuperC18, 3 µm, 100 * 2.1 mm
Detector	Shimadzu LCMS-8040 Detection: ESI positive, MRM: m/z 304.1 → 241.0; 304.1 → 121.1; 304.1 → 161.1
Flow rate	0.4 mL/min
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 50% B 5.00 min 100% B 7.00 min 100% B 7.01 min 50% B Run time: 9.00 min
Retention time	Approx. 3.62 min

Calculations

Pre-tests for quantal data with binominal distribution included testing of (1) monotonicity (trend analysis by contrasts, $p \leq 0.05$) and - where appropriate - (2) extra-binomial variance (Tarone's test, $p \leq 0.01$). As a result, a significant linear trend was found for both time points. Extra-binomial variances were not found. The usage of the Step-down Cochran-Armitage ($p \leq 0.05$, on-sided greater) was justified.
Effect concentrations were estimated by concentration-response modelling using Weibull transformed data with maximum likelihood regression. 95% confidence intervals were determined using Fieller's theorem.

Statistical evaluation was performed using ToxRat professional, version 3.3.0 (Ratte 2018).

Results and discussions

Validity criteria:

- Dissolved oxygen concentration at the end of the test in control and test vessels ≥ 3 mg O_2/L (observed: ≥ 8.17 mg O_2/L)
- Immobility in control daphnids ≤ 10% and no other signs of disease or stress (observed: 0%, no other signs of disease or stress)

The study was considered to be valid since both validity criteria were met.

Test substance concentrations are presented in the table below.

Table A2.2.1.1.2-1. Concentrations and % recovery rates of ethofumesate in test media

Product nominal (mg product/L)	Active substance nominal (mg a.s./L)	0 h (fresh media)		48 h (old media)	
		Mean (mg a.s./L)	% recovery	Mean (mg a.s./L)	% recovery
100.00	46.81	47.17	101	49.278	105
50.06	23.43	23.98	102	25.064	107
25.03	11.72	11.51	98	12.302	105
12.46	5.832	5.865	101	6.280	108
6.29	2.943	2.880	98	3.258	111
Control	Control	Not detected	-	Not detected	-

LOQ – 0.7246 mg/L

Since recoveries of ethofumesate, the active substance of AG-E1-500 SC1, were within 98-111% of nominal, the biological results were based on nominal test substance concentrations.

Observations of immobility of daphnids are presented in the table below.

Table A2.2.1.1.2-2. Observations of immobility in acute *Daphnia* toxicity test with AG-E1-500 SC.

Concentration product (mg product/L)	Immobilised *Daphnia* (number)			Immobility of *Daphnia* (%)		
	3 h	24 h	48 h	3 h	24 h	48 h
100.00	0	11	20	0.0	55.0*	100.0*
50.06	0	2	13	0.0	10.0*	65.0*
25.03	0	0	0	0.0	0.0	0.0
12.46	0	0	0	0.0	0.0	0.0
6.29	0	0	0	0.0	0.0	0.0
Control	0	0	0	0.0	0.0	0.0

*significantly different from the control (Step-down Cochran-Armitage (p ≤ 0.05, on-sided greater)

The calculated study endpoints are given in the following table.

Table A2.2.1.1.2-3. Study endpoints of acute *Daphnia* toxicity test with AG-E1-500 SC1.

Parameter	Estimated value (mg product/L / mg a.s./L)	
	24 h	48 h
NOEC	25.03 / 11.72	25.03 / 11.72
LOEC	50.06 / 23.44	50.06 / 23.44
EC_{10} (95% confidence interval)	53.63 (24.80 – 67.81) / 25.11 (11.61 – 31.74)	31.39 (23.24 – 36.42) / 14.69 (10.88 – 17.05)
EC_{20} (95% confidence interval)	67.41 (41.86 – 80.70) / 31.56 (19.60 – 37.78)	36.66 (29.66 – 41.42) / 17.16 (13.89 – 19.39)
EC_{50} (95% confidence interval)	95.19 (79.20 – 122.34) / 44.56 (37.08 – 57.27)	46.33 (40.93 – 52.67) / 21.69 (19.16 – 24.66)

At the end of the 48-hour exposure, no immobile *Daphnia* were found in the control and at the test concentrations of 6.29-25.03 mg product/L. At the test concentrations of 50.06 and 100.00 mg

product/L, 65.0% and 100.0% immobile *Daphnia* were observed. Based on these results, the 48-hour NOEC, LOEC, EC_{10}, EC_{20} and EC_{50} were determined as 25.03, 50.06, 31.39 (23.24 – 36.42 95% confidence interval), 36.66 (29.66 – 41.42 95% confidence interval) and 46.33 (40.93 – 52.67 95% confidence interval) mg product/L, respectively.

No visible abnormalities were observed at any assessment. Abnormal responses of non-surviving/immobile daphnids were not recorded.

Conclusion

In this test to determine the acute toxicity of AG-E1-500 SC1 to *Daphnia magna*, the EC_{50} was determined to be 46.33 mg product/L (40.93 – 52.67 mg product/L 95% confidence interval) and the NOEC was 25.03 mg product/L, based on nominal test substance concentrations.

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Growth inhibition of algae

Comments of zRMS:	The study was conducted in line with OECD 201 with no deviations. The mean measured concentrations of the active substance were maintained within 80-120% of nominal. All the validity criteria were met and the study is considered acceptable the following endpoints relevant for the risk assessment: E_rC_{50} = 9.52 mg product/L (based on nominal concentration) E_rC_{20} = 7.86 mg product/L (based on nominal concentration) E_rC_{10} = 7.11 mg product/L (based on nominal concentration) NOE_rC = 6.62 mg product/L (based on nominal concentration) E_yC_{50} = 7.97 mg product/L (based on nominal concentration) E_yC_{20} = 7.33 mg product/L (based on nominal concentration) E_yC_{10} = 6.94 mg product/L (based on nominal concentration) NOE_yC = 6.62 mg product/L (based on nominal concentration)

Reference:	**KCP 10.2/03**
Report	Effects of AG-E1-500 SC1 on *Desmodesmus subspicatus* in an algal growth inhibition test, Renner P., 2020b, 20 48 AAL 0001
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL

Expiry date	01/2021
Reference substance	Potassium dichromate was routinely tested at concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L to verify the test system sensitivity. Based on the results of the most recent reference substance test, the E_rC_{50} and E_yC_{50} were determined as 1.07 (0.83 – 1.38 95% confidence interval) and 0.45 (0.37 – 0.53 95% confidence interval) mg/L, respectively.

Test organism:

Test species	*Desmodesmus subspicatus*, (Chodat) Hegewald et Schmidt, cultures in logarithmic growth phase
Origin	In-house culture, originally obtained from SAG Culture Collection of Algae, Nikolausberger Weg 18, 37073 Goettingen, Germany strain: 86.81 SAG
Cell concentration at test start	5 x 10^3 cells/mL
Acclimation	Axenic stock cultures were grown in culturing vessels for 4 days prior to test initiation; cultivation was performed in glass flasks with medium as used in the test; algae were kept at the same temperature and light conditions as in the test.

Test conditions:

Test substance concentrations	4.10, 5.21, 6.62, 8.40, 10.67 mg product/L (nominal) A stock solution (= highest test substance concentration) was prepared by dissolving AG-E1-500 SC1 in test medium directly before test start. Dilutions of this stock solutions were performed with test medium to obtain the lower test concentrations.	
Control	Untreated test medium	
Test duration	72 h	
Test medium	OECD 201 medium:	
	Macronutrients: NH_4Cl	15.0 mg/L
	$MgCl_2$ x 6 H_2O	12.0 mg/L
	$CaCl_2$ x 2 H_2O	18.0 mg/L
	$MgSO_4$ x 7 H_2O	15.0 mg/L
	KH_2PO_4	1.6 mg/L
	$NaHCO_3$	50.0 mg/L
	Trace elements: H_3BO_3	185 µg/L
	$MnCl_2$ x 4 H_2O	415 µg/L
	$ZnCl_2$	3.0 µg/L
	$CoCl_2$ x 6 H_2O	1.5 µg/L
	$CuCl_2$ x 2 H_2O	0.01 µg/L
	Na_2MoO_4 x 2 H_2O	7.0 µg/L
	$FeCl_3$ x 6 H_2O	64.0 µg/L
	Na_2EDTA x 2 H_2O	100 µg/L
	(reagents of analytical grade)	
Test type	Static	
Test medium pH	8.18 (before test start) 8.10-8.19 measured at 0 h 8.05-8.49 measured at 72 h	
Water temperature	22.6-22.7°C	
Shaking	Rotary shaker at approximately 85 rpm	
Test vessel	250 mL Erlenmeyer flasks with air-permeable stoppers	
Test volume	100 mL	
No. test vessels per test substance concentration	control group: 6 treated group: 3 (additional vessel for analysis and retain specimen)	

Light intensity	74 µEm^{-2}/s^{-1} (mean) measured at 400-700 nm once before test start Differences from the selected light intensity over the test area did not exceed the range ± 15%.
Photoperiod	24 h light

Observations:

Water quality	pH – 0, 72 h
Light intensity	Test start
Water temperature	Continuous
Algae observations	Biomass (cell counts) – test start, 24, 48 and 72 h Using a Neubauer counting chamber
Qualitative observations	Test solution appearance – test start, 24, 48 and 72 h Algae cell shape – test start, 24, 48 and 72 h
Test substance concentration	0, 72 h

Analytical method:

Method type	HPLC-MS
Equipment	Shimadzu LC-20ADXR pumps, Shimadzu DGU-20A3R degasser, Shimadzu SIL-20ACXR autosampler, Shimadzu CTO-20A column oven, Shimadzu CBM-20A controller, Shimadzu LabSolutions Version 5.86 data system
Column	ACE Excel 3 SuperC18, 3 µm, 100 * 2.1 mm
Detector	Shimadzu LCMS-8040 Detection: ESI positive, MRM: m/z 304.1 → 241.0; 304.1 → 121.1; 304.1 → 161.1
Flow rate	0.4 mL/min
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 50% B 5.00 min 100% B 7.00 min 100% B 7.01 min 50% B Run time: 9.00 min
Retention time	Approx. 3.62 min

Calculations

Based on the cell counts at 0, 24, 48 and 72 hours, the growth rates and yield as well as their percentage inhibition were calculated in accordance with OECD 201 (2011).
Based on the outcome of pre-tests on normality (Shapiro-Wilk's test, $\alpha = 0.05$) and homogeneity (Levene's test, $\alpha = 0.05$) of the data and a trend analysis by contrasts ($\alpha = 0.05$), the usage of Williams t-test ($\alpha = 0.05$, one-sided smaller) was justified for all yield data and the 72-hour growth rate data, as all data was normally distributed, variances were homogenous and monotonicity criteria were fulfilled. For the 24-hour and 48-hour growth rate endpoints, the Welch's t-test ($\alpha = 0.05$, one-sided smaller) was used as variances were not homogenous.
Effect concentrations were determined by concentration-response modelling. For estimation of the growth rate endpoints, 3-parameter normal cumulative distribution functions (CDF) were used, and for estimation of the yield endpoints, Weibull analysis (maximum likelihood regression) was used. 95% confidence intervals were determined using Monte-Carlo simulation and Fieller's theorem, respectively. The goodness of fit for the 3-parameter normal CDFs was justified by the modelling parameters b_0, b_1 and b_2 and lack of fit analysis in case of the employed non-linear regression. The fit of the Weibull model was justified by the chi^2 fit measure (p(Chi^2) and significance of the concentration-response relationship (p(F)).

Results and discussions

Validity criteria:

- Minimum 16-fold biomass increase in the control culture during the 72-hour test period (observed: 76.5-fold biomass increase)
- Mean coefficient of variation for section-by-section specific growth rates in control cultures must not exceed 35% (observed: 22.4%)
- Coefficient of variation of average specific growth rates in replicate control cultures must not exceed 7% (observed: 1.9%)

The study was considered to be valid as all validity criteria were met.

Test substance concentrations are presented in the table below.

Table A2.2.1.1.3-1. Concentrations and % recovery rates of ethofumesate in test media

Product nominal (mg product/L)	Active substance nominal (mg a.s./L)	0 h (fresh media)		72 h (old media)	
		Mean (mg a.s./L)	% recovery	Mean (mg a.s./L)	% recovery
10.67	4.995	5.321	107	5.626	113
8.40	3.934	4.374	111	4.374	111
6.62	3.097	3.374	109	3.447	111
5.21	2.438	2.654	109	2.722	112
4.10	1.920	2.062	107	2.084	109
Control	Control	-	-	-	-

LOQ – 0.959 mg/L

Since recoveries of ethofumesate, the active substance of AG-E1-500 SC1, were within 107-113% of nominal, the biological results were based on nominal test substance concentrations.

The mean number of algal cells at each time point and concentration are presented in the table below.

Table A2.2.1.1.3-2. Algal cell counts (x 10^4/mL) following exposure to AG-E1-500 SC1.

Concentration product (mg product/L)	Test duration (h)			
	0	24	48	72
10.67	0.50	0.42	1.08	2.08
8.40	0.50	0.75	3.67	9.50
6.62	0.50	2.83	12.25	39.00
5.21	0.50	2.92	12.17	39.17
4.10	0.50	3.17	12.08	38.08
Control	0.50	2.83	12.58	38.25

The calculated growth rates and percentage inhibition of growth rates in comparison to the control are presented in the table below.

Table A2.2.1.1.3-3. Growth rates of algae and their percentage inhibition.

Concentration product (mg product/L)	24 h		48 h		72 h	
	Growth rate μ (day^{-1})	% Inhibition	Growth rate μ (day^{-1})	% Inhibition	Growth rate μ (day^{-1})	% Inhibition
10.67	-0.231	100.0*[a)]	0.373	76.9*	0.473	67.2*
8.40	0.405	76.6*	0.994	38.3*	0.981	32.1*
6.62	1.731	0.1	1.598	0.9	1.452	-0.5
5.21	1.758	-1.5	1.596	1.0	1.454	-0.6
4.10	1.841	-6.3	1.591	1.3	1.444	0.1
Control	1.732	-	1.612	-	1.445	-

Note: Negative values indicate an increase in growth relative to the control
* Significantly different from the control (Welsh's t-Test, $p < 0.05$ or William's t-Test, $p < 0.05$)
a) Effect > 100% (biased by no cell counts compared to test start)

The calculated yield and percentage inhibition of yield in comparison to the control are presented in the table below.

Table A2.2.1.1.3-4. Yield of algae and its percentage inhibition.

Concentration product (mg product/L)	24 h		48 h		72 h	
	Yield y	% Inhibition	Yield y	% Inhibition	Yield y	% Inhibition
10.67	-0.083	100.0*[a)]	0.583	95.2*	1.583	95.8*
8.40	0.250	89.3	3.167	73.8*	9.000	76.2*
6.62	2.333	0.0	11.750	2.8	38.500	-2.0
5.21	2.417	-3.6	11.667	3.4	38.667	-2.4
4.10	2.667	-14.3	11.583	4.1	37.583	0.4
Control	2.333	-	12.083	-	37.750	-

Note: Negative values indicate an increase in growth relative to the control
* Significantly different from the control (William's t-Test, $p < 0.05$)
a) Effect > 100% (biased by no cell counts compared to test start)

The calculated study endpoints are given in the following table.

Table A2.2.1.1.3-5. Study endpoints of algae growth inhibition test with AG-E1-500 SC1.

Parameter	Estimated value (mg product/L / mg a.s./L)	
	Endpoints for growth rate	Endpoints for yield
72-h NOEC	6.62 / 3.097	6.62 / 3.097
72-h LOEC	8.40 / 3.934	8.40 / 3.934
72-h EC_{10} (95% confidence interval)	7.11 (6.89 – 7.34) / 3.33 (3.23 – 3.44)[a)]	6.94 (6.79 – 7.06) / 3.25 (3.18 – 3.31)[a)]
72-h EC_{20} (95% confidence interval)	7.86 (7.63 – 8.10) / 3.68 (3.57 – 3.79)[a)]	7.33 (7.22 – 7.43) / 3.43 (3.38 – 3.48)[a)]
72-h EC_{50} (95% confidence interval)	9.52 (9.16 – 9.88) / 4.33 (4.29 – 4.63)[a)]	7.97 (7.90 – 8.04) / 3.73 (3.70 – 3.76)[a)]

a) Calculated by the applicant based on actual active substance concentration and density

At the end of the 72-hour exposure, growth rates of algae as well as yield of algae were statistically significantly inhibited at the two highest test concentrations of 8.40 and 10.67 mg product/L. Therefore, the LOEC and NOEC were determined as 8.40 and 6.62 mg product/L, respectively, both based on growth rate and yield.

72-h E_rC_{10}, E_rC_{20} and E_rC_{50} were calculated to be 7.11 (6.89 – 7.34 95% confidence interval), 7.86 (7.63 – 8.10 95% confidence interval) and 9.25 (9.16 – 9.88 95% confidence interval) mg product/L, respectively, and 72-h E_yC_{10}, E_yC_{20} and E_yC_{50} were calculated to be 6.94 (6.79 – 7.06 95% confidence interval), 7.33 (7.22 – 7.43 95% confidence interval) and 7.97 (7.90 – 8.04 95% confidence interval) mg product/L, respectively. Despite having tested concentrations with a spacing factor of 1.27, the concentration-response curve was still very steep.

No abnormalities were found regarding test solutions, i.e. test solutions were not cloudy and precipitations were not noticed. The cell shape was not altered in the control and any test concentration.

Conclusion

In this test to determine the growth inhibition of AG-E1-500 SC1 in the green algae *Desmodesmus subspicatus*, the 72-h NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on growth rate were calculated to be 6.62, 7.11 (6.89 – 7.34 95% confidence interval), 7.86 (7.63 – 8.10 95% confidence interval) and 9.525 (9.16 – 9.88 95% confidence interval) mg product/L, respectively. The 72-h NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on yield were determined as 6.62, 6.94 (6.79 – 7.06 95% confidence interval), 7.33 (7.22 – 7.43 95% confidence interval) and 7.97 (7.90 – 8.04 95% confidence interval) mg product/L, respectively.

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Effects on aquatic plants

Comments of zRMS:	The study was conducted in line with OECD 239 with no deviations. The mean measured concentrations of the active substance were maintained within 80-120% of nominal. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: Growth rate based on shoot fresh weight: E_rC_{50} = 0.257 mg product/L (based on nominal concentration) E_rC_{20} = 0.104 mg product/L (based on nominal concentration) E_rC_{10} = 0.065 mg product/L (based on nominal concentration) NOE_rC = 0.0641 mg product/L (based on nominal concentration) Growth rate based on shoot dry weight: E_rC_{50} > 0.400 mg product/L (based on nominal concentration) E_rC_{20} = 0.182 mg product/L (based on nominal concentration) E_rC_{10} = 0.093 mg product/L (based on nominal concentration) NOE_rC = 0.0641 mg product/L (based on nominal concentration) Growth rate based on total shoot length: E_rC_{50} > 0.400 mg product/L (based on nominal concentration) E_rC_{20} = 0.167 mg product/L (based on nominal concentration) E_rC_{10} = 0.085 mg product/L (based on nominal concentration) NOE_rC = 0.0102 mg product/L (based on nominal concentration) Growth rate based on main shoot length: E_rC_{50} = 0.290 mg product/L (based on nominal concentration) E_rC_{20} = 0.091 mg product/L (based on nominal concentration) E_rC_{10} = 0.046 mg product/L (based on nominal concentration) NOE_rC = 0.0256 mg product/L (based on nominal concentration) Yield based on shoot fresh weight: E_yC_{50} = 0.183 mg product/L (based on nominal concentration) E_yC_{20} = 0.088 mg product/L (based on nominal concentration) E_yC_{10} = 0.060 mg product/L (based on nominal concentration) NOE_yC = 0.0641 mg product/L (based on nominal concentration)

	Yield based on shoot dry weight: E_yC_{50} > 0.400 mg product/L (based on nominal concentration) E_yC_{20} = 0.121 mg product/L (based on nominal concentration) E_yC_{10} = 0.061 mg product/L (based on nominal concentration) NOE_yC = 0.0641 mg product/L (based on nominal concentration) Yield based on total shoot length: E_yC_{50} = 0.257 mg product/L (based on nominal concentration) E_yC_{20} = 0.051 mg product/L (based on nominal concentration) E_yC_{10} = 0.022 mg product/L (based on nominal concentration) NOE_yC = 0.0641 mg product/L (based on nominal concentration) Yield based on main shoot length: E_yC_{50} = 0.129 mg product/L (based on nominal concentration) E_yC_{20} = 0.037 mg product/L (based on nominal concentration) E_yC_{10} = 0.018 mg product/L (based on nominal concentration) NOE_yC = 0.0641 mg product/L (based on nominal concentration)

Reference:	**KCP 10.2/04**
Report	Effects of AG-E1-500 SC1 on *Myriophyllum spicatum* in a static water-sediment system, Renner P., 2020c, 20 48 AMS 0001
Guideline(s):	Yes, OECD 239 (2014)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	Not applicable

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	3,5-dichlorophenol was routinely tested at concentrations of 0.95, 1.71, 3.09, 5.56 and 10.0 mg/L to verify the test system sensitivity. Based on the results of the most recent reference substance test, the E_rC_{50} and E_yC_{50} values for total shoot length were determined as 5.13 (4.77 – 5.51 95% confidence interval) and 4.28 (3.70 – 4.96 95% confidence interval) mg/L, respectively.

Test organism:

Test species	*Myriophyllum spicatum* L.
Origin	In-house culture, originally obtained from Federal Environment Agency Division IV, Schichauweg 58, 12307 Berlin, Germany
Acclimation	Pre-culture plants were prepared 1 – 2 weeks before test initiation in artificial sediment with fresh medium, cultivated under test conditions.

Test conditions:

Test substance concentrations	0.0102, 0.0256, 0.0641, 0.160, 0.400 mg product/L (nominal) A stock solution (= highest test substance concentration) was prepared by dissolving AG-E1-500 SC1 in test medium directly before test start. Dilutions of this stock solutions were performed with test medium to obtain the lower test concentrations.
Control	Untreated test medium
Test duration	14 d
Test medium	Smart and Barko medium: Macroelements: $CaCl_2$ x 2 H_2O 91.7 mg/L $MgSO_4$ x 7 H_2O 69.0 mg/L $NaHCO_3$ 58.4 mg/L $KHCO_3$ 15.4 mg/L
Test sediment	4% (dry weight) peat as close to pH 5.5 – 6 as possible 20% (dry weight) kaolin clay (kaolinite content >30%) 76% (dry weight) fine quartz sand (.50% particle between 50 – 200 µm) 2.035% (dry weight) total organic carbon 200 mg/kg (dry sediment) ammonium chloride aqueous 200 mg/kg (dry sediment) sodium phosphate aqueous 10 kg (dry weight) sediment was mixed with 4.7L deionised water properties of artificial sediment at the start of the experimental phase: moisture content: 31.53 %; pH: 7.41
Test type	Static
Test medium pH ~~at test start~~	7.79 at start 7.47-7.87 during the test
O_2	8.71-9.67 mg/L during the test
Water temperature	19.7 – 21.7°C
Test vessel	2 L glass beakers with lids
Plant pots	160 mL glass beakers
Test volume	1800 mL
No. replicates	1 plant per plant pot 3 plant pots per replicate 6 control replicates 4 treated group replicates Additional vessels for analysis and retained specimens
Light intensity	Mean 125 µE/m^2/s^{-1} Differences from the selected light intensity over the test area did not exceed the range ± 15%.
Photoperiod	16 h light

Observations:

Light intensity	Test start
Water temperature	Continuous
Macrophyte observations: Shoot length, total shoot length, no. lateral branches Fresh and dry weight	 0, 14 d 0, 14 d
Qualitative observations: Change in plant development Test medium appearance Root appearance	 0, 7, 14 d 0, 7, 14 d 0, 14 d
Test substance concentration	0, 14 d (overlying water, sediment and pore water)
pH and O_2	0, 7, 14 d

Analytical method:

Method type	HPLC-MS
Equipment	Shimadzu LC-20ADXR pumps, Shimadzu DGU-20A3R degasser, Shimadzu SIL-20ACXR autosampler, Shimadzu CTO-20A column oven, Shimadzu CBM-20A controller, Shimadzu LabSolutions Version 5.86 data system
Column	ACE Excel 3 SuperC18, 3 µm, 100 * 2.1 mm
Detector	Shimadzu LCMS-8040 Detection: ESI positive, MRM: m/z 304.1 → 241.0; 304.1 → 121.1; 304.1 → 161.1
Flow rate	0.4 mlL/min
Mobile phase	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol 0.00 min 50% B 5.00 min 100% B 7.00 min 100% B 7.01 min 50% B Run time: 9.00 min
Retention time	Approx. 3.62 min

Calculations

A sequence of pretesting was performed before final statistical testing. These pre-tests included testing of normal distribution (Shapiro-Wilks test, $\alpha = 0.01$), variance homogeneity (Levene's test, $\alpha = 0.01$) and monotonicity (trend analysis by contrasts, $\alpha = 0.05$). Outlier tests were not performed.

NOEC/LOEC determinations based on the statistical pre-tests were calculated using the Williams t-test ($\alpha = 0.05$, one-sided smaller).

Effect concentrations (ECx) were estimated using concentration-response modelling. Non-linear regression models (3-parameter normal or logistic cumulative distribution function) were employed. The goodness of fit for each model was justified by the significance of the modelling parameter (b1, b2, b3), R^2 values and visual observations. 95% confidence intervals were determined by Monte-Carlo estimation.

Statistical analysis was performed using ToxRat Professional Version 3.3.0 (RATTE, 2018).

Results and discussions

Validity criteria:

- Total shoot length and shoot fresh weight in control plants must at least double during the exposure phase (observed increase 4.8 for total shoot length and 3.1 for shoot fresh weight)
- Mean coefficient of variation for yield fresh weight ≤ 35 % (observed 17.6%)
- Control plants must be free from chlorosis and contamination by other organisms (yes)
- Test solutions, plants and sediment shall be free from bacterial, algal, fungal films (yes)

The study was considered to be valid as all validity criteria were met.

Test substance concentrations are presented in the table below.

Table A2.2.1.1.3-1. Concentrations and % recovery rates of ethofumesate in test media

Product nominal (mg product/L)	Dilution factor	Active substance nominal (mg a.s./L)	0 h (fresh media)		14 d (old media)	
			Mean (mg a.s./L)	% recovery	Mean (mg a.s./L)	% recovery
Control	2	-	nd	-	nd	-
0.0102	2	4.784	4.974	104	4.801	100
0.0256	2	12.00	12.17	101	11.70	97
0.0641	2	30.00	31.83	106	29.77	99
0.160	2	74.88	77.76	104	75.52	101
0.400	4	187.3	193.6	103	183.5	98

LOQ – 2.379 µg/L, nd not detected

Since recoveries of ethofumesate, the active substance of AG-E1-500 SC1, were within 80 - 120% of nominal, the biological results were based on nominal test substance concentrations.

The percentage inhibition of yield and growth rates for fresh weight, dry weight, shoot length and total shoot length in comparison to the control are presented in the table below.

Table A2.2.1.1.3-3. Percentage inhibition of yield and growth rates of *Myriophyllim spicatum*

Concentration product (mg product/L)	Fresh weight		Dry weight	
	Yield % inhibition	Growth rate % inhibition	Yield % inhibition	Growth rate % inhibition
Control	-	-	-	-
0.0102	3.1	2.3	1.5	1.3
0.0256	6.4	4.1	9.7	6.8
0.0641	-1.6	-1.1	-18.4	-12.0
0.160	56.6*	42.6*	25.7*	17.7*
0.400	72.7*	61.5*	25.4*	18.4*
	Shoot length		Total shoot length	
	Yield % inhibition	Growth rate % inhibition	Yield % inhibition	Growth rate % inhibition
Control	-	-	-	-
0.0102	1.6	2.7	3.8	3.5
0.0256	7.7	4.4	15.2	8.2*
0.0641	11.2*	8.0*	5.4	4.6*
0.160	57.8*	38.3*	40.6*	22.5*
0.400	74.1*	56.7*	60.5*	40.0*

Note: Negative values indicate an increase in growth relative to the control
* Significantly different from the control (William's t-Test, $p < 0.05$)

The calculated study endpoints are given in the following table.

Table A2.2.1.1.3-5. Study endpoints of *Myriophyllum spicatum* test with AG-E1-500 SC1.

Endpoint	Fresh weight		Dry weight	
	Yield	**Growth rate**	**Yield**	**Growth rate**
LOEC	0.160	0.160	0.160	0.160
NOEC	0.0641	0.0641	0.0641	0.0641
EC_{10} (95% CI)	0.060 (0.025-0.140)	0.065 (0.029-0.145)	0.061 (0.011-0.336)	0.093 (0.030-0.292)
EC_{20} (95% CI)	0.088 (0.039-0.201)	0.104 (0.048-0.230)	0.121 (0.021-0.735)	0.182 (0.054-0.653)
EC_{50} (95% CI)	0.183 (0.067-0.502)	0.257 (0.095-0.683)	>0.400 ~~0.452* (0.031-5.834)~~	>0.400 ~~0.661* (0.076-5.310)~~
	Shoot length		**Total shoot length**	
	Yield % inhibition	**Growth rate % inhibition**	**Yield % inhibition**	**Growth rate % inhibition**
LOEC	0.0641	0.0641	0.160	0.0256
NOEC	0.0256	0.0256	0.0641	0.0102
EC_{10} (95% CI)	0.018 (0.010-0.026)	0.046 (0.027-0.066)	0.022 (0.011+0.044)	0.085 (0.051-0.141)
EC_{20} (95% CI)	0.037 (0.024-0.049)	0.091 (0.064-0.117)	0.051 (0.031-0.084)	0.167 (0.099-0.286)
EC_{50} (95% CI)	0.129 (0.101-0.158)	0.290 (0.246-0.335)	0.257 (0.191-0.345)	>0.400 ~~0.612* (0.276-1.308)~~

CI – 95 % confidence intervals (lower – upper)
~~* value considered unreliable (extrapolated)~~

At the end of the 14-day exposure, yield and growth rates based on fresh weight and dry weight were statistically significantly inhibited at the two highest test concentrations of 0.16 and 0.4 mg product/L. Therefore, the LOEC and NOEC were determined as 0.16 and 0.0641 mg product/L, respectively. Yield and growth rates based on shoot length and total shoot length were more sensitive to AG-E1-500 SC1. The LOEC and NOEC for yield and growth rate based on shoot length were 0.0641 and 0.0256 mg product/L, respectively. The LOEC and NOEC for yield based on total shoot length were 0.160 and 0.0641 mg product/L, respectively. The LOEC and NOEC for growth rate based on total shoot length were 0.0256 and 0.0102 mg product/L, respectively.
ErC_{10}, ErC_{20}, ErC_{50}, EyC_{10}, EyC_{20} and EyC_{50} values based on fresh weight, dry weight, shoot length and total shoot length are presented in the table above with 95% confidence intervals. Effects on several endpoints appear suddenly with high inhibition rates alongside increasing concentrations (e.g. fresh weight growth rate). Other endpoints barely reach 20 % inhibition (e.g. dry weight growth rate). This led to difficulty with statistical model fitting and as a result, some endpoints were estimated by extrapolation. These values should be considered unreliable. However, the most sensitive endpoints were estimated within the limitations of the model, and are therefore considered reliable.

Conclusion

In this test to determine the effects of AG-E1-500 SC1 in the aquatic plant *Myriophyllum spicatum*, the 14-d NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on fresh weight were calculated to be 0.0641, 0.065, 0.104, 0.257 mg product/L, respectively. The 14-d NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on fresh weight were determined as 0.0641, 0.060, 0.088, 0.183 mg product/L, respectively. The 14-d NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on dry weight were calculated to be 0.0641, 0.093, 0.182, >0.4 mg product/L, respectively. The 14-d NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on dry weight were determined as 0.0641, 0.061, 0.121, >0.4 mg product/L, respectively. The 14-d NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on shoot length were calculated to be 0.0256, 0.046, 0.091, 0.290 mg product/L, respectively. The 14-d NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on shoot length were determined as 0.0256, 0.018, 0.037, 0.129 mg product/L, respectively. The 14-d NOEC, E_rC_{10}, E_rC_{20} and E_rC_{50} based on total shoot length were calculated to be 0.0102, 0.085, 0.1667, >0.4 mg product/L, respectively. The 14-d NOEC, E_yC_{10}, E_yC_{20} and E_yC_{50} based on total shoot length were determined as

0.0641, 0.022, 0.051, 0.257 mg product/L, respectively.

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

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

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

KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was performed in line with OECD 213 with a minor deviation. It was noted that during the test the relative humidity fell to 46% which is below the recommended minimum of 50%. However, all the validity criteria were met and this deviation is considered to have no impact on the outcome of the study. Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: 48h LD_{50} > 500.0 µg product/bee (corresponding to > 234.1 µg a.s./bee)

Reference:	**KCP 10.3.1.1.1/01**
Report	Acute toxicity of AG-E1-500 SC1 to the honeybee *Apis mellifera* L. under laboratory conditions, Franke M., 2020, 20 48 BAA 0004
Guideline(s):	OECD 213 (1998)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Dimethoate EC 400 400 g dimethoate/L (nominal), 429 g dimethoate/L (actual)

Test organism:

Test species	Honeybee – *Apis mellifera* L. Buckfast (Hymenoptera, Apoidea)
Origin	Apiary: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany. All bees used in the test derived from a healthy, disease-free and queen-right bee colony. The bees were taken from a hive that had not received chemical treatments for at least one month. The honeybees were reared in the hive until they were used for testing. The colony was free from *Varroa destructor*, *Nosema* and foulbrood disease/infection.
Collection	Bees were collected in the morning of use (application). Ten bees each were transferred into each test cage without anaesthesia.

Age at test start	Female, adult worker bees; normally, at an age of about 3 to 5 weeks (forager bees)
No. bees per replicate	10
No. replicates per test substance, reference substance or control	3

Test conditions:

Test item dosage	Offered dose rates (chosen based on the results of a non-GLP range-finding test): 31.3, 62.5, 125.0, 250.0 and 500.0 µg product/bee, equivalent to 14.6, 29.3, 58.5, 117.0 and 243.1 µg a.s./bee, based on analysed content of a.s. Actually consumed dose rates: 31.3, 62.5, 125.0, 250.0 and 500.0 µg product/bee, equivalent to 14.6, 29.3, 58.5, 117.0 and 243.1 µg a.s./bee, based on analysed content of a.s. Application volume: 200 µL/cage with 10 bees The test solutions were prepared with 50% (w/v) sucrose solution.
Reference item dosage	Offered dose rates: 0.215, 0.308, 0.440 and 0.628 µg product/bee, equivalent to 0.086, 0.123, 0.175 and 0.250 µg a.s./bee, based on analysed content of a.s. Actually consumed dose rates: 0.215, 0.308, 0.440 and 0.628 µg product/bee, equivalent to 0.086, 0.123, 0.175 and 0.250 µg a.s./bee, based on analysed content of a.s. Application volume: 200 µL/cage with 10 bees The test solutions were prepared with 50% (w/v) sucrose solution.
Control	50% (w/v) sucrose solution Application volume: 200 µL/cage with 10 bees
Administration	Groups of 10 bees per cage were provided with 200 µL test solution in a glass ampoule (half-open on its longitudinal axis, 5 cm long). The feeding tubes were introduced through a hole in the roof of the cage. Due to their social feeding behaviour (trophallaxis), honeybees of a distinct group are assumed to receive approximately the same amount of food (about 20 µL/bee) and consequently the same dose of test or reference item. Approximately 2 hours after application when the application solution was obviously consumed, the feeding tubes were removed and the exact quantity of consumed test solution was determined.
Test duration	48 hours after application
Test units	Disposable cardboard cages (95 mm x 50 mm x 65 mm, length x width x height) with holes in the bottom side for ventilation and a glass plate in front
Temperature	25 ± 2°C (nominal), 23-24°C (actual)
Relative humidity	Approximately 50-70% (nominal), 46-59% (actual)
Illumination	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Ventilation	By the air-conditioning equipment of the climate chamber
Feeding	After obviously complete consumption of the test solutions containing the control, test item or reference item (approximately 2 hours after application), the feeding tubes were replaced by tubes with untreated food (50% (w/v) sucrose solution), which was offered *ad libitum* placed on the floor of the test cage.

Observations:

Mortality	4, 24 and 48 hours after application
Behaviour	4, 24 and 48 hours after application Number of bees/replicate that were healthy (normal), affected (impaired locomotion) or moribund; furthermore, any other deviations in behaviour of treated bees were described (e.g. abnormal amount/colour of excretion)

Statistics

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (Ratte, 2018) was used.

Mortality in the test item and reference item treatment groups was analysed for statistical significance using Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($p \leq 0.05$).

For the test item treatment, no LD_{50} could be calculated since no significant mortality occurred for any tested dose. For the reference item treatment, the LC_{50} was calculated by Probit analysis (linear maximum likelihood regression).

Results and discussions

Validity criteria:

- Control mortality: ≤ 10% after 48 hours (observed: 0.0%)
- Reference mortality: 0.10 – 0.35 µg a.s./bee after 24 hours (observed: 0.124 µg a.s./bee)

The study was considered to be valid as all validity criteria were met.

Observations of mortality and behaviour are presented in the table below.

Table 10.3.1.1.1-1. Mortality and behaviour of bees following oral exposure to AG-E1-500 SC1.

Treatment [dosage unit]	Dosage (consumed)	Mean mortality [a] [%]			Behavioural abnormalities Σ A M								
		4 h	24 h	48 h	4 h			24 h			48 h		
Control[b]	-	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
AG-E1-500 SC1 [µg prod./bee]	500.0	0.0	0.0	6.7	0	0	0	0	0	0	0	0	0
	250.0	0.0	3.3	3.3	0	0	0	0	0	0	0	0	0
	125.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
	62.5	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
	31.3	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Reference item [µg a.s./bee]	0.250	6.7	96.7*	100.0*	16	16	0	0	0	0	0	0	0
	0.175	0.0	83.3*	86.7*	4	4	0	0	0	0	0	0	0
	0.123	0.0	53.3*	60.0*	0	0	0	0	0	0	0	0	0
	0.086	0.0	13.3	16.7*	0	0	0	0	0	0	0	0	0

A: affected (impaired locomotion)
M: moribund
a) Mortality results are averages based on 3 replicates consisting of 10 bees each; no correction of mortality (according to Schneider-Orelli 1947) was performed since control mortality was 0.0%.
b) 50% (w/v) sucrose solution
* Significant difference in pairwise comparison between treatment group and control by Fisher's Exact Binominal Test after Bonferroni-Holm correction for mortality data (α = 0.05, one sided greater)

After 48 hours, no mortality was observed in the control group. In the test item treatment groups, mortality was 0.0%, except for the two highest treatments of 250.0 and 500.0 µg product/bee, in which mortality was 3.3% and 6.7% after 48 hours, respectively. No statistically significant effect on mortality was determined in any test item treatment group when compared to the control by Fisher's Exact Binominal Test after Bonferroni-Holm correction for mortality data (α = 0.05, one sided

greater). Therefore, the 48-hour oral LD_{50} was estimated to be > 500.0 µg product/bee which corresponds to > 234.1 µg a.s./bee. No effects on behaviour compared to the control were observed at the tested doses up to 500.0 µg product/bee within the 48-hour oral testing.

In the reference item treatment groups, mortality was between 13.3% and 96.7% after 24 hours. The 24-hour oral LD_{50} was calculated as 0.124 µg a.s./bee.

Conclusion

In this test on acute oral toxicity of AG-E1-500 SC1 in the honeybee *Apis mellifera* L., the 48-hour oral LD_{50} was > 500.0 µg product/bee which corresponds to > 234.1 µg a.s./bee.

KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was performed in line with OECD 214 with minor deviations. It was noted that during the test the relative humidity fell to 46% which is below the recommended minimum of 50%. It was also noted that in order to ensure a more reliable dispersion of the test item, the application volume was 2 µL per bee instead of 1 µL per bee recommended by the guideline. However, all the validity criteria were met and these deviations are considered to have no impact on the outcome of the study. Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: 48h LD_{50} > 500.0 µg product/bee (corresponding to > 234.1 µg a.s./bee)

Reference:	**KCP 10.3.1.1.2/01**
Report	Acute toxicity of AG-E1-500 SC1 to the honeybee *Apis mellifera* L. under laboratory conditions, Franke M., 2020, 20 48 BAA 0004
Guideline(s):	OECD 214 (1998)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Dimethoate EC 400 400 g dimethoate/L (nominal), 429 g dimethoate/L (actual)

Test organism:

Test species	Honeybee – *Apis mellifera* L. Buckfast (Hymenoptera, Apoidea)
Origin	Apiary: BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany. All bees used in the test derived from a healthy, disease-free and queen-right bee colony. The bees were taken from a hive that had not received chemical treatments for at least one month. The honeybees were reared in the hive until they were used for testing. The colony was free from *Varroa destructor*, *Nosema* and foulbrood disease/infection.
Collection	Bees were collected in the morning of use (application). Ten bees each were transferred into each test cage without anaesthesia.
Age at test start	Female, adult worker bees; normally, at an age of about 3 to 5 weeks (forager bees)
No. bees per replicate	10
No. replicates per test substance, reference substance or control	3

Test conditions:

Test item dosage	31.3, 62.5, 125.0, 250.0 and 500.0 µg product/bee, equivalent to 14.6, 29.3, 58.5, 117.0 and 243.1 µg a.s./bee, based on analysed content of a.s. The dose rates were chosen based on the results of a non-GLP range-finding test. Application volume: 2 µL/bee The test solutions were prepared with aqueous 1% (v/v) Tween®80.
Reference item dosage	0.265, 0.353, 0.471 and 0.628 µg product/bee, equivalent to 0.106, 0.141, 0.188 and 0.250 µg a.s./bee, based on analysed content of a.s. Application volume: 2 µL/bee The test solutions were prepared with aqueous 1% (v/v) Tween®80.
Control	- Deionised water, application volume: 2 µL/bee - Aqueous Tween®80 solution (1%, v/v), application volume: 2 µL/bee
Administration	Before application, bees in each test cage were anaesthetised with CO_2 for approximately 30 seconds. Anaesthetised bees were removed from the cages to a large petri dish and turned around with the forceps for thoracic application of a single 2 µL/bee droplet of the control (deionised water or Tween®80 solution), test item or reference item solutions. The doses were placed on the dorsal thorax using an Eppendorf Micropipette. After application, bees were carefully transferred one by one into the test cages by means of forceps.
Test duration	48 hours after application
Test units	Disposable cardboard cages (95 mm x 50 mm x 65 mm, length x width x height) with holes in the bottom side for ventilation and a glass plate in front
Temperature	25 ± 2°C (nominal), 23-24°C (actual)
Relative humidity	Approximately 50-70% (nominal), 46-59% (actual)
Illumination	Constant darkness throughout the test (diffuse artificial light only during handling and assessments)
Ventilation	By the air-conditioning equipment of the climate chamber
Feeding	Food (50% (w/v) sucrose solution) was offered *ad libitum* in feeding tubes placed on the floor of the test cage immediately after application.

Observations:

Mortality	4, 24 and 48 hours after application
Behaviour	4, 24 and 48 hours after application Number of bees/replicate that were healthy (normal), affected (impaired locomotion) or moribund; furthermore, any other deviations in behaviour of treated bees were described (e.g. abnormal amount/colour of excretion)

Statistics

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (Ratte, 2018) was used.

Mortality in the test item and reference item treatment groups was analysed for statistical significance using Fisher's Exact Binomial Test with Bonferroni-Holm Correction ($p \leq 0.05$).

For the test item treatment, no LD_{50} could be calculated since no significant mortality occurred for any tested dose. For the reference item treatment, the LC_{50} was calculated by Probit analysis (linear maximum likelihood regression).

Results and discussions

Validity criteria:

- Control mortality: ≤ 10% after 48 hours (observed: 0.0%)
- Reference mortality: 0.10 – 0.30 µg a.s./bee after 24 hours (observed: 0.154 µg a.s./bee)

The study was considered to be valid as all validity criteria were met.

Observations of mortality and behaviour are presented in the table below.

Table 10.3.1.1.2-1. Mortality and behaviour of bees following contact exposure to AG-E1-500 SC1.

Treatment [dosage unit]	**Dosage (consumed)**	**Mean mortality[a)] [%]**			**Behavioural abnormalities Σ A M**								
		4 h	**24 h**	**48 h**	**4 h**			**24 h**			**48 h**		
Control - DW	-	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Control - TS	-	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
AG-E1-500 SC1 [µg prod./bee]	500.0	0.0	0.0	3.3	0	0	0	0	0	0	0	0	0
	250.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
	125.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
	62.5	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
	31.3	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0
Reference item [µg a.s./bee]	0.250	3.3	93.3*	96.7*	16	16	0	0	0	0	0	0	0
	0.188	0.0	76.7*	80.0*	4	4	0	0	0	0	0	0	0
	0.141	0.0	43.3*	50.0*	0	0	0	0	0	0	0	0	0
	0.106	0.0	6.7	6.7	0	0	0	0	0	0	0	0	0

DW: deionised water
TS: aqueous Tween®80 solution, 1% (v/v)
A: affected (impaired locomotion)
M: moribund
a) Mortality results are averages based on 3 replicates consisting of 10 bees each; no correction of mortality (according to Schneider-Orelli 1947) was performed since control mortality was 0.0%.
* Significant difference in pairwise comparison between treatment group and control by Fisher's Exact Binominal Test after Bonferroni-Holm correction for mortality data ($\alpha = 0.05$, one sided greater)

After 48 hours, no mortality was observed in the control group. In the test item treatment groups, mortality was 0.0%, except for the highest treatment of 500.0 µg product/bee, in which mortality was 3.3% after 48 hours. No statistically significant effect on mortality was determined in any test item treatment group when compared to the control by Fisher's Exact Binominal Test after Bonferroni-Holm correction for mortality data ($\alpha = 0.05$, one sided greater). Therefore, the 48-hour contact LD_{50} was estimated to be > 500.0 µg product/bee which corresponds to > 234.1 µg a.s./bee. No effects on behaviour compared to the control were observed at the tested doses up to 500.0 µg product/bee within the 48-hour contact testing.

In the reference item treatment groups, mortality was between 6.7% and 93.3% after 24 hours. The 24-hour contact LD_{50} was calculated as 0.154 µg a.s./bee.

Conclusion

In this test on acute contact toxicity of AG-E1-500 SC1 in the honeybee *Apis mellifera* L., the 48-hour contact LD_{50} was > 500.0 µg product/bee which corresponds to > 234.1 µg a.s./bee.

KCP 10.3.1.2 Chronic toxicity to bees

Comments of zRMS:	The study was performed in line with OECD 245 with a minor deviation. It was noted that behavioural abnormalities in the reference item group were not recorded as it was assumed that moribund and affected bees would die by the end of the test. The reference substance is known to be toxic to honeybees and adverse effects are expected. This deviation is considered to have no impact on the test results since behavioural abnormalities in the toxic standard group are not taken into account in derivation of the endpoints. The endpoints are expressed as nominal concentrations since the measured concentrations of the active substance in the diet were maintained at 80-120% of nominal during the study period. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 10-d LDD_{50} > 115.77 µg a.s./bee/day 10-d LC_{50} > 8067.4 mg a.s./kg food NOEDD = 115.77 µg a.s./bee/day NOEC = 8067.4 mg a.s./kg food

Reference:	**KCP 10.3.1.2/01**
Report	AG-E1-500 SC 1: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee, *Apis mellifera* L. under Laboratory Conditions. Ansaloni T., 2020a, S19-20080
Guideline(s):	OECD 245 (2017)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 511 g ethofumesate/L (actual)
Appearance	Sight beige liquid
Density	1.12 g/mL (20°C)
Expiry date	01/2021
Reference substance	BAS 152 11I Batch No. FRE-001578, 429.0 g dimethoate/L

Test organism:

Test species	Honey bee (*Apis mellifera* L.)
Origin	Bees originated from healthy colonies sited in a commercial apiary registered to the Local Government Administration (No. 176-V-026).
Collection	Two days before test initiation, frames with capped cells were transferred to a bioclimatic chamber. One day before test initiation, bees were randomly transferred to test units.
Age at test start	1 – 2 days old
No. bees per replicate	10
No. replicates per test substance, reference substance or control	4

Test conditions:

Test item dosage	0, 210, 294, 411.6, 576.24, 806.74 µg a.s./bee/day, equivalent to 0, 1764.71, 2470.59, 3458.82, 4842.35, 6779.33 mg a.s./L diet. The highest test substance concentration was prepared by diluting the appropriate amount of test item in 50% (w/v) sucrose solution. Aliquots were diluted in 50% (w/v) sucrose solution to prepare the lower doses. All treated diets were prepared daily.
Reference item dosage	0.107 µg a.s./bee/day, equivalent to 1.07 mg a.s./L diet. A stock solution was prepared each day by diluting the appropriate amount of reference item in 50% (w/v) sucrose solution.
Control	Untreated 50% (w/v) sucrose solution.
Administration	1 mL/replicate/day of each test substance, reference substance or control solution was offered to bees *ad libitum*, in 5 mL syringes.
Test duration	10 days
Test units	Bees were kept in cages made of stainless steel, 8.5 cm x 4.5 cm x 6.5 cm, lined with filter paper.
Temperature	32.4 – 34.2 °C
Relative humidity	57.0 – 67.9 %
Illumination	24-hour darkness, except during application and assessments

Observations:

Mortality	Daily before renewal of feeding solutions
Behavioural abnormalities	Daily before renewal of feeding solutions

Analytical method:

Method type	LC-MS/MS
Equipment	Thermo Accela 1250 HPLC pump with Thermo Accela Open autosampler, HPLC guard column (KJ0-4282, Phenomenex) with 4 mm C18 cartridge (AJ0-4287, Phenomenex)

Column	Phenomenex Luna 5m Phenyl-Hexyl., 150 mm x 2 mm, 5 µm (Part No. 00F-4257-B0)
Column temperature	40ºC
Detector	Thermo TSQ Vantage triple quadrupole system Ionisation type: ESI Polarity: Positive ion mode Ion mass transition: 287 → 121 m/z (quantifier); 287 → 77 m/z
Flow rate	0.5 mL/min
Mobile phase	A: Water C: Methanol D: Water + 1% formic acid 0.00 min 60% A/35% C/5% D 3.00 min 5% A/90% C/5% D 5.00 min 5% A/90% C/5% D 5.01 min 60% A/35% C/5% D 7.00 min 60% A/35% C/5% D Run time: 9.00 min
Retention time	Approx. 4.3 min
Sample preparation	Samples were diluted in water and extracted with acetonitrile. Magnesium sulphide, sodium chloride and sodium citrate were added, then the samples were centrifuged. Resultant supernatants were diluted with acetonitrile/water (1:1 v/v).

Calculations

The cumulative mortality [%] for each treatment group was calculated from the number of dead larvae in relation to the number of introduced test organisms.
Consumption of feed solutions was calculated by dividing the total daily consumption per replicate by the number of bees in the replicate for that 24-hour assessment interval. The mean consumption per treatment was calculated by averaging the results for all replicates.
The evaporation of feed solution was determined by weighing syringes of feed solution that were left in cages without bees. The evaporation of feed solution was taken into account when calculating the consumption of feed solution.

Statistics

Statistical calculations were made with the statistical program ToxRatPro Version 3.2.1.
NOEDD/NOEC values were calculated by comparing mortality to the control using a Chi^2 2x2 Table Test with Bonferroni Correction ($\alpha = 0.05$, one-sided greater).

Results and discussions

Validity criteria:

- Control mean mortality was ≤ 15% at test end (observed: 5 %)
- Reference mean mortality was ≥ 50% at test end (observed: 100 %)

The study was considered to be valid as all validity criteria were met.

Test substance concentrations in the larval diet are presented in the table below.

Table A2.3.1.2-1. Concentrations and % recovery rates of ethofumesate in diet solutions

Treatment	Active substance nominal (mg a.s./L kg diet)	Day	Active substance actual (mg a.s./L kg diet)	% of nominal
Highest test item dosage	6779.33	0	6070	90
		1	6370	94
		2	6020	89
		3	6160	91
		4	6100	90
		5	6340	94
		6	6120	90
		7	6110	90
		8	6610	98
		9	6030	89
Lowest test item dosage	1764.71	0	1830	104
		1	1750	99
		2	1680	95
		3	1680	95
		4	1650	93
		5	1640	93
		6	1710	97
		7	1720	97
		8	1830	104
		9	1700	96
Control	0	0	<LOD	-
		1	<LOD	-
		2	<LOD	-
		3	<LOD	-
		4	<LOD	-
		5	<LOD	-
		6	<LOD	-
		7	<LOD	-
		8	<LOD	-
		9	<LOD	-

LOD – 51.3 mg a.s./kg

Since recoveries of ethofumesate, the active substance of AG-E1-500 SC1, were within 89-104 % of nominal, the biological results were based on nominal test substance concentrations.

Cumulative mortality and corrected cumulative mortality in the control, the test item and reference item treatment groups as well as the study endpoints are presented in the tables below.

Table A2.3.1.2-2. Cumulative mortality of honey bees following exposure to AG-E1-500 SC1.

Nominal dose [µg a.s./bee/day]	Total No. bees dosed	Final mortality (% cumulative)	Standard error	Corrected mortality (% cumulative)
Control	40	5.0	5.00	n/a
210.00	40	5.0	2.89	0.00
294.00	40	15.0	11.9	10.53
411.60	40	7.5	4.76	2.63
576.24	40	7.5	2.50	2.63
806.74	40	15.0	5.00	10.53
Reference (0.17)	40	100	0	100

n/a not applicable

Table A2.3.1.2-3. Behavioural abnormalities of honey bees following exposure to AG-E1-500 SC1.

Nominal dose [µg a.s./bee/day]	% of affected bees									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Control	0	0	0	0	0	0	0	0	0	0
210.00	0	0	0	0	0	0	0	2.50 [a]	0	0
294.00	5.13 [a]	0	0	2.70 [a]	0	0	0	0	0	0
411.60	0	0	0	2.50 [a]	0	0	0	0	0	0
576.24	0	0	0	0	0	0	0	0	0	0
806.74	0	0	0	5.00 [a]	0	0	0	2.50 [a]	2.56 [m]	2.94 [m]

a = affected, m = moribund

Table A2.3.1.2-4. Consumption of feed solutions by honey bees following exposure to AG-E1-500 SC1.

Nominal dose [µg a.s./bee/day]	Mean consumed solution			Mean consumed dose [µg a.s./bee]	
	[mg/bee/day]	Standard deviation	Standard error	Daily	Cumulative (10 days)
Control	20.17	8.02	1.27	n/a	n/a
210.00	19.08	6.30	1.00	33.66	336.62
294.00	19.91	6.19	0.98	49.18	491.78
411.60	18.11	5.89	0.93	62.64	626.38
576.24	19.10	5.34	0.84	92.50	924.95
806.74	17.08	6.42	1.02	115.77	1157.66
Reference (0.17)	13.80	6.93	1.79	0.0124	0.047*

n/a not applicable, * cumulative (4 days)

Table A2.3.1.2-5. Endpoints of the honey bee chronic test on D10.

Endpoint	[µg a.s./bee/day]
10-d NOEDD[a)]	115.77
10-d LOEDD[a)]	>115.77
10-d LDD_{10}	Not estimated[b)]
10-d LDD_{20}	Not estimated[b)]
10-d LDD_{50}	>115.77
Endpoint	**[mg a.s./kg diet]**
10-d NOEC	8067.4
10-d LOEC	>8067.4
10-d LC_{10}	Not estimated[b)]
10-d LC_{20}	Not estimated[b)]
10-d LC_{50}	>8067.4

a) Chi^2 2x2 Table Test with Bonferroni Correction
b) No statistically significant concentration/response was found

In the control group, cumulative mean mortality was 5%. Cumulative mean mortality in the test item doses of 210, 294, 411.6, 576.24 and 806.74 µg a.s./bee/day was 5%, 15%, 7.5%, 7.5% and 15%, respectively, at D10 of the test. In the reference group, cumulative mean mortality was 100%.

Transient behaviour abnormalities were observed in all test substance groups except 576.24 µg a.s./bee/day. At the highest test substance concentration of 806.74 µg a.s./bee/day 2.56% and 2.94% of bees were observed as moribund at D9 and D10 of the test, respectively. No affected bees were observed in the control group throughout the test.

Mean 50% (w/v) sucrose solution consumption was 20.17, 19.08, 19.91, 18.11, 19.1 and 17.08 mg/bee/day in the test item doses of 0, 210, 294, 411.6, 576.24 and 806.74 µg a.s./bee/day, respectively. This was equivalent to mean daily doses of 0, 33.66, 49.18, 62.64, 92.50 and 115.77 µg

a.s./bee/day. Mean consumption of reference treated 50% (w/v) sucrose solution was 13.80 mg/bee/day. This was equivalent to a mean daily dose of 0.0124 µg a.s./bee/day.

Based on the study results at D10, the NOEDD, LOEDD and LDD_{50} were determined to be 115.77, >115.77 and >115.77 µg a.s./bee/day, respectively. The corresponding NOEC, LOEC and LC_{50} were 8067.4, >8067.4 and >8067.4 mg a.s./kg~~L~~ diet, respectively. The D10 LDD_{10} / LC_{10} and LDD_{20} / LC_{20} could not be estimated due to the lack of a statistically significant ~~clear~~ dose/response relationship.

Conclusion

In this chronic oral test with AG-E1-500 SC1 in the honey bee *Apis mellifera* L., the 10-day NOEDD, LOEDD and LDD_{50} were determined to be 115.77, >115.77 and >115.77 µg a.s./bee/day, respectively, with corresponding NOEC, LOEC and LC_{50} of 8067.4, >8067.4 and >8067.4 mg a.s./kg~~L~~ diet, respectively. The 10-day LDD_{10} / LC_{10} and LDD_{20} / LC_{20} could not be estimated due to the lack of a clear dose/response relationship.

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

Comments of zRMS:	The study was performed in line with OECD 239 with minor deviations. It was noted that the temperature in the incubator was slightly higher than 35ºC (max 35.6ºC) for intervals of more than two consecutive hours during day 1 to day 15 (D1-D15). The relative humidity in the incubator was partly below 90% during D1-D7 (min 43.4%) and partly below 75% during D7-D15 (min 55.0%). The change of humidity conditions was on D7 instead of D8, because the earlier reduction of humidity in the step between larval and pre-pupal stage ensured normal development of the organisms. It was noted in the study report that the Test Facility experience had proven that the reduction of the humidity conditions on D7 was suitable and no adverse effects on the outcome of the study were to be expected. The reported deviations to the guideline are considered not to have impacted the outcome of the study since all the validity criteria were met. The endpoints are expressed as nominal concentrations since the measured concentrations of the active substance were maintained at 80-120% of nominal. Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment: 22-d ED_{50} = 164.11 µg a.s./larva 22-d NOED = 140.00 µg a.s./larva 22-d EC_{50} = 1065.65 mg a.s./kg food 22-d NOEC = 909.09 mg a.s./kg food

Reference:	**KCP 10.3.1.3/01**
Report	AG-E1-500 SC 1: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, Ansaloni T., 2020b, S19-20081
Guideline(s):	OECD 239 (2016)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 511 g ethofumesate/L (actual)
Appearance	Sight beige liquid
Density	1.12 g/mL (20°C)
Expiry date	01/2021
Reference substance	BAS 152 I Batch No. COD-002332, 99.7% w/w

Test organism:

Test species	Honey bee (*Apis mellifera* L.)
Origin	The larvae originated from three different bee hives maintained at the test facility. The colonies were examined for reportable bee epidemics by an authorised bee specialist and were inspected periodically according to the standard bee-keeping practices by an experienced apiarist. The hives used for honey bee larvae collection were adequately fed, healthy, as far as possible parasite-free and queen-right. No chemical substances (such as antibiotics, anti-Varroa treatments, pesticides, etc.) had been used in the hive within 4 weeks preceding the start of the test.
Collection	At day 1 (D1), three combs were transferred to the laboratory using an insulated container in order to avoid temperature variation. Once in the laboratory, first instar larvae were selected for grafting. On D1, the test was initiated with excess larvae with 3 reserve plates. Before the first application of the test item on D3, it was assured that all larvae used for the test were of similar size and alive.
Age at test start	Synchronized first instar (L1) larvae Four days prior to grafting of larvae (D-3), in order to synchronize the age of larvae used for the test, the queens of at least three colonies were confined in their own colony in an excluder cage containing a comb with empty cells. Three days prior to the grafting (D-2), maximum 30 hours after encaging, the queens were released from the cages. The combs containing eggs were left in the excluder cages during the incubation stage until hatching on D1.
No. bees per replicate	16
No. replicates per test substance, reference substance or control	3 (each replicate contained larvae from a different hive)

Test conditions:

Test item dosage	Cumulative doses: 41.48, 62.22, 93.33, 140.00 and 210.00 µg a.s./larva, The test item stock and application solutions were prepared in deionised water. Just before feeding from D3 until D6, the application solutions were added to the diet using a micropipette. The volume of application solution in the diet was 10% of the final diet volume. Feeding volume: D3: 20 µL D4: 30 µL D5: 40 µL

	D6: 50 µL Based on the cumulative feeding volume and the density of the diet, the cumulative doses per larva were calculated.
Reference item dosage	Cumulative dose: 7.39 µg dimethoate/larva, The reference item stock and application solution were prepared in deionised water. Addition of the application solution to the diet and feeding volume was as for the test item.
Control	Diet B and C to which 10% of deionised water without test/reference item were added Feeding volume: as for the test item
Administration	Each larva was fed once a day (except on D2) with a standardized amount of artificial diet until day 6. For feeding, a multi stepper pipette was used. Care was taken to avoid touching and drowning the larvae when feeding them. Food was dropped next to the larva, along the wall of the grafting cell.
Test duration	22 days
Test units	Larvae were transferred into crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm. Cells were initially sterilised by emerging for 30 min in ethanol 70% (v/v), and then dried. Each cell was placed into a well of a sterile 48-well cellular culture plate (Greiner Bio One), and the prepared experimental units were placed under UV light for 15 minutes. The open plates of the control group, of all test item groups and the reference item group were individually placed into hermetically sealed Plexiglas desiccators, containing dishes filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from D1 to D7, when the well plates were transferred to another Plexiglas desiccator, containing a saturated sodium chloride (NaCl) solution in order to keep the established relative humidity until D15. All desiccators were placed into the same incubator with forced air circulation. After the assessment on D15, the test units were allocated into an emergence box (plastic polypropylene approximately 18 x 13 x 7 cm) and placed inside a climatic chamber. Each emergence box was supplied with 50% (w/v) aqueous sucrose solution *ad libitum*.
Grafting of larvae	On D1, 20 µL of diet A were dropped into each grafting cell of the well plate. Using a grafting tool, one larva was delicately transferred from the comb to each cell on the surface of the diet. Larvae were grafted in excess, to replace non-suitable larvae with individuals from the reserve plates on D3.
Temperature	Nominal: 34-35°C, deviations remaining within 23-40°C allowed for ≤ 0.5 h once daily Actual: D1-D7: 34.4-35.3°C D7-D15: 34.6-35.6°C D15-D22: 32.9-35.0°C
Relative humidity	Nominal: 95% ± 5% at D1-D7, 80% ± 5% at D7-D15, 50-80% at D15-D22 Actual: D1-D7: 43.4-98.0% D7-D15: 55.0-83.4% D15-D22: 56.1-70.3%
Illumination	During the entire test period, the bee larvae were kept under constant darkness except during feeding and assessments.
Feeding	The diet was prepared with deionised water using the following ingredients: - Diet A (D1, volume administered: 20 µL/larva): 50% weight

	of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose - Diet B (D3, volume administered: 20 µL/larva): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose - Diet C (from D4 to D6, volume administered: 30 µL/larva, 40 µL/larva and 50 µL/larva, respectively): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose Batches of diet B and C were prepared with less water than required taking into account the volume of solvent (i.e. 10% of deionised water) that would be added later when spiking with the application solutions or deionised water (control).

Observations:

Mortality	Daily from D4 to D8 and on D15 (before feeding) On D15, larvae that had not transformed into pupae were recorded as dead. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group.
Behavioural abnormalities	Daily from D4 to D8 and on D15 (before feeding)
Presence of uneaten food	On D8 during assessment of mortality
Emergence rate	D22 With assistance of a stereo microscope, when necessary, larvae were recorded as dead if no respiration (movement of spiracles) was observed.

Analytical method:

Method type	LC-MS/MS
Equipment	Thermo Accela 1250 HPLC pump with Thermo Accela Open autosampler, HPLC guard column (KJ0-4282, Phenomenex) with 4 mm C18 cartridge (AJ0-4287, Phenomenex)
Column	Phenomenex Luna 5m Phenyl-Hexyl., 150 mm x 2 mm, 5 µm (Part No. 00F-4257-B0)
Detector	Thermo TSQ Vantage triple quadrupole system Ionisation type: ESI Polarity: Positive ion mode Ion mass transition: 287 → 121 m/z (quantifier); 287 → 77 m/z
Flow rate	0.5 mL/min
Mobile phase	A: Water C: Methanol D: Water + 1% formic acid 0.00 min 60% A/35% C/5% D 3.00 min 5% A/90% C/5% D 5.00 min 5% A/90% C/5% D 5.01 min 60% A/35% C/5% D 7.00 min 60% A/35% C/5% D Run time: 9.00 min
Retention time	Approx. 4.2 min

Calculations

The cumulative mortality [%] for each treatment group was calculated from the number of dead larvae in relation to the total number of larvae per treatment group across all replicates after re-grafting on D3. Mortality during the pupation phase was evaluated on D15 and on D22. The cumulative pupae mortality [%] for each treatment group was calculated from the number of larvae that had not transformed into pupae on D15 and those bees without emergence on D22 in relation to the total number of entered pupae after pre-pupae stage on D8. The adult emergence [%] for each treatment group was calculated from the number of emerged bees on D22 in relation to the total number of larvae per treatment group after selection on D3.

The cumulative mortality for each test item group and the reference item group were expressed as percentage of the control populations after an adjustment according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

Statistics

Statistical calculations were made with the statistical program ToxRatPro Version 3.2.1.

Step-down Cochran-Armitage test procedure was used to calculate the 22-day NOED / NOEC and LOEC / LOED values.

The LD_{50}/LC_{50} endpoints with 95% confidence intervals were calculated by using the Trimmed Spearman-Karber procedure.

Since no clear concentration/response in the required interval was found, reliable $LD_{10/20}$ / $LC_{10/20}$ values could not be estimated.

Results and discussions

Validity criteria:

- Control cumulative larval mortality (D3-D8): ≤ 15% across all replicates in control group (observed: 6.25%)
- Control adult emergence rate on D22: ≥ 70% across all replicates in control group (observed: 77.08%)
- Reference cumulative larval mortality: ≥ 50% across all replicates on D8 (observed: 91.67%)

The study was considered to be valid as all validity criteria were met.

Test substance concentrations in the larval diet are presented in the table below.

Table A2.3.1.3-1. Concentrations and % recovery rates of ethofumesate in larval diet

Treatment	Active substance nominal (mg a.s./kg diet)	Day	Active substance actual (mg a.s./kg diet)	% of nominal
Highest test item dosage	1363.64	3	1360	100
		4	1190	87
		5	1240	91
		6	1250	92
Lowest test item dosage	269.36	3	271	101
		4	252	94
		5	228	85
		6	226	84
Control	0	3	< LOD	-
		4	< LOD	-
		5	< LOD	-
		6	< LOD	-

LOD – 7.32 mg a.s./kg (larval diet)

Since recoveries of ethofumesate, the active substance of AG-E1-500 SC1, were within 84-101% of nominal, the biological results were based on nominal test substance concentrations.

Cumulative mortality, corrected cumulative mortality and emergence rate in the control, the test item and reference item treatment groups as well as the study endpoints are presented in the tables below.

Table A2.3.1.3-2. Cumulative mortality of honey bee larvae following exposure to AG-E1-500 SC1.

Treatment	Dose		Cumulative mortality [%]						
			D4	D5	D6	D7	D8	D15	D22
Control	-	-	2.08	4.17	6.25	6.25	6.25	22.92	22.92
AG-E1-500 SC1 (ethofumesate)	210.00	[µg a.s./larva]	6.25	12.50	18.75	20.83	20.83	70.83	75.00
	140.00		2.08	4.17	10.42	10.42	10.42	31.25	33.33
	93.33		2.08	6.25	12.50	12.50	12.50	33.33	33.33
	62.22		2.08	2.08	2.08	2.08	2.08	29.17	31.25
	41.48		2.08	2.08	4.17	4.17	6.25	27.08	27.08
Reference item (dimethoate)	7.39	[µg dimethoate/larva]	25.00	58.33	81.25	87.50	91.67	97.92	97.92

Table A2.3.1.3-3. Cumulative mortality in the test item and reference item treatment groups corrected by the control group.

Treatment	Dose		Corrected cumulative mortality [%]						
			D4	D5	D6	D7	D8	D15	D22
Control	-	-	-	-	-	-	-	-	-
AG-E1-500 SC1 (ethofumesate)	210.00	[µg a.s./larva]	4.26	8.70	13.33	15.56	15.56	62.16	67.57
	140.00		0.00	0.00	4.44	4.44	4.44	10.81	13.51
	93.33		0.00	2.17	6.67	6.67	6.67	13.51	13.51
	62.22		0.00	-2.17	-4.44	-4.44	-4.44	8.11	10.81
	41.48		0.00	-2.17	-2.22	-2.22	0.00	5.41	5.41
Reference item (dimethoate)	7.39	[µg dimethoate/larva]	23.40	56.52	80.00	86.67	91.11	97.30	97.30

Table A2.3.1.3-4. Emergence rate of honey bee larvae following exposure to AG-E1-500 SC1.

Treatment	Dose		Emergence rate [%] D22
Control	-	-	77.08
AG-E1-500 SC1 (ethofumesate)	210.00	[µg a.s./larva]	25.00
	140.00		66.67
	93.33		66.67
	62.22		68.75
	41.48		72.95
Reference item (dimethoate)	7.39	[µg dimethoate/larva]	2.08

Table A2.3.1.3-5. Endpoints of the honey bee chronic larvae test on D22.

Endpoint	**[µg a.s./larva/developmental period]**
22-d NOED[a)]	140.00
22-d LOED[a)]	210.00
22-d ED_{10} (95% confidence interval)	Not estimated[b)]
22-d ED_{20} (95% confidence interval)	Not estimated[b)]
22-d ED_{50} (95% confidence interval)[c)]	164.11 (149.88-179.69)
Endpoint	**[mg a.s./kg diet]**
22-d NOEC[a)]	909.09
22-d LOEC[a)]	1363.64
22-d EC_{10} (95% confidence interval)	Not estimated[b)]
22-d EC_{20} (95% confidence interval)	Not estimated[b)]
22-d EC_{50} (95% confidence interval)[c)]	1065.65 (973.25-1166.82)

a) Step-down Cochran-Armitage test procedure
b) No concentration/response was found in the interval of interest
c) Trimmed Spearman-Karber procedure

In the control group, cumulative mean mortality from D4 until D8 was 6.25%. Cumulative mean mortality in the test item doses of 41.48, 62.22, 93.33, 140.00 and 210.00 µg a.s./larva, was 6.25%, 2.08%, 12.50%, 10.42% and 20.83%, respectively, at D8 of the test. Five larvae in the highest treatment of 1363.64 mg a.s./kg diet with presence of uneaten food were observed at day 8 (D8). In the reference item group, cumulative larval mortality was 91.67% by D8.

At D15 of the test, cumulative mean mortality was 27.08%, 29.17%, 33.33%, 31.25% and 70.83%, in the test item doses of 41.48, 62.22, 93.33, 140.00 and 210.00 µg a.s./larva, respectively.

On D22, cumulative mean mortality in the control group was 22.92% and thus, adult emergence rate was 77.08% of the initially grafted larvae. Cumulative mean mortality in the test item doses of 41.48, 62.22, 93.33, 140.00 and 210.00 µg a.s./larva, was 27.08%, 31.25%, 33.33%, 33.33% and 75.00%, respectively, at the end of the test (D22). Consequently, the mean emergence rates were 72.92%, 68.75%, 66.67%, 66.67% and 25.00%, respectively. No affected emerged bees were recorded on D22.

Based on the study results at D22, the NOED, LOED and ED_{50} were determined to be 140.00, 210.00 and 164.11 (95% confidence interval 149.88-179.69) µg a.s./larva/developmental period, respectively. The corresponding NOEC, LOEC and EC_{50} were 909.09, 1363.64 and 1065.65 (95% confidence interval 973.25-1166.82) mg a.s./kg diet, respectively. The 22-day ED_{10} / EC_{10} and ED_{20} / EC_{20} could not be estimated due to the lack of a clear dose/response relationship in the interval of interest.

Conclusion

In this chronic larvae test with AG-E1-500 SC1 in the honey bee *Apis mellifera* L., the 22-day NOED, LOED and ED_{50} were determined to be 140.00, 210.00 and 164.11 µg a.s./larva/developmental period, respectively, with corresponding NOEC, LOEC and EC_{50} of 909.09, 1363.64 and 1065.65 mg a.s./kg diet. The 22-day ED_{10} / EC_{10} and ED_{20} / EC_{20} could not be estimated due to the lack of a clear dose/response relationship in the interval of interest.

A 2.3.1.2 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.3 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.4 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

Acute Effects on *Typhlodromus pyri*

Comments of zRMS:	The study was performed in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} > 9452 mL product/ha (5000 g a.s./ha, based on analysed content of the a.s.)

Reference:	**KCP 10.3.2/01**
Report	Effects of AG-E1-500-SC1 on the predatory mite *Typhlodromus pyri* Scheuten in a laboratory test, Röhlig U., 2020a, 20 48 NTL 0001
Guideline(s):	Blümel, S., Bakker, F.M., Baier, B., Brown, K., Candolfi, M.P., Goßmann, A., Grimm, C., Jäckel, B., Nienstedt, K., Schirra, K.J., Ufer, A. and Waltersdorfer, A.: Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Candolfi, M. P., Blümel, S., Forster, R., Bakker, F. M., Grimm, C., Hassan, S. A., Heimbach, U., Mead-Briggs, M. A., Reber, B., Schmuck, R., Vogt, H. (eds): Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC, BART and EPPO Joint Initiative, IOBC/WPRS publication 2000, 121-143. Grimm, C., Schmidli, H., Bakker, F.M., Brown, K., Campbell, P., Candolfi, M., Chapman, P., Harrison, E.G., Mead-Briggs, M., Schmuck, R. and Ufer, A.: Use of standard toxicity tests with *Typhlodromus pyri* and *Aphidius rhopalosiphi* to establish a dose-response relationship. J. Pest Science, 74, 72-84, 2001.
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500-SC1 (= AG-E1-500 SC1)
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	DANADIM PROGRESS (Dimethoate EC 400) 400 g dimethoate/L (nominal), 429 g dimethoate/L (actual)

Test organism:

Test species	*Typhlodromus pyri* SCHEUTEN
Origin	"Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Age at test start	Protonymphs (< 24 hours)
No. protonymphs per replicate	20
No. replicates per test substance, reference substance or control	5

Sex ratio	All surviving mites from the mortality phase were used for the reproduction test differentiated according to their sex. The sex ratio was 0.55, 0.55, 0.54, 0.54, 0.52 and 0.55 for the control and the 500, 890, 1580, 2810 and 5000 g a.s./ha treatment, respectively, at the beginning of the reproduction test (calculated by the applicant).

Test conditions:

Test item concentration	500, 890, 1580, 2810 and 5000 g a.s./ha (equivalent to 945.2, 1682, 2987, 5312 and 9452 mL product/ha, based on analysed content of a.s.) Application in 200 L water/ha
Reference item concentration	15 mL product/ha (6 g dimethoate/ha) Application in 200 L water/ha
Application method	Spray by single Lechler ES 90-015 nozzle in an application cabin (tracksprayer by Schachtner, 71640 Ludwigsburg, Germany), 3.4 bar pressure, 2.25 km/h spraying speed
Test duration	Day 0 to 7 mortality test Day 7 to 14 reproduction test
Test arena	Two glass plates (cover glasses: 50 mm x 22 mm stuck together along their longitudinal sides) with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray (inside dimensions: about 165 mm x 120 mm x 60 mm) filled with tap water up to a height of approximately 15 mm
Temperature	25 ± 2°C (nominal), 23-27°C (actual)
Relative humidity	60-90% (nominal), 67-73% (actual)
Light intensity	1970 lux
Photoperiod	16 hours light, 8 hours dark
Food and feeding regime	pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1, at each assessment day (days 3, 7, 9 and 11)

Observations:

Mortality and no. escapers	days 3, 7, 9, 11 and 14
No. eggs and hatched juveniles	days 9, 11 and 14

Statistics

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (Ratte, 2018) was used.

Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Chi²-2x2 Table Test after Bonferroni-Holm as a distribution-free test which does not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$.

Reproduction was analysed for statistical significance using Williams-t-test, following Shapiro-Wilk's test for normal distribution, Levene's test procedure for variance homogeneity and a trend analysis by contrasts to test the data for monotonicity of rate/response.

Mortality and effect on reproduction in all test item treatment groups was less than 50% compared to the control group, hence, a calculation of the LR_{50} (median lethal rate) and the ER_{50} (median effect rate) was not possible.

Results and discussions

Validity criteria:

- Control mortality: ≤ 20% (dead and escaped mites) on day 7 (observed: 2.0%)
- Corrected reference mortality: 50-100% on day 7 (observed: 87.8%)
- Control reproduction: ≥ 4 eggs per female (observed: 6.77 eggs per female)

The study was considered to be valid as all validity criteria were met.

Observations of mortality are presented in the table below.

Table 2.3.2.1-1. Observations of mortality of *T. pyri* following exposure to AG-E1-500 SC1.

Treatment	Days after application	Total number of dead mites (including escapers)	Mortality at day 7 (uncorrected) [%]	Corrected[a] mortality at day 7 [%]
Control	3	0	2	-
	7	2		
	14	13		
AG-E1-500 SC1 500 g a.s./ha	3	0	3	1.0
	7	3		
	14	17		
AG-E1-500 SC1 890 g a.s./ha	3	0	2	0
	7	2		
	14	12		
AG-E1-500 SC1 1580 g a.s./ha	3	0	3	1.0
	7	3		
	14	14		
AG-E1-500 SC1 2810 g a.s./ha	3	0	1	-1.0
	7	1		
	14	11		
AG-E1-500 SC1 5000 g a.s./ha	3	0	3	1.0
	7	3		
	14	15		
Reference substance 6 g dimethoate/ha	3	58	88	87.8
	7	88		
	14	Not assessed		

Note: No statistically significant differences in mortality between the test substance treatments and the control were observed (Multiple Sequentially-rejective Chi²-2x2 Table Test after Bonferroni-Holm procedure, α = 0.05, one-sided greater).

a) Corrected according to Abbott (1925)

Observations of reproduction are presented in the table below.

Table 2.3.2.1-2. Observations of reproduction of *T. pyri* following exposure to AG-E1-500 SC1.

Treatment	No. of surviving females at day 7	No. of surviving males at day 7	Cumulative no. of eggs (day 7-14)	Cumulative no. of larvae (day 7-14)	Reproduction rate (mean no. of eggs[a] per female, day 7-14)	Effect on reproduction[b] [%]
Control	54	44	333	8	6.77	-
AG-E1-500 SC1 500 g a.s./ha	53	44	319	7	6.59	2.7
AG-E1-500 SC1 890 g a.s./ha	53	45	315	9	6.57	3.0
AG-E1-500 SC1 1580 g a.s./ha	52	45	333	5	6.96	-2.8
AG-E1-500 SC1 2810 g a.s./ha	51	48	291	6	6.20	8.4
AG-E1-500 SC1 5000 g a.s./ha	53	44	176	1	3.55*	47.6
Reference subst. 6 g dimethoate/ha	8	4	not determined			

a) Eggs including larvae

b) Change in mean number of eggs per female, relative to control. A positive value indicates a decrease and a negative

value indicates an increase, relative to the control.
* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$)

After 7 days, mortality of 2.0% was observed in the water-treated control. In the test item treatments, mortality ranged between 1.0% and 3.0%. This resulted in corrected mortality rates between -1.0% and 1.0%. No statistically significant effect on mortality was determined at any treatment rate when compared to the control (Multiple Sequentially-rejective Chi²-2x2 Table Test after Bonferroni-Holm procedure, $\alpha = 0.05$, one-sided greater). The LR_{50} was estimated to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha). The NOER (no observed effect rate) for mortality was determined to be 5000 g a.s./ha (equivalent to 9452 mL product/ha).

The reproduction rate in the control was 6.77 eggs/female. The reproduction rates in the test item treated groups were between 6.96 and 3.55 eggs/female. Thus, an effect on reproduction between -2.8% and 47.6% was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at test item rates up to and including 2810 g a.s./ha (equivalent to 5312 mL product/ha; Williams-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha). The NOER (no observed effect rate) for reproduction was 2810 g a.s./ha (equivalent to 5312 mL product/ha).

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.
The reference item caused 88.0 % mortality in exposed mites, resulting in a corrected mortality of 87.8 %.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to AG-E1-500 SC1 on mortality and reproduction of *Typhlodromus pyri*, the LR_{50} and ER_{50} were both determined to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha).

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Acute Effects on *Aphidius rhopalosiphi*

Comments of zRMS:	The study was performed in line with the respective guideline with no deviations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} > 9452 mL product/ha (5000 g a.s./ha, based on analysed content of the a.s.)

Reference:	**KCP 10.3.2/02**
Report	Effects of AG-E1-500-SC1 on the parasitic wasp *Aphidius rhopalosiphi* (De Stefani-Perez) in a laboratory test, Röhlig U., 2020b, 20 48 NAL 0001
Guideline(s):	Mead-Briggs, M. et al.: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae). In: Candolfi, M. P., Blümel, S., Forster, R., Bakker, F. M., Grimm, C., Hassan, S. A., Heimbach, U., Mead-Briggs, M. A., Reber, B., Schmuck, R., Vogt, H. (eds): Guidelines to evaluate side-effects of plant protection products to non-target arthropods, IOBC, BART and EPPO Joint Initiative, IOBC/WPRS publication 2000, 121-143. Grimm, C., Schmidli, H., Bakker, F.M., Brown, K., Campbell, P., Candolfi, M., Chapman, P., Harrison, E.G., Mead-Briggs, M., Schmuck, R. and Ufer, A.: Use of standard toxicity tests with *Typhlodromus pyri* and *Aphidius rhopalosiphi* to establish a dose-response relationship. J. Pest Science, 74, 72-84, 2001.

Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500-SC1 (= AG-E1-500 SC1)
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	DANADIM PROGRESS (Dimethoate EC 400) 400 g dimethoate/L (nominal), 411.2 g dimethoate/L (actual)

Test organism:

Test species	*Aphidius rhopalosiphi* (De Stefani-Perez)
Origin	"Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Age at test start	< 48 hours after hatching
No. adults per replicate	Mortality phase: 10 (7 females + 3 males) Reproduction phase: 1 female
No. replicates per test substance, reference substance or control	Mortality phase: 4 Reproduction phase: 15
Sex ratio	Mortality phase: 7 male:3 female (0.7)

Test conditions:

Test item concentration	500, 890, 1580, 2810 and 5000 g a.s./ha (equivalent to 945.2, 1682, 2987, 5312 and 9452 mL product/ha, based on analysed content of a.s.) Application in 200 L water/ha
Reference item concentration	0.3 mL product/ha (0.12 g dimethoate/ha) Application in 200 L water/ha
Application method	Spray by single Lechler ES 90-015 nozzle in an application cabin (tracksprayer by Schachtner, 71640 Ludwigsburg, Germany), 3.4 bar pressure, 2.25 km/h spraying speed
Test duration	Day 0 to 2 exposure phase (48-hour mortality test) Day 2 to 3 parasitisation phase (24-hour parasitisation of cereal aphids by the wasps) Day 3 to 14 reproduction phase (reproduction test after removal of the wasps)
Test arena	Mortality phase: Two square glass plates (13 cm x 13 cm), held apart by an aluminium frame (13 cm x 13 cm x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min) Reproduction phase (including parasitisation): Acrylic cylinder (about 11 cm Ø, 20 cm high) with approx. 20 wheat seedlings (*Triticum*) e.g. variety "Tambor" (8 days old) planted in a pot containing potting soil, infested with > 100 adult and nymphal aphids (*Rhopalosiphum padi*, reared in the laboratory of the test facility) and covered at the top of the cylinder with gauze

Temperature	20 ± 3°C ~~12 ± 2°C~~ (nominal), 18-22°C (actual)
Relative humidity	50 ~~60~~-90% (nominal), 60-77% (actual)
Light intensity	1080 lux (exposure phase) 2210 lux (parasitisation phase) 6740 lux (reproduction phase)
Photoperiod	16 hours light, 8 hours dark
Food and feeding regime	Mortality phase: 1:3 v/v solution of honey and water

Observations:

Mortality and condition	2, 24 and 48 hours after start of exposure
No. of parasitized aphids (mummies)	Day 14 (end of reproduction phase)

Statistics

For statistical calculation of the results, the computer program ToxRat Professional 3.3.0 (Ratte, 2018) was used.

Mortality was analysed for statistical significance using the Chi²-2x2 Table Test as distribution-free test which does not require testing for normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$.

Reproduction capacity was analysed for statistical significance using Williams-t-test, following Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity and Trend analysis by contrasts to test the data for monotonicity of rate/response.

Since there were only minor effects on mortality and reproduction in the test item treatment groups, a calculation of the LR_{50} (median lethal rate) and ER_{50} (median effect rate) was not possible.

Results and discussions

Validity criteria:

- Control mortality: ≤ 13% (48 hours) (observed: 2.5%)
- Corrected reference mortality: > 50% and preferably < 100% (48 hours) (observed: 100%)
- Control reproduction: ≥ 5 mummies per female (observed: 20.7) and no more than two wasps producing zero values (observed: two wasps producing zero values)

The study was considered to be valid as all validity criteria were met.

Observations of mortality and condition are presented in the table below.

Table 2.3.2.2-1. Observations of mortality and condition of *A. rhopalosiphi* following exposure to AG-E1-500 SC1.

Treatment	No. of dead wasps after 48 hours	No. of moribund wasps after 48 hours	No. of surviving wasps after 48 hours	Mortality after 48 hours (uncorrected) [%]	Corrected[a)] mortality after 48 hours [%]
Control	1	0	39	2.5	-
AG-E1-500 SC1 500 g a.s./ha	0	0	40	0	-2.6
AG-E1-500 SC1 890 g a.s./ha	1	0	39	2.5	0
AG-E1-500 SC1 1580 g a.s./ha	0	0	40	0	-2.6
AG-E1-500 SC1 2810 g a.s./ha	1	0	39	2.5	0
AG-E1-500 SC1 5000 g a.s./ha	1	0	39	2.5	0
Reference substance 0.12 g dimethoate/ha	37	3	0	100	100

Note 1: No statistically significant differences in mortality between the test substance treatments and the control were observed (Chi²-2x2 Table Test, $\alpha = 0.05$).
Note 2: After 48 hours of exposure, no affected wasps (additional condition criterion) were observed in any treatment group.
a) Corrected according to Abbott (1925)

Observations of reproduction are presented in the table below. The effects of the reference substance dimethoate on reproduction of *A. rhopalosiphi* could not be tested because all of the female wasps were either dead or moribund at the end of the mortality phase.

Table 2.3.2.2-2. Observations of reproduction of *A. rhopalosiphi* following exposure to AG-E1-500 SC1.

Treatment	No. of females used for reproduction test	Mean no. of mummies/female[a)] at end of reproduction phase	Effect on reproductive capacity[b)] [%]
Control	15	20.7	-
AG-E1-500 SC1 500 g a.s./ha	15	20.2	2.4
AG-E1-500 SC1 890 g a.s./ha	15	21.6	-4.3
AG-E1-500 SC1 1580 g a.s./ha	15	21.1	-1.9
AG-E1-500 SC1 2810 g a.s./ha	15	19.8	4.3
AG-E1-500 SC1 5000 g a.s./ha	15	20.5	1.0

Note: No statistically significant differences in reproduction between the test substance treatments and the control were observed (Williams-t-test, $\alpha = 0.05$).
a) The mean number of mummies/female was calculated from the number of mummies per surviving female.
b) Change in mean number of mummies per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase, relative to the control.

After 48 hours, mortality was 2.5% in the water-treated control. In the test item treatments, mortality ranged between 0% and 2.5%. This resulted in corrected mortality rates of between -2.6% and 0%. No statistically significant effects on mortality were determined in all test item treatment groups (Chi²-2x2 Table Test, $\alpha = 0.05$). The LR_{50} was estimated to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha). The NOER (no observed effect rate) for mortality was 5000 g a.s./ha (equivalent to 9452 mL product/ha).

The mean number of mummies produced per female in the test item treatment groups ranged between 19.8 and 21.6, compared to the control value of 20.7 mummies/female. No statistically significant effects on reproductive capacity were determined in any of the test item treatment groups (Williams-t-test, $\alpha = 0.05$). The ER_{50} was estimated to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha).

The NOER (no observed effect rate) for reproduction was determined to be 5000 g a.s./ha (equivalent to 9452 mL product/ha).

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusion

In this standard laboratory test (use of glass plates as substrate) to determine the effects of exposure to AG-E1-500 SC1 on mortality and reproduction of *Aphidius rhopalosiphi*, the LR_{50} and ER_{50} were both determined to be > 5000 g a.s./ha (equivalent to > 9452 mL product/ha).

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was performed in line with OECD 222 with no deviations. It was noted that the test design was relevant to derive both NOEC and EC_x values as there were 8 concentrations tested with 4 replicates per treatment and 8 replicates for control, and the spacing factor did not exceed 1.8. Reliability of the EC_{10} value has been evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673 based on endpoints expressed in terms of the active substance: • NW (normalised width) of 0.59 was calculated, which results with rating "fair" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} (60.8 mg a.s./kg dws) is lower than $EC_{20,low}$ (68.2 mg a.s./kg dws), • the dose-response curve is medium with steepness of 0.51 (i.e. between 0.33 and 0.66); it should be however noted that EC_{50} was greater than the maximum concentration tested and for this reason calculation of the steepness of the dose-response curve is not fully reliable. Taking into account the provided above indications, the calculated EC_{10} is considered to be not fully reliable. Nevertheless, the lower NOEC is recommended for the risk assessment, so reliability of EC_{10} is of lesser importance in this case. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC (mortality, biomass) = 271.2 mg product/kg dw soil (corresponding to 120 mg a.s./kg dw soil) NOEC (reproduction) = 120.5 mg product/kg dw soil (corresponding to 53.3 mg a.s./kg dw soil) EC_{10} (reproduction) = 137.4 mg product/kg dw soil (corresponding to 60.8 mg a.s./kg dw soil) EC_{20} (reproduction) = 183.2 mg product/kg dw soil (corresponding to 81.1 mg a.s./kg dw soil) EC_{50} (reproduction) >271.2 mg product/kg dw soil (corresponding to >120.0 mg a.s./kg dw soil)

Reference:	**KCP 10.4.1.1/01**
Report	Effects of AG-E1-500 SC1 on the reproduction of the earthworm *Eisenia andrei* in artificial soil, Friedrich S., 2020a, 20 48 TEC 0002
Guideline(s):	OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A

Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Maypon Flow (Carbendazim, SC 500)

Test organism:

Test species	Earthworm *Eisenia andrei* (Bouché, 1972)
Origin	Reared under ambient laboratory conditions in the test facility (original source: "W. Neudorff GmbH KG", An der Mühle 3, 31860 Emmerthal, Germany)
Age at test start	Adult worms (approximately 4 months old with clitellum)
Weight at test start	302-458 mg/worm
Acclimatisation	At least 24 hours in test substrate (with food)
Diet	Air-dried and finely ground horse manure One day after application, 5 g of manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (approximately 5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food. As a final feed, 5 g of manure were added to each test vessel at day 28 after adult earthworms had been removed and the soil had been returned to the original test vessels.

Test conditions:

Test substance concentration	7.0, 10.5, 15.8, 23.7, 35.6, 53.3, 80.0 and 120.0 mg a.i./kg dry soil, corresponding with 15.9, 23.8, 35.7, 53.6, 80.4, 120.5, 180.8 and 271.2 mg product/kg dry soil (based on product density and nominal content of a.s.) On the day of the test start, a stock solution of the test item (= application solution of the highest test concentration)was prepared by dispersion in deionised water. This stock solution was serially diluted with deionised water to prepare the further application solutions.
Reference item concentration	The reference item Maypon Flow (Carbendazim, SC 500) was tested in a separate study (reported 30 January 2020) at 5 and 10 mg product/kg dry soil.
Control	Deionised water
Test substrate	Artificial soil was prepared with the following constituents: - 10% sphagnum peat (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried) - 20% kaolinite clay (kaolinite content > 30%) - 0.5% calcium carbonate - 69.5% industrial quartz sand (> 50% of particles between 50 and 200 µm)
No. test organisms per replicate (test vessel)	10
No. replicates per treatment	8 for the control, 4 for the test item treatments

Application method	One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. On the day of the test start, the test item was introduced to the soil by adding 60 mL of the appropriate test solution to portions (750 g wet weight) of the pre-moistened soil. This brought the soil to its final water content (40-60% of the soil maximum water holding capacity (WHC)). The control substrate contained the corresponding amount of deionised water only. Each test vessel was then filled with the treated soil (810 g wet weight corresponding to 600 g dry weight). Groups of 10 worms were randomly assigned to each test vessel. The individually weighed worms were placed on the surface of the soil and after approximately 30 minutes, the test vessels were closed with lids.
Test duration	56 days After 28 days, adult worms were removed from the test vessels and the soil was returned to the original test vessels for reproduction assessment during the second 28 days of the test.
Test vessels	Plastic vessel (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid permeable to air and light
Temperature	19.0-20.0°C
Illumination	16 h light (light intensity: 640 lux) : 8 h dark photoperiod
Water content of artificial soil	Test start: 34.9% - 35.0% (equivalent to 56.0% – 56.2% WHC) Test end: 34.2% – 34.8% (equivalent to 54.9% – 55.9% WHC)
pH of artificial soil	Test start: 6.04 – 6.10 Test end: 5.82 – 5.90

Measurements:

Adult mortality	At day 28
Behavioural effects and pathological symptoms of adults (including feeding activity)	Weekly until day 28
Biomass development of adults	The fresh weight of worms was weighed individually at test start and per replicate after 28 days.
Reproduction	The number of hatched juvenile earthworms per replicate was determined at day 56. The test vessels were placed in a water bath set to 50°C - 60°C and left for a period of approximately 20 minutes which forced the living juvenile earthworms to the soil surface. The juvenile earthworms were removed from the soil surface and counted by hand. Afterwards, the soil from each test vessel was carefully checked for any remaining juveniles left in the soil.
Temperature	Continuously by a data logger
pH of the soil samples	At test start and end
Water content of the soil samples	At test start and end

Calculations

The endpoints of the test were mortality, change in biomass (difference in fresh weight of surviving worms between test start and 28 days after treatment) and inhibition of reproduction (reduction in the number of juveniles present). The arithmetic mean and standard deviation per treatment for each endpoint were calculated.

The statistical analysis was performed with the software ToxRat Professional ToxRat Professional 3.2.1 (Ratte 2015). The EC_{10}, EC_{20} and EC_{50} values (reduction in number of juveniles) were estimated using Logit analysis using the maximum likelihood method. Confidence limits (95%) for the EC_x values were computed by normal approximation. For determining the NOEC values, the Williams-t-

test was used to compare the control with the test item treatment groups. For statistical evaluation of the biomass endpoint, the change in mean fresh weight of surviving worms per replicate was used.

Results and discussions

Validity criteria:

- Each control replicate produces ≥ 30 juveniles (observed: 236-342 juveniles)
- The coefficient of variation of the reproduction rate per replicate in the control is ≤ 30% (observed: 14.2%)
- Mean mortality of adults in the control is ≤ 10% (observed: 0%)

The study was considered to be valid as all validity criteria were met.

Observations of mortality, body weight change and reproduction are presented in the table below.

Table A2.4.1.1-1. Effects of AG-E1-500 SC1 on mortality, body weight change and reproduction of *Eisenia andrei*

Treatment		**Mortality after 28 days of exposure (%)**	**Mean biomass change (day 0-28) (%)**	**Reproduction after 56 days**	
(mg a.s./kg dry soil)	**(mg product/kg dry soil)**			**(mean number of juveniles/replicate)**	**Reduction compared to control (%)**
0 (control)	0 (control)	0.0	27.2	280.1	-
7.0	15.9	0.0	25.9	282.8	-0.9
10.5	23.8	0.0	28.7	274.3	2.1
15.8	35.7	0.0	30.1	276.8	1.2
23.7	53.6	0.0	27.0	284.5	-1.6
35.6	80.4	0.0	23.9	282.3	-0.8
53.3	120.5	0.0	24.6	286.0	-2.1
80.0	180.8	0.0	29.3	206.3*	26.4
120.0	271.2	0.0	26.5	166.0*	40.7
Endpoints (mg a.s./kg dry soil) (95% confidence limits) / (mg product/kg dry soil) (95% confidence limits)					
EC_{10} (reproduction)[a)]			60.8 (45.5 – 81.3) / 137.4 (102.7 – 183.8)		
EC_{20} (reproduction)[a)]			81.1 (68.2 – 96.3) / 183.2 (154.2 – 217.6)		
EC_{50} (reproduction)[a)]			> 120.0 / > 271.2		
NOEC (reproduction)			53.3 / 120.5		
NOEC (mortality, biomass change)			120.0 / 271.2		
LC_{50} (mortality)			> 120.0 / > 271.2		

Note: There were no significant differences in biomass change (Williams-t-test, α = 0.05, one-sided smaller).
Negative values for reduction of reproduction = increase, relative to control
No effects on behaviour (including feeding activity) of the worms were observed during the test. The mean number of unhatched cocoons per replicate was 1.6 in the control and 1.8, 1.8, 1.8, 1.5, 1.8, 1.8, 2.0 and 1.3 in the test item treatments of 7.0, 10.5, 15.8, 23.7, 35.6, 53.3, 80.0 and 120.0 mg a.s./kg dry soil, respectively, at the end of the study (day 56).

* Statistically significantly different compared to control (Williams-t-test, α = 0.05, one-sided smaller)

a) Based on Logit analysis

Mortality during the first 28 days of the test was 0.0% in the control and in all test item treatments. No effects on behaviour (including feeding activity) of the worms were observed during the test.

The mean biomass change of adult worms from day 0 to day 28 was 27.2% in the control and ranged between 23.9% and 30.1% in the test item treatments. There were no significant differences in biomass change (Williams-t-test, α = 0.05, one-sided smaller) for any test item treatment in comparison with the control.

The mean number of juveniles per replicate at the end of the study (day 56) was 280.1 in the control group. In the test item treatments, the mean number of juveniles per replicate ranged between 274.3

and 286.0 at the test concentrations of 7.0 to 53.3 mg a.s./kg dry soil and was 206.3 at the second highest test concentration of 80.0 mg a.s./kg dry soil and 166.0 at the highest test concentration of 120.0 mg a.s./kg dry soil. The mean number of juveniles per replicate was statistically significantly reduced at the two highest test concentrations of 80.0 and 120.0 mg a.s./kg dry soil (Williams-t-test, α = 0.05, one-sided smaller).

In a separate study with the reference item Maypon Flow (Carbendazim, SC 500), the number of juveniles was reduced by 53% and 99% at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 162 and 5), respectively, after 8 weeks of test duration when compared to the control (mean number of juveniles = 347). The effects on the reduction of reproduction showed that the test system was sensitive.

Conclusion

In this test on sub-lethal effects of AG-E1-500 SC1 on the earthworm *Eisenia andrei*, the EC_{10}, EC_{20}, and EC_{50} for reproduction were determined to be 60.8, 81.1 and > 120.0 mg a.s./kg dry soil (137.4, 183.2 and > 271.2 mg product/kg dry weight), respectively. The NOEC for reproduction was determined as 53.3 mg a.s./kg dry soil (120.5 mg product/kg dry soil). The NOEC for mortality and biomass change was 120.0 mg a.s./kg dry soil (271.2 mg product/kg dry soil).

A 2.4.1.1 KCP 10.4.1.2 Earthworms - field studies

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

Effects on *Folsomia candida*

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 based on endpoints expressed in terms of the active substance: • NW (normalised width) of 0.70 was calculated, which results with rating "fair" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} (167 mg a.s./kg dws) is lower than $EC_{20,low}$ (206 mg a.s./kg dws), • the dose-response curve is medium with steepness of 0.334 (i.e. between 0.33 and 0.66); it should be however noted that EC_{50} was greater than the maximum concentration tested and for this reason calculation of the steepness of the dose-response curve is not fully reliable. Taking into account the provided above indications, the calculated EC_{10} is considered to be not fully reliable. Nevertheless, the lower NOEC is recommended for the risk assessment, so reliability of EC_{10} is of lesser importance in this case. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC (mortality) = 628 mg product/kg d.w. soil (corresponding to 278 mg a.s./kg d.w. soil) NOEC (reproduction) = 349 mg product/kg d.w. soil (corresponding to 154 mg a.s./kg d.w. soil)

	EC_{10} (reproduction) = 378 mg product/kg d.w. soil (corresponding to 167 mg a.s./kg d.w. soil) EC_{20} (reproduction) = 577 mg product/kg d.w. soil (corresponding to 255 mg a.s./kg d.w. soil) EC_{50} (reproduction) > 1130 mg product/kg d.w. soil (corresponding to >500 mg a.s./kg d.w. soil)

Reference:	**KCP 10.4.2.1/01**
Report	Effects of AG-E1-500 SC1 on the reproduction of the collembolan *Folsomia candida*, Friedrich S., 2020b, 20 48 TCC 0003
Guideline(s):	OECD 232 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Boric acid (analysed purity: 100.8%)

Test organism:

Test species	Collembolan *Folsomia candida* (Willem)
Origin	Reared under ambient laboratory conditions in the test facility (original source: "Biologische Bundesanstalt (BBA)", Berlin-Dahlem, Germany)
Age at test start	Juvenile collembolans, 9 – 12 days old
Acclimatisation	The synchronised culture was bred at test temperature and a 12 h light (light intensity: 400-800 lux): 12 h dark photoperiod.
Diet	Granulated dry yeast Approximately 2 mg granulated dry yeast were added to the test vessels at the start of the test and after 14 days.

Test conditions:

Test substance concentration	8, 15, 26, 48, 86, 154, 278 and 500 mg a.s./kg dry soil, corresponding with 18, 33, 60, 108, 194, 349, 628 and 1130 mg product/kg dry soil (based on product density and nominal content of a.s.) On the day of the test start (immediately before application), a stock solution of the test item (= application solution of the highest test concentration) was prepared by dispersion in deionised water. This stock solution was serially diluted with deionised water to prepare the further application solutions.
Reference item concentration	The reference item boric acid was tested in a separate study (dated 19 August 2019) at 44, 67, 100, 150 and 225 mg a.s./kg dry soil.
Control	Deionised water

Test substrate	Artificial soil was prepared with the following constituents: - 5% sphagnum peat (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried) - 20% kaolinite clay (kaolinite content > 30%) - 0.3% calcium carbonate - 74.7% industrial quartz sand (> 50% of particles between 50 and 200 µm)
No. test organisms per replicate (test vessel)	10
No. replicates per treatment	8 for the control (+ 2 replicates without collembolans for physico-chemical measurement purposes) 4 for the test item treatments (+ 2 replicates without collembolans for physico-chemical measurement purposes)
Application method	Two days before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. On the day of the test start, the test item was introduced to the soil by adding 25 mL of the appropriate test solution to each prepared amount of artificial soil. This brought the soil to its final water content (40-60% of the soil maximum water holding capacity (WHC)). The control substrate contained the corresponding amount of deionised water only. After thorough mixing, 30 g (dry weight equivalent) of the test substrate was placed into each vessel. Ten test organisms were introduced to each vessel, using an aspirator. The test containers were tightly covered with a lid and briefly opened twice a week for aeration.
Test duration	28 days
Test vessels	Glass container (approximately 150 mL) covered with a lid; surface area of soil: 18.9 cm²
Temperature	19.4-21.6°C
Illumination	16 h light (light intensity: 600 lux) : 8 h dark photoperiod
Water content of artificial soil	Test start: 24.9% - 25.0% (equivalent to 57.8% – 58.0% WHC) Test end: 24.3% – 24.7% (equivalent to 56.4% – 57.3% WHC)
pH of artificial soil	Test start: 5.97 – 6.04 Test end: 5.83 – 5.88

Measurements:

Adult mortality and reproduction	At day 28, the parental and juvenile collembolans in the test and control vessels were counted. Furthermore, observations on obvious physiological or pathological symptoms or distinct changes in behaviour were made. The test substrate of each replicate was poured into an individual container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring, the number of parental and juvenile collembolans floating on the surface was determined. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system. The efficiency of the extraction method was determined to be 98% in a separate extraction run using vessels containing a known number of juveniles kept in untreated test substrate.
Temperature	Continuously by a data logger
pH of the soil samples	At test start and end

Water content of the soil samples	At test start and end The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2% of the initial water content.

Calculations

The endpoints of the test were adult mortality and inhibition of reproduction (reduction in the number of juveniles per replicate).
The statistical analysis was performed with the software ToxRat Professional ToxRat Professional 3.2.1 (Ratte 2015). Multiple Sequentially-rejective Fisher Tests after Bonferroni-Holm (mortality) and Williams Multiple Sequential t-test (reproduction) were used to compare the test item treatment groups with the control in order to determine NOEC values for mortality and reproduction. The EC_{10}, EC_{20} and EC_{50} values for reproduction (reduction in number of juveniles) were estimated by Probit analysis using the maximum likelihood method. Confidence limits (95%) of the EC_x values were computed by normal approximation.

Results and discussions

Validity criteria:

- Mean adult mortality in the control ≤ 20% (observed: 2.5%)
- Mean number of juveniles per replicate in the control ≥ 100 (observed: 1303/replicate)
- Coefficient of variation of reproduction per replicate in the control ≤ 30% (observed: 11.5%)

The study was considered to be valid as all validity criteria were met.

Observations of adult mortality and reproduction are presented in the table below.

Table A2.4.2.1-1. Effects of AG-E1-500 SC1 on mortality and reproduction of *Folsomia candida*

Treatment		**Adult mortality after 28 days of exposure (%)**	**Reproduction after 28 days**	
(mg a.s./kg dry soil)	**(mg product/kg dry soil)**		**(mean number of juveniles/replicate)**	**Reduction compared to control (%)**
0 (control)	0 (control)	2.5	1303[a)]	-
8	18	2.5	1280	1.8
15	33	2.5	1328	-1.9
26	60	2.5	1307	-0.3
48	108	0.0	1293	0.8
86	194	2.5	1278	1.9
154	349	0.0	1266	2.9
278	628	5.0	929*	28.7
500	1130	20.0*	757*	41.9
Endpoints (mg a.s./kg dry soil) (95% confidence limits) / (mg product/kg dry soil) (95% confidence limits)				
EC_{10} (reproduction)[b)]		167 (119 – 236) / 378 (268 – 533)		
EC_{20} (reproduction)[b)]		255 (206 –316) / 577 (466 – 714)		
EC_{50} (reproduction)[b)]		> 500 / > 1130		
NOEC (reproduction)		154 / 349		
NOEC (mortality)		278 / 628		
LC_{50} (mortality)		> 500 / > 1130		

Note: No effects on behaviour of the collembolans were observed during the test.
Negative values for reduction of reproduction = increase, relative to control

* Statistically significantly different compared to control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, α = 0.05, one-sided greater; Williams Multiple Sequential t-test for reproduction, α = 0.05, one-sided smaller)

a) Coefficient of variation: 11.5%

b) Based on Probit analysis

Adult mortality at the end of the test (day 28) was 2.5% in the control and in the range of 0.0% and 5.0% in the test item treatments of 8 to 278 mg a.s./kg dry soil. At the highest Test substance concentration of 500 mg a.s./kg dry soil, adult mortality was 20.0% which was statistically significantly reduced in comparison to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). No effects on behaviour of the collembolans were observed during the test.

The mean number of juveniles per replicate at the end of the study (day 28) was 1303 in the control group. In the test item treatments, the mean number of juveniles per replicate ranged between 1266 and 1328 at the test concentrations of 8 to 154 mg a.s./kg dry soil and was 929 at the second highest test concentration of 278 mg a.s./kg dry soil and 757 at the highest test concentration of 500 mg a.s./kg dry soil. The mean number of juveniles per replicate was statistically significantly reduced at the two highest test concentrations of 278 and 500 mg a.s./kg dry soil (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study with the reference item boric acid and collembolans of the same source culture, the EC_{50} (reproduction) was determined to be 103 mg a.s./kg dry soil. The LC_{50} was calculated as 161 mg a.s./kg dry soil. The NOEC for mortality and for reproduction were both determined to be 44 mg a.s./kg dry soil. The EC_{50} (reproduction) was close to the value of 100 mg a.s./kg dry soil as stated in OECD 232 (2016) and therefore showed that the test system was sensitive.

Conclusion

In this test on sub-lethal effects of AG-E1-500 SC1 on the collembolan *Folsomia candida*, the EC_{10}, EC_{20}, and EC_{50} for reproduction were determined to be1 167, 255 and > 500 mg a.s./kg dry soil (378, 577 and > 1130 mg product/kg dry weight), respectively. The NOEC for reproduction was determined as 154 mg a.s./kg dry soil (349 mg product/kg dry soil). The NOEC for mortality was 278 mg a.s./kg dry soil (628 mg product/kg dry soil) and the LC_{50} was estimated to be > 500 mg a.s./kg dry soil (> 1130 mg product/kg dry soil).

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Effects on *Hypoaspis aculeifer*

Comments of zRMS:	The study was performed in line with OECD 226 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). Since no effects >10% were observed, calculation of EC_{10} was not possible and is expected to be >1130 mg product/kg dw, the maximum concentrations tested. All the validity criteria were met and overall the study is considered acceptable with the following endpoints relevant for the risk assessment: 14d NOEC (reproduction) = 1130 mg product/kg dw soil (corresponding to 500 mg a.s./kg dw soil) 14d EC_{50} (reproduction) >1130 mg product/kg dw soil (corresponding to >500 mg a.s./kg dw soil) 14d EC_{10} (reproduction) >1130 mg product/kg dw soil (corresponding to >500 mg a.s./kg dw soil)

Reference:	**KCP 10.4.2.1/02**
Report	Effects of AG-E1-500 SC1 on the reproduction of the predatory mite *Hypoaspis aculeifer*, Friedrich S., 2020c, 20 48 THC 0002
Guideline(s):	OECD 226 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Dimethoate (analysed purity: 98.8% ± 0.5%)

Test organism:

Test species	Predatory mite *Hypoaspis aculeifer* (Canestrini)
Origin	A synchronised culture was reared at the test facility. The test organisms were originally obtained from "Katz Biotech AG", Baruth, Germany.
Age at test start	Adults from a synchronised culture with an age of 28 to 30 days
Acclimatisation	The synchronised culture was bred at a temperature of 21-24°C and a 16 h light: 8 h dark photoperiod (artificial light; 2 - 3000 lux).
Diet	At the beginning and during the test (every 2 – 3 days), the predatory mites were fed *ad libitum* with *Tyrophagus putrescentiae* (Schrank), originally obtained from "Bayer CropScience AG", Monheim am Rhein, Germany, and reared in the test facility.

Test conditions:

Test substance concentration	8, 15, 26, 48, 86, 154, 278 and 500 mg a.s./kg dry soil, corresponding with 18, 33, 60, 108, 194, 349, 628 and 1130 mg product/kg dry soil (based on product density and nominal content of a.s.) On the day of the test start, a stock solution of the test item (= application solution of the highest test concentration) was prepared by dispersion in deionised water. This stock solution was serially diluted with deionised water to prepare the further application solutions.
Reference item concentration	The reference item dimethoate was tested in a separate study (dated 23 September to 28 October 2019). The tested concentrations are not reported.
Control	Deionised water

Test substrate	Artificial soil was prepared with the following constituents: - 5% sphagnum peat (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried) - 20% kaolinite clay (kaolinite content > 30%) - 0.25% calcium carbonate - 74.75% industrial quartz sand (> 50% of particles between 50 and 200 µm) The mixed constituents were pre-moistened with deionised water two days before test start.
No. test organisms per replicate (test vessel)	10
No. replicates per treatment	8 for the control (+ 2 replicates for physico-chemical measurement purposes) 4 for the test item treatments (+ 2 replicates for physico-chemical measurement purposes)
Application method	On the day of the test start, the test item was introduced to the soil by adding 20 mL of the appropriate test solution to each prepared amount of artificial soil (223.16 g wet weight, corresponding to 200 g dry weight). This brought the soil to its final water content of approximately 50% of the maximum water holding capacity (WHC). The control substrate contained the corresponding amount of deionised water only. After thorough mixing, 24.32 g of the artificial soil (20 g dry weight equivalent) were placed into each test vessel. At test start (within 2 hours after treatment of the soil), ten adult females were introduced into each test vessel by means of a moistened brush. Thereafter, the food mite *Tyrophagus putrescentiae* was added (approximately 20 mg per vessel) and the test vessels were closed.
Test duration	14 days
Test vessels	160 mL WECK-jar with glass lid (inside dimensions: 4.7 cm in diameter, 8 cm high) The test vessels were aerated in combination with feeding of the mites, i.e. every 2 – 3 days.
Temperature	19.4-21.4°C
Illumination	16 h light (light intensity: 571 lux) : 8 h dark photoperiod
Water content of artificial soil	Test start: 20.34% - 21.12% (equivalent to 47.12% – 48.92% WHC) Test end: 20.33% – 21.13% (equivalent to 47.10% – 48.95% WHC)
pH of artificial soil	Test start: 6.3 – 6.5 Test end: 5.9 – 6.0

Measurements:

Adult mortality and reproduction	On day 14, surviving *Hypoaspis aculeifer* mites and juveniles were extracted from each test replicate using a MacFadyen high-gradient extractor (heat / light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (1 mm mesh size) on the bottom. Beneath the canister was a funnel attached to a collecting flask with 25 mL of a fixing liquid (70% ethanol). A temperature gradient was created between the upper part (where the samples were) and the lower part of the system (where the collecting flasks were placed) during 48 hours. Adult and juvenile mites moved down through the soil substrate away from the heat source, until they fell from the substrate into the funnel / fixing liquid. Following extraction, all juveniles and adults present in the fixing

	liquid were counted. The extraction efficiency of the extractor was determined to be 91.5% in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate.
Temperature	Continuously recording
pH of the soil samples	At test start and end
Water content of the soil samples	At test start and end The water content was maintained throughout the test by reweighing the additional test vessels and any water loss was compensated.

Calculations

The endpoints of the test were adult mortality and reproductive output (calculated in % compared to control).
The statistical analysis was performed with the software ToxRat Professional ToxRat Professional 3.2.1 (Ratte 2015). Multiple Sequentially-rejective Fisher Tests after Bonferroni-Holm (mortality) and Dunnett's Multiple t-test Procedure (reproduction) were used to compare the test item treatment groups with the control in order to determine NOEC values for mortality and reproduction.

Results and discussions

Validity criteria:

- Mean adult mortality in the control ≤ 20% (observed: 5.0%)
- Mean number of juveniles per replicate in the control ≥ 50 (observed: 287.1/replicate)
- Coefficient of variation of reproduction per replicate in the control ≤ 30% (observed: 7.9%)

The study was considered to be valid as all validity criteria were met.

Observations of adult mortality and reproduction are presented in the table below.

Table A2.4.2.1-2. Effects of AG-E1-500 SC1 on mortality and reproduction of *Hypoaspis aculeifer*

Treatment		**Adult mortality after 14 days of exposure (%)**	**Reproduction after 14 days**	
(mg a.s./kg dry soil)	**(mg product/kg dry soil)**		**(mean number of juveniles/replicate)**	**Reproduction compared to control (% of control)**
0 (control)	0 (control)	5.0	287.1[a)]	100
8	18	2.5	302.8	105
15	33	2.5	299.3	104
26	60	10.0	293.5	102
48	108	2.5	280.0	98
86	194	5.0	297.8	104
154	349	2.5	309.8	108
278	628	0.0	298.8	104
500	1130	2.5	271.3	94
Endpoints (mg a.s./kg dry soil) / (mg product/kg dry soil)				
EC_{10} (reproduction)		> 500 / > 1130		
EC_{20} (reproduction)		> 500 / > 1130		
EC_{50} (reproduction)		> 500 / > 1130		
NOEC (reproduction)		500 / 1130		
NOEC (mortality)		500 / 1130		
LC_{50} (mortality)		> 500 / > 1130		

Note: No statistically significant differences in the test item treatments compared to the control were observed for adult mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and

reproduction (Dunnett's Multiple t-test Procedure, α = 0.05, one-sided smaller).
a) Coefficient of variation: 7.9%

Adult mortality at the end of the test (day 14) was 5.0% in the control and in the range of 0.0% and 10.0% in all test item treatments of 8 to 500 mg a.s./kg dry soil. No statistically significant differences in the test item treatments compared to the control were observed for adult mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater). Differences in the behaviour and the morphology of the mites between the test item treatment groups and the control could not be observed.

The mean number of juveniles per replicate at the end of the study (day 14) was 287.1 in the control group. In all test item treatments of 8 to 500 mg a.s./kg dry soil, the mean number of juveniles per replicate ranged between 271.3 and 309.8. No statistically significant differences in the test item treatments compared to the control were observed for reproduction (Dunnett's Multiple t-test Procedure, α = 0.05, one-sided smaller).

In a separate study with the reference item dimethoate, the EC_{50} (reproduction) was determined to be 6.3 mg a.s./kg dry soil. This is in the range of 3.0 and 7.0 mg a.s./kg dry soil stated by OECD 226 (2016). The results of the reference test demonstrated the sensitivity of the test system.

Conclusion

In this test on sub-lethal effects of AG-E1-500 SC1 on the predatory mite *Hypoaspis aculeifer*, the EC_{10}, EC_{20}, and EC_{50} for reproduction as well as the LC_{50} could not be calculated but were estimated to be all > 500 mg a.s./kg dry soil (> 1130 mg product/kg dry soil). The NOEC for reproduction and the NOEC for mortality were both determined as 500 mg a.s./kg dry soil (1130 mg product/kg dry soil).

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was performed fully in line with OECD 216 with no deviations. All the 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 15.07 mg product/kg dw soil (corresponding to 7.05 mg a.s./kg dw soil, based on analysed content of the active substance and product relative density of 1.13 g/mL).

Reference:	**KCP 10.5/01**
Report	Effects of AG-E1-500-SC1 on the activity of soil microflora (Nitrogen transformation test), Persdorf U., 2020, 20 48 SMN 0003
Guideline(s):	OECD 216 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500-SC1 (= AG-E1-500 SC1)
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021
Reference substance	Dicyandiamide (purity: 99.9%, batch No.: 10201776)

Test soil:

Origin	Wassergut Canitz, Canitz, Saxony, Germany 12.694435960 degrees East 51.403774567 degrees North
Cultivation	At soil removal (2019): fallow ground Pre-cultivation (2018): fallow ground
History	Fertilizer application: none since 2003 Plant protection product application: none since 1990
Batch	4/2019
Soil sampling	From the top 20 cm 4 October 2019
Soil preparation	Carefully dried at room temperature and passed through a 2-mm mesh sieve (4-8 October 2019)
Soil storage	At approximately 4°C (8-28 October 2019)
Soil conditioning	At test conditions (28 October – 11 November 2019)

Physico-chemical properties	pH (H_2O): 6.1 C_{org}: 1.45% Microbial biomass: 53.66 mg C/100 g dry soil = 3.70% of C_{org} N_{min}: 1.71 mg/100 g dry soil Total-N: 0.15% Particle size distribution Clay (< 0.002 mm): 10.2% Silt (0.002-0.050 mm): 36.9% Sand (0,05-2.0 mm): 52.9% Classification (USDA): Sandy loam Water content: 11.25 g/100 g dry soil Maximum water holding capacity: 37.74 g/100 g dry soil Cation exchange capacity: 9.5 cmol+/kg dry soil NO_3-N-content (determined within the study): 4.57 mg/ 100 g dry soil

Test conditions:

Test item concentration	1.51 and 15.07 mg product/kg dry soil, corresponding with 1 and 10 L product/ha assuming a soil density of 1.5 g/cm³ and a soil depth of 5 cm
Reference item concentration	50, 100 and 200 mg/kg dry soil, tested in a separate study together with a control (test performed 22 October – 19 November 2019)
Control	Deionised water
No. replicates per treatment	3
Application method	Soil samples equivalent to 200 g dry weight were weighed per replicate. The soil was mixed with 0.5% (i.e. 1.0 g/200 g dry soil, C/N ratio of lucerne meal: 13.2/1). A stock solution of the test item in deionised water was prepared at 0.7535 mg/mL. The stock solution was used as application solution of the high dose. For the low dose, an application solution was prepared by 1:10 dilution of the stock solution with deionised water. An aliquot of 4.00 mL of the respective application solution was mixed with the soil by means of a hand stirrer. 7.46 mL of deionised water were added to the soil samples to achieve a water content of approximately 45% of the maximum water holding capacity. The controls received 11.46 mL of deionised water.
Test duration	28 days
Test vessels	Wide-mouth glass flasks (500 mL) The screw caps of the flasks used permitted air exchange.
Temperature	20 ± 2°C (nominal), 19.2-20.5°C (actual)
Illumination	Constant darkness
Water content of the soil	17.29-17.81 g/100 g dry soil (equivalent to 45.81-47.20% of the maximum water holding capacity)
pH of soil	6.0 (in test item treatments and control at test start and end)

Measurements:

NH_4-N-, NO_3-N- and NO_2-N-contents of the soil samples	Soil sub-samples (equivalent to 10 g dry soil) were taken at 3 hours (day 0), 7, 14 and 28 days after application. The soil samples were extracted with 50 mL 1 M KCl solution and NH_4-N-, NO_3-N- and NO_2-N-contents determined by an autoanalyser (SEAL Analytics). Ammonium reacts with salicylate and dichloroisocyanuric acid to form an indophenole blue compound, that is colorimetrically measured at a wavelength of 660 nm. Nitrate is reduced to nitrite by hydrazinesulphate. The nitrite reacts with sulphanilamide in an acidic solution to form a diazocompound which is then coupled with naphthylamine. The

	intensity of the formed azodye is colorimetrically measured at a wavelength of 550 nm. The nitrite contents are determined without nitrate reduction. The nitrate contents are calculated as the difference between the nitrate/nitrite sum and the nitrite contents.
pH of the soil samples	At test start and end
Water content of the soil samples	At test start (after application) and weekly thereafter, adjusted to 40-50% of the maximum water holding capacity as necessary

Calculations

The mean NO_3-N-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. The NH_4-N- and NO_2-N-contents were recorded.

Furthermore, the nitrogen transformation rate per time interval and the nitrogen transformation rate per day (day 0-7, 7-14, 14-28) were calculated for each treatment group based on the measured NO_3-N-contents.

The % deviations in nitrogen transformation rate between the control and the test item treatment groups were calculated.

Results and discussions

Validity criterion:

- Variation in replicate control samples: ≤ ± 15% (observed coefficient of variation: 2.1-7.2%)

The study was considered to be valid as the validity criterion was met.

NO_3-N-contents and nitrogen transformation rates are presented in the table below.

Table 10.5-1. NO_3-N-contents and nitrogen transformation rates after treatment of soil with AG-E1-500 SC1.

Sampling [days]	Treatment group	Repl.	NO_3-N-content [mg NO_3-N/100 g dry soil] Measured values	Mean ± SD	Sampling interval [days]	Nitrate transformation rate [mg NO_3-N/kg dry soil/day]	Deviation from control [%]
0	Control	1	4.50	4.70 ± 0.19			
		2	4.87				
		3	4.74				
	test item 1.51 mg/kg	1	4.51	4.67 ± 0.15			
		2	4.80				
		3	4.71				
	test item 15.07 mg/kg	1	4.73	4.81 ± 0.07			
		2	4.86				
		3	4.85				
7	Control	1	7.99	8.17 ± 0.17	0-7	4.96	-
		2	8.20				
		3	8.33				
	test item 1.51 mg/kg	1	7.91	8.12 ± 0.19		4.92	-0.7
		2	8.18				
		3	8.27				
	test item 15.07 mg/kg	1	7.95	8.09 ± 0.22		4.68	-5.6
		2	7.98				
		3	8.34				
14	Control	1	8.18	8.61 ± 0.40	7-14	0.63	-
		2	8.68				
		3	8.98				
	test item 1.51 mg/kg	1	8.99	9.23 ± 0.29		1.58	+151.5
		2	9.55				

		3	9.14				
	test item 15.07 mg/kg	1	9.58	9.73 ± 0.16		2.34	+272.7
		2	9.71				
		3	9.90				
28	Control	1	9.51	10.31 ± 0.74	14-28	1.21	-
		2	10.97				
		3	10.44				
	test item 1.51 mg/kg	1	10.46	11.03 ± 0.50		1.29	+6.5
		2	11.39				
		3	11.24				
	test item 15.07 mg/kg	1	11.53	11.31 ± 0.21		1.13	-6.5
		2	11.30				
		3	11.11				

Note: The coefficient of variation of NO_3-N-contents in replicate control samples was in the range of 2.1-7.2% for all samplings.
SD: Standard deviation

At the end of the 28-day exposure, the deviations in nitrogen transformation rate (time interval 14-28 days) between the test item treatments at 1.51 and 15.07 mg product/kg dry soil and the control were +6.5% and -6.5%, respectively, and were thus below the trigger of 25%. Therefore, no adverse effects of the test item on nitrogen transformation in the test soil were observed up to 15.07 mg product/kg dry soil.

In a separate study, the reference item dicyandiamide caused inhibitions of nitrogen transformation rate of -62.0% and -74.3% at 100 and 200 mg dicyandiamide/kg dry soil, respectively, determined after 28 days of exposure for the time interval of 14-28 days. Therefore, the test system was shown to be suitable.

The NH_4-N-contents were 0.76-0.80 mg/100 g dry soil in the test item treatment groups and the control at test start (3 hours) and < LOQ (0.10 mg/100 g dry soil) in any test group for the consecutive samplings. The NO_2-N-contents were < LOQ (0.10 mg/100 g dry soil) for all test groups and samplings. Therefore, NH_4-N- and NO_2-N-contents were not used for evaluation of the results.

Conclusion

In this nitrogen transformation test, the test item AG-E1-500 SC1 caused no adverse effects (deviation from control < 25%) on soil nitrogen transformation (measured as nitrogen transformation rate per day) at the end of the 28-day incubation period when tested up to 15.07 mg product/kg dry soil.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

KCP 10.6.2 Testing on non-target plants

Seedling Emergence

Comments of zRMS:	The study was performed in line with OECD 208 with a minor deviation. It was noted that for a technical reason the relative humidity was below the recommended minimum value of 45% on 11 days during the experimental phase in the greenhouse. The longest deviation occurred on one day and lasted for approximately 13 hours. The minimum value for the relative humidity was 23.1 %. As there was a normal control development without visible negative effects, this deviation is considered to have no negative impact on the outcome and the integrity of the study. The analytical measurements of the stock solutions in both trials (L1 and L3; for details see the study summary below) showed that the concentrations of the active substance were within 80-120% of nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: $ER_{50,\ shoot\ dry\ weight}$ = 0.098 L product/ha (wheat) It is noted that endpoints based on phytotoxic effects were not calculated, although they are required in line with agreements taken during the Central Zone harmonisation meetings. Nevertheless, analysis of information on phytotoxic effects available in the study report indicates that effects on emergence, mortality, shoot height and shoot dry weight correspond with phytotoxic effects observed on tested plants and for this reason it is not expected that calculation of endpoints based on phytotoxicity would provide any adverse information that would need to be considered in the risk assessment. For this reason no additional calculations were requested form the Applicant.

Reference:	**KCP 10.6.2/01**
Report	AG-E1-500 SC1: Effects on the Seedling Emergence and Seedling Growth of Non-Target Terrestrial Plant Species under Greenhouse Conditions, Duffner A., 2020a, S19-22437
Guideline(s):	OECD 208 (2006)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021

Test organism:

Test species and origin	Dicotyledonous species:	
	Fagopyrum esculentum (buckwheat)	Bingenheimer
	Glycine max (soybean)	Saatbau Linz
	Helianthus annuus (sunflower)	Bingenheimer
	Lepidium sativum (garden cress)	Wildsameninsel
	Linum usitatissimum (flax)	Templiner Kräuter
	Medicago sativa (lucerne)	Templiner Kräuter
	Solanum lycopersicum (tomato)	Hild
	Virgina radiate (mung bean)	Plötschke
	Monocotyledonous species:	
	Hordeum vulgare (barley)	Partnerbio
	Triticum aestivum (wheat)	Bioland Hof Jeebel
Number of seeds per pot	2 Dicotyledons, 4 Monocotyledons	
Number of pots per treatment	10 Dicotyledons, 5 Monocotyledons	
Planting	Seeds were placed in soil at an appropriate depth and approximately equally spaced.	
Fertiliser	0.2% solution of Hakaphos Blau at 7-day intervals, twice for trial L1 and 3 times for trial L3	
Watering	Watering was done regularly to the plant saucer.	

Test soil:

Supplier	EBRD GmbH & Co. KG	
Test	Trial L1	Trial L3
Soil batch	Göbrichen_2019_1	Göbrichen_2020_01
Soil type	Loamy sand	Loamy Sand
Physico-chemical properties Soil Texture: TOC: Organic matter: pH: Conductivity:	 76.7% sand, 20.2% silt, 3.1% clay 0.17% 0.29% 7.97 77.6 µS/cm	 73.7% sand, 23.7% silt, 2.7% clay 0.26% 0.45% 7.66 106.1 µS/cm

Test conditions:

Trial	L1	L3
Test design	Eight dicotyledonous and two monocotyledonous species were sown in pots and the pots were sprayed with AG-E1-500 SC1 at five application rates per plant species in two sets of trials (L1 and L3). In Trial L3 the plant species *Lepidium sativum*, *Hordeum vulgare* and *Triticum aestivum* were exposed to lower test item rates than in Trial L1 in order to achieve the study objectives.	
Test item concentration	0, 0.48, 1.06, 2.34, 5.15, 11.34 L product/ha	0, 0.009, 0.020, 0.045, 0.099, 0.218, 0.480 L product/ha
Spray volume	200 L/ha	200 L/ha
Plant species treated	*Fagopyrum esculentum, Glycine max, Helianthus annuus, Solanum lycopersicum, Vigna radiata, Linum usitatissimum, Medicago sativa* ~~All species~~	*Hordeum vulgare, Triticum aestivum* 0.009, 0.020, 0.045, 0.099 and 0.218 L product/ha ~~0.009 – 0.218 L product/ha~~ *Lepidium sativum* 0.020, 0.045, 0.099, 0.218 and 0.480 L product/ha ~~0.020 – 0.480 L product/ha~~
Control	Tap water	
Application method	Plants were sown, then pots were treated with test substance or water control. Test substance was diluted to appropriate concentrations using tap water as close to the time of application as possible. Application was by laboratory track-sprayer.	
Test duration	21 days after emergence of 50% of seedlings in the control	
Observations	Mortality and phytotoxicity on days 7, 14 and 21 Germination was checked 5-7 days after sowing Growth stage, shoot height and shoot dry weight were assessed at test end	
Test vessels	15cm diameter plastic pots containing approx. 1.5 kg soil	
Test location	Greenhouse in Neulingen-Göbrichen, Germany	
Temperature	15.07 – 28.36 ºC mean 22.04ºC	18.50 – 33.48 ºC* mean 23.68ºC
Relative humidity	49.21 – 75.79 % mean 57.80%	23.12 – 85.31 % mean 60.63%
Light intensity	300 – 350 µmol/m²/s	350 – 390 µmol/m²/s
Photoperiod	16 hours light	
Mortality assessment	Plants were considered dead if completely dried out without any green compartment	
Phytotoxicity assessment	Symptoms were graded as follows: Up to 20% = slight symptoms Up to 40% = moderate symptoms Up to 60% = severe symptoms Up to 80% = symptoms on nearly the total plant Up to but not including 100% = moribund plants Symptoms were described as follows: CH = chlorosis LD = leaf deformation NE = necrosis ST = stunted growth	

*short term deviation (< 2 hours)

Analytical method:

Method type	HPLC-UV
Equipment	Jasco LG-2080-02 with Jasco autosampler AS-2050 Plus and Jasco Diodenarray-Detektor MD-2010 Plus
Column	YMC-Pack ODS-A 150 x 4.6 mm, 5µm
Column temperature	60ºC
Flow rate	1.3 mL/min
Mobile phase	Acetonitrile/water 66:34 v/v
Wavelength	228 nm
Injection volume	20 µL
Retention time	Approx. 3 min
Sample preparation	Samples were diluted in acetonitrile 1:10, then further diluted in mobile phase 1:100.
Analytical rate verification	Analysis of the test item solution from the highest application rates (11.34 L/ha in Trial L1 and 0.480 L/ha in Trial L3) and the control solution (C) of both trials.

Calculations

The sum of emerged seedlings was calculated for each treatment group. Percentage seedling emergence was calculated in relation to the number of seeds initially sown. Inhibition of emergence was calculated based on the sum of emerged seedlings in the treatment groups compared to the control group.
Percentage mortality was calculated from the cumulative number of dead seedlings in relation to the total number of emerged plants.
Means and standard deviations were calculated for shoot height. Percent inhibition of shoot height per treatment group was calculated by comparison to the control group.
Total shoot weight per replicate was divided by the number of surviving plants per replicate to determine the average shoot weight per plant. Means and standard deviations were calculated per treatment group. Percent inhibition of shoot weight per treatment group was calculated by comparison to the control group.

Statistics

Statistical evaluation was performed using ToxRat professional, version 3.3.0 (Ratte 2018).
Seedling emergence and mortality data were analysed with Multiple Fisher's Exact Test with Bonferroni-Holm adjustment ($\alpha = 0.05$) or Cochran-Armitage Test with/without Rao-Scott adjustment ($\alpha = 0.01$).
Shoot weight and height data were analysed with Shapiro-Wilks Test and Leven-Test followed by William's test or Dunett's T-test, Welch's T-test, Jonckheere-Terpstra Test or Multiple Median Chi^2-test with Bonferroni-Holm adjustment.
Effect rates were calculated using Probit analysis.

Results and discussions

Validity criteria:

- Seedling emergence in controls is at least 70% (observed: 80 – 100%)
- Control seedlings do not exhibit visible signs of phytotoxicity and normal variation in growth and morphology (observed: no phytotoxicity and normal growth and morphology variation)
- Mean survival of emerged control seedlings is at least 90% for the duration of the study (observed: 100%)
- Environmental conditions per species are identical and growing media are the same (observed: conditions and growing media per species were the same)

The study was considered to be valid as the validity criteria were met.

Analytical verification of test substance concentrations in control and highest dose test solutions are summarised in the table below.

Table A2.6.1.1-1. Dose verification of test solutions containing AG-E1-500 SC1.

Test solution	Application rate [L product/ha]	Nominal concentration [g a.s./L]*	Measured concentration [g a.s./L]	% recovery
L1 control	0	0	<LOD	n/a
L1 highest test conc.	11.34	29.994	25.7	85.7
L3 control	0	0	<LOD	n/a
L3 highest test conc.	0.480	1.270	1.24	97.6

LOD = 0.048 µg a.s./mL, n/a not applicable
*****Based on an application volume of 200 L/ha and the analysed content of active ingredient

Seedling emergence, post-emergence mortality, observations of phytotoxicity, growth stage (BBCH) at test end, shoot height and shoot dry weight results are summarised in the tables below.

Table A2.6.1.1-2. Seedling emergence of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Total emerged plants	% emerged plants	% inhibition
Fagopyrum esculentum			
Control	18	90	n/a
0.48	19	95	-5.6
1.06	20	100	-11.1
2.34	19	95	-5.6
5.15	18	90	0
11.34	20	100	-11.1
Glycine max			
Control	20	100	n/a
0.48	18	90	10.0
1.06	17	85	15.0
2.34	20	100	0
5.15	16	80	2.0
11.34	19	95	5.0
Helianthus annuus			
Control	18	90	n/a
0.48	19	95	-5.6
1.06	18	90	0
2.34	18	90	0
5.15	17	85	5.6
11.34	20	100	-11.1
Lepdium sativum			
Control	18	90	n/a
0.20	18	90	0
0.045	18	90	0
0.099	18	90	0
0.218	19	95	-5.6
0.480	19	95	-5.6
Linum usitatissimum			
Control	18	90	n/a
0.48	4 [a]	20	77.8
1.06	3 [a]	15	83.3
2.34	0 [a]	0	100
5.15	0 [a]	0	100
11.34	0 [a]	0	100
Medicago sativa			
Control	16	80	n/a
0.48	16	80	0
1.06	16	80	0
2.34	10 [b]	50	37.5
5.15	8 [b]	40	50.0

Application rate [L product/ha]	Total emerged plants	% emerged plants	% inhibition
11.34	4 [b]	20	75.0
Solanum lycopersicum			
Control	20	100	n/a
0.48	20	100	0
1.06	20	100	0
2.34	19	95	5.0
5.15	17	85	15.0
11.34	16	80	20.0
Vigna radiata			
Control	18	90	n/a
0.48	20	100	-11.1
1.06	19	95	-5.6
2.34	20	100	-11.1
5.15	20	100	-11.1
11.34	20	100	-11.1
Hordeum vulgare			
Control	18	90	n/a
0.009	20	100	-11.1
0.020	20	100	-11.1
0.045	20	100	-11.1
0.099	19	95	-5.6
0.218	19	95	-5.6
Triticum aestivum			
Control	20	100	n/a
0.009	18	90	10.0
0.020	18	90	10.0
0.045	20	100	0
0.099	19	95	5.0
0.218	14	70	30.0

n/a not applicable, [a] statistically significantly different (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater, $\alpha = 0.05$), [b] statistically significantly different (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$), negative values indicate enhanced growth compared to the control

Table A2.6.1.1-3. Post-emergence mortality of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	No. emerged plants	No. dead plants	% mortality
Fagopyrum esculentum			
Control	18	0	0
0.48	19	0	0
1.06	20	0	0
2.34	19	0	0
5.15	18	0	0
11.34	20	4	20.0
Glycine max			
Control	20	0	0
0.48	18	0	0
1.06	17	2	11.8
2.34	20	1	5.0
5.15	16	1	6.3
11.34	19	3	15.8
Helianthus annuus			
Control	18	0	0
0.48	19	0	0
1.06	18	0	0
2.34	18	0	0
5.15	17	0	0
11.34	20	0	0
Lepdium sativum			
Control	18	0	0
0.20	18	0	0
0.045	18	0	0

Application rate [L product/ha]	No. emerged plants	No. dead plants	% mortality
0.099	18	0	0
0.218	19	0	0
0.480	19	0	0
Linum usitatissimum			
Control	18	0	0
0.48	4 [a]	0	0
1.06	3 [a]	0	0
2.34[1]	- 0 [a]	- n/a	- n/a
5.15[1]	- 0 [a]	- n/a	- n/a
11.34[1]	- 0 [a]	- n/a	- n/a
Medicago sativa			
Control	16	0	0
0.48	16	0	0
1.06	16	1	6.3
2.34	10 [b]	0	0
5.15	8 [b]	0	0
11.34	4 [b]	4 [a]	100
Solanum lycopersicum			
Control	20	0	0
0.48	20	0	0
1.06	20	0	0
2.34	19	0	0
5.15	17	0	0
11.34	16	4	25.0
Vigna radiata			
Control	18	0	0
0.48	20	0	0
1.06	19	0	0
2.34	20	0	0
5.15	20	0	0
11.34	20	0	0
Hordeum vulgare			
Control	18	0	0
0.009	20	0	0
0.020	20	0	0
0.045	20	0	0
0.099	19	0	0
0.218	19	3	15.8
Triticum aestivum			
Control	20	0	0
0.009	18	0	0
0.020	18	0	0
0.045	20	0	0
0.099	19	0	0
0.218	14	4	28.6

n/a not applicable, [a] statistically significantly different (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater, $\alpha = 0.05$), [b] statistically significantly different (Cochran-Armitage test, one-sided greater, $\alpha = 0.05$), negative values indicate enhanced growth compared to the control; [1] no plant emerged

Table A2.6.1.1-4. Phytotoxicity of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Minimum [%]	Maximum [%]	Mean [%]	Symptoms
Fagopyrum esculentum				
Control	0	0	0	n/a
0.48	20	40	32	ST, LD, NE
1.06	30	50	40	ST, LD, NE
2.34	60	70	61	ST, LD, NE
5.15	50	70	69	ST, LD, NE
11.34	70	90	79	ST, LD, NE
Glycine max				
Control	0	0	0	n/a
0.48	40	50	49	ST, LD, NE
1.06	50	70	63	ST, LD, NE
2.34	70	90	80	ST, LD, NE
5.15	70	90	81	ST, LD, NE
11.34	80	90	83	ST, LD, NE
Helianthus annuus				
Control	0	0	0	n/a
0.48	0	0	0	n/a
1.06	0	0	0	n/a
2.34	0	0	0	n/a
5.15	0	30	8	CH, LD
11.34	0	30	7	CH, LD
Lepdium sativum				
Control	0	0	0	n/a
0.20	0	0	0	n/a
0.045	0	0	0	n/a
0.099	10	10	10	ST
0.218	20	30	23	ST, CH, LD
0.480	30	60	35	ST, CH, LD
Linum usitatissimum				
Control	0	0	0	n/a
0.48	70	70	70	ST, LD
1.06	70	70	70	ST, LD
2.34[1]	n/a	n/a	n/a	n/a
5.15[1]	n/a	n/a	n/a	n/a
11.34[1]	n/a	n/a	n/a	n/a
Medicago sativa				
Control	0	0	0	n/a
0.48	60	60	60	ST, LD
1.06	60	70	69	ST, LD
2.34	60	80	78	ST, LD
5.15	80	90	81	ST, LD
11.34[2]	n/a	n/a	n/a	n/a
Solanum lycopersicum				
Control	0	0	0	n/a
0.48	50	50	50	LD, NE, CH, ST
1.06	60	60	60	LD, NE, CH, ST
2.34	60	60	60	LD, NE, CH, ST
5.15	70	80	76	LD, NE, CH, ST
11.34	80	90	84	LD, NE, CH, ST
Vigna radiata				
Control	0	0	0	n/a
0.48	0	0	0	n/a
1.06	0	30	5	LD
2.34	0	70	23	LD, ST
5.15	10	60	43	LD, ST
11.34	30	70	50	LD, ST, CH
Hordeum vulgare				
Control	0	0	0	n/a
0.009	0	0	0	n/a
0.020	0	0	0	n/a

Application rate [L product/ha]	Minimum [%]	Maximum [%]	Mean [%]	Symptoms
0.045	0	0	0	n/a
0.099	0	40	6	ST, LD
0.218	0	70	28	ST, LD
Triticum aestivum				
Control	0	0	0	n/a
0.009	0	0	0	n/a
0.020	0	0	0	n/a
0.045	10	10	10	LD
0.099	10	20	12	ST, LD
0.218	30	40	32	ST, LD

n/a not applicable
[1] No plant emerged
[2] No plant survived
Phytotoxicity symptoms: Chlorosis (CH), Leaf deformation (LD), Necrosis (NE), Rolled leaves (RL), Stunted growth (ST)

Table A2.6.1.1-5. Growth stage (BBCH) at test end of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Minimum growth stage [BBCH]	Maximum growth stage [BBCH]
Fagopyrum esculentum		
Control	53	53
0.48	14	14
1.06	14	14
2.34	12	12
5.15	12	12
11.34	12	12
Glycine max		
Control	14	14
0.48	14	14
1.06	14	14
2.34	13	13
5.15	13	13
11.34	12	12
Helianthus annuus		
Control	16	16
0.48	16	16
1.06	16	16
2.34	16	16
5.15	16	16
11.34	16	16
Lepdium sativum		
Control	18	18
0.20	18	18
0.045	18	18
0.099	18	18
0.218	18	18
0.480	18	18
Linum usitatissimum		
Control	22	22
0.48	12	18
1.06	10	10
2.34[1]	n/a	n/a
5.15[1]	n/a	n/a
11.34[1]	n/a	n/a
Medicago sativa		
Control	16	16
0.48	14	14
1.06	12	12
2.34	12	12
5.15	11	11
11.34[2]	n/a	n/a
Solanum lycopersicum		

Application rate [L product/ha]	Minimum growth stage [BBCH]	Maximum growth stage [BBCH]
Control	14	14
0.48	14	14
1.06	14	14
2.34	14	14
5.15	12	12
11.34	12	12
Vigna radiata		
Control	14	14
0.48	14	14
1.06	14	14
2.34	14	14
5.15	14	14
11.34	12	12
Hordeum vulgare		
Control	21	21
0.009	21	21
0.020	21	21
0.045	21	21
0.099	21	21
0.218	21	21
Triticum aestivum		
Control	22	22
0.009	22	22
0.020	22	22
0.045	22	22
0.099	22	22
0.218	22	22

n/a not applicable
[1] No plant emerged
[2] No plant survived

Table A2.6.1.1-6. Shoot height and shoot dry weight at test end of plants exposed to AG-E1-500 SC1.

Application rate	Shoot height			Shoot dry weight		
[L product/ha]	Mean [cm]	± SD [cm]	% inhibition	Mean [g]	± SD [g]	% inhibition
Fagopyrum esculentum						
Control	35.8	5.5	n/s	0.400	0.085	n/a
0.48	28.7 [c]	3.5	19.8	0.193 [a]	0.068	51.8
1.06	21.9 [c]	3.6	38.8	0.111 [a]	0.034	72.3
2.34	17.6 [c]	4.5	50.8	0.071 [a]	0.024	82.3
5.15	11.8 [c]	4.7	67.0	0.040 [a]	0.012	90.0
11.34	8.6 [c]	1.6	76.0	0.036 [a]	0.012	91.0
Glycine max						
Control	45.9	7.4	n/a	1.019	0.143	n/a
0.48	24.8 [a]	3.3	46.0	0.612 [b]	0.160	39.9
1.06	16.2 [a]	6.2	64.7	0.438 [b]	0.29	57.0
2.34	8.5 [a]	1.6	81.5	0.268 [b]	0.063	73.7
5.15	6.9 [a]	2.2	85.0	0.213 [b]	0.073	79.1
11.34	4.7 [a]	1.7	89.8	0.199 [b]	0.038	80.5
Helianthus annuus						
Control	7.5	0.7	n/a	0.662	0.101	n/a
0.48	7.3	1.1	2.7	0.628	0.164	5.1
1.06	7.5	0.7	0	0.774	0.214	-16.9
2.34	7.8	0.8	-4.0	0.660	0.123	0.3
5.15	6.8 [c]	0.9	9.3	0.549 [e]	0.091	17.1
11.34	6.6 [c]	0.5	12.0	0.670	0.096	-1.2
Lepdium sativum						
Control	10.3	1.1	n/a	0.477	0.068	n/a
0.20	9.6	0.8	6.8	0.506	0.111	-6.1
0.045	9.3	1.5	9.7	0.422	0.090	11.5
0.099	9.2 [c]	1.5	10.7	0.403	0.092	15.5
0.218	8.8 [c]	1.0	14.6	0.384 [c]	0.130	19.5

Application rate [L product/ha]	Shoot height			Shoot dry weight		
	Mean [cm]	± SD [cm]	% inhibition	Mean [g]	± SD [g]	% inhibition
0.480	8.3 [c]	1.5	19.4	0.272 [c]	0.079	43.0
Linum usitatissimum						
Control	18.6	1.4	n/a	0.087	0.031	n/a
0.48	8.5 [c]	3.0	54.3	0.025 [c]	0.010	71.3
1.06	2.7 [c]	0.6	85.5	0.005 [c]	0.002	94.3
2.34[1]	n/a	n/a	n/a	n/a	n/a	n/a
5.15[1]	n/a	n/a	n/a	n/a	n/a	n/a
11.34[1]	n/a	n/a	n/a	n/a	n/a	n/a
Medicago sativa						
Control	15.0	2.8	n/a	0.053	0.022	n/a
0.48	3.4 [b]	1.0	77.3	0.016 [a]	0.006	69.8
1.06	2.1 [b]	1.6	86.0	0.010 [a]	0.005	81.1
2.34	1.3 [b]	0.8	91.3	0.006 [a]	0.003	88.7
5.15	1.0 [b]	0	93.3	0.004 [a]	0.003	92.5
11.34[2]	n/a	n/a	n/a	n/a	n/a	n/a
Solanum lycopersicum						
Control	12.0	0.5	n/a	0.410	0.043	n/a
0.48	9.6 [c]	0.8	20.0	0.109 [d]	0.020	73.4
1.06	7.6 [c]	0.6	36.7	0.065 [d]	0.016	84.1
2.34	7.1 [c]	1.0	40.8	0.049 [d]	0.016	8.0
5.15	3.4 [c]	1.2	71.7	0.015 [d]	0.008	96.3
11.34	3.9 [c]	0.9	67.5	0.016 [d]	0.007	96.1
Vigna radiata						
Control	16.5	2.0	n/a	0.620	0.120	n/a
0.48	15.7	1.8	4.8	0.473 [b]	0.079	23.7
1.06	15.3	2.0	7.3	0.415 [b]	0.068	33.1
2.34	13.9 [c]	1.8	15.8	0.525	0.186	15.3
5.15	11.0 [c]	0.9	33.3	0.344 [b]	0.101	44.5
11.34	10.8 [c]	1.9	34.5	0.283 [b]	0.077	54.4
Hordeum vulgare						
Control	42.1	2.6	n/a	0.351	0.082	n/a
0.009	40.4	3.0	4.0	0.259	0.017	26.2
0.020	39.4	1.9	6.4	0.294	0.044	16.2
0.045	41.6	1.8	1.2	0.323	0.045	8.0
0.099	38.0 [c]	3.1	9.7	0.309	0.030	12.0
0.218	33.9 [c]	5.8	19.5	0.221 [c]	0.057	37.0
Triticum aestivum						
Control	36.4	1.6	n/a	0.322	0.014	n/a
0.009	33.7	0.8	7.4	0.363	0.066	-12.7
0.020	34.2	1.4	6.0	0.382	0.016	-18.6
0.045	36.0	4.1	1.1	0.223 [c]	0.059	30.7
0.099	33.3 [c]	2.4	8.5	0.171 [c]	0.056	46.9
0.218	27.9 [c]	4.0	23.4	0.071 [c]	0.037	78.0

n/a not applicable, SD: Standard Deviation., Statistically significant: [a] Multiple Median Chi^2-test with Bonferroni-Holm adjustment, [b] Multiple Welch's t-test with Bonferroni-Holm adjustment, [c] Williams` test, [d] Jonckheere-Terpstra test, [e] Multiple Mann-Whitney U-test with Bonferroni-Holm adjustment; all tests one-sided smaller, $\alpha = 0.05$, negative values indicate that there was an enhanced effect compared to the control

[1] No plant emerged

[2] No plant survived

Calculated LOER, NOER and ER_{50} values for the different parameters for each plant species are presented in the table below.

Table A2.6.1.1-7. LOER, NOER and ER_{50} for various endpoints for plants exposed to AG-E1-500 SC1.

Endpoint	*Fagopyrum esculentum* (95% CI)	*Glycine max* (95% CI)	*Helianthus annuus* (95% CI)	*Lepidium sativum* (95% CI)	*Linum usitatissimum* (95% CI)	*Medigo sativa* (95% CI)	*Solanum lycopersicum* (95% CI)	*Vigna radiate* (95% CI)	*Hordeum vulgare* (95% CI)	*Triticum aestivum* (95% CI)
Seedling emergence										
LOER	>11.34	>11.34	>11.34	>0.480	0.48[a]	2.34[b]	>11.34	>11.34	>0.218	>0.218
NOER	11.34	11.34	11.34	0.480	ND (<0.48)	1.06	11.34	11.34	0.218	0.218
ER_{50}	>11.34 (ND)	>11.34 (ND)	>11.34 (ND)	>0.480 (ND)	<0.48 (ND)	2.88 (1.77 - 5.06)	>11.34 (ND)	>11.34 (ND)	>0.218 (ND)	>0.218 (ND)
Post-emergence mortality										
LOER	>11.34	>11.34	>11.34	>0.480	>1.06	11.34[a]	>11.34	>11.34	>0.218	>0.218
NOER	11.34	11.34	11.34	0.480	1.06	5.15	11.34	11.34	0.218	0.218
ER_{50}	>11.34 (ND)	>11.34 (ND)	>11.34 (ND)	>0.480 (ND)	>1.06 (ND)	s.n.r	>11.34 (ND)	>11.34 (ND)	>0.218 (ND)	>0.218 (ND)
Shoot height										
LOER	0.48[aa]	0.48[bb]	5.15[aa]	0.099[aa]	0.48[aa]	0.48[c]	0.48[aa]	2.34[aa]	0.099[aa]	0.099[aa]
NOER	ND (<0.48)	ND (<0.48)	2.34	0.045	ND (<0.48)	ND (<0.48)	ND (<0.48)	1.06	0.045	0.045
ER_{50}	2.16	0.53	>11.34 (ND)	>0.480 (ND)	s.n.r	<0.48 (ND)	s.n.r	>11.34	>0.218 (ND)	>0.218 (ND)
Shoot dry weight										
LOER	0.48[bb]	0.48[c]	>11.34*	0.218[aa]	0.48[aa]	0.48[bb]	0.48[d]	0.48[c88]	0.218[aa]	0.045[aa]
NOER	ND (<0.48)	ND (<0.48)	11.34	0.099	ND (<0.48)	ND (<0.48)	ND (<0.48)	ND (<0.48)	0.099	0.020
ER_{50}	s.n.r	0.69 (0.38 - 1.02)	>11.34 (ND)	>0.480 (ND)	s.n.r	<0.48 (ND)	<0.48 (ND)	s.n.r	>0.218 (ND)	0.098 (0.031 - 0.632)

s.n.r statistically not reliable; ND – not determinable;
LOER determined with: [a] Cochran-Armitage test wih Rao-Scott adjustment, [b] Cochran-Armitage test, both tests one-sided greater, $\alpha = 0.05$,
LOER determined with: [aa] Williams` test, [bb] Multiple Median Chi^2-test with Bonferroni-Holm adjustment, [c] Multiple Welch's t-test with Bonferroni-Holm adjustment; [d] Jonckheere-Terpstra test; all tests one-sided smaller, $\alpha = 0.05$
* For Helianthus annuus a statistically significant difference was observed only in the second highest application rate, which was not considered for the LOER since the dry weight at the highest rate was again not statistically significant.
** All treatment groups showed a statistically significant difference except of one application rate (2.34 L prod. / ha). Therefore 0.48 L prod./ha was considered as LOER.

At the end of the 21-day exposure, statistically significantly different effects on seedling emergence were observed in *Linum usitatissimum* at all test substance concentrations and *Medicago sativa* at 2.34, 5.15 and 11.34 L product/ha.
Statistically significantly different effects on post-emergence mortality were observed in *Medicago sativa* at 11.34 L product/ha.
Symptoms of phytotoxicity were observed in all plant species.
Test substance effects on the BBCH at test end were observed in *Fagopyrum esculentum, Glycine max, Linum usitatissimum, Medicago sativa, Solanum lycopersicum* and *Vigna radiata*.
Statistically significantly different effects on shoot height and shoot dry weight were observed in *Fagopyrum esculentum, Glycine max, Linum usitatissimum, Medicago sativa* and *Solanum lycopersicum* at all test substance concentrations. Statistically significantly different effects on shoot height were observed in *Helianthus annuus* at 5.15 and 11.34 L product/ha, *Lepidium sativum* at 0.099, 0.218 and 0.480 L product/ha, *Hordeum vulgare* and *Triticum aestivum* at 0.099 and 0.218 L product/ha. Statistically significantly different effects on shoot dry weight were observed in

Helianthus annuus at 5.15 L product/ha, *Lepidium sativum* at 0.218 and 0.480 L product/ha, *Vigna radiata* at all test substance concentrations except 0.234 L product/ha, *Hordeum vulgare* at 0.218 L product/ha and *Triticum aestivum* at 0.045, 0.099 and 0.218 L product/ha.

Conclusion

In a vegetative vigour test with AG-E1-500 SC1 at various application rates on 10 plant species, the lowest ER_{50} for risk assessment was 0.098 L product/ha for effects on shoot dry weight in *Triticum aestivum.*

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Vegetative Vigour

Comments of zRMS:	The study was performed in line with OECD 227 with a minor deviation. It was noted that for a technical reason the relative humidity was below the recommended minimum value of 45% on 10 days during the experimental phase in the greenhouse. The longest deviation occurred on one day and lasted for approximately 13.08 hours. The minimum value for the relative humidity was 23.1 %. As there was a normal control development without visible negative effects, this deviation is considered to have no negative impact on the outcome and the integrity of the study. The analytical measurements of the stock solutions in both trials (L1 and L3; for details see the study summary below) showed that the concentrations of the active substance were within 80-120% of nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: $ER_{50,\ shoot\ dry\ weight}$ = 0.37 L product/ha (lucerne) The phytotoxicity data were analysed by the zRMS and it was noted that for several species the ER_{50} values based on phytotoxic effects would be potentially below the ER_{50} of 0.37 L/ha based on shoot dry weight: 1. *Glycine max*: effects at 50% were observed already at 0.22 L/ha. Based on that, the ER_{50} would be at this exposure level (0.22 L/ha). 2. *Lepidium sativum*: effects at 50% would be expected between rates 0.247 and 0.741 L/ha (mean effects observed at these rates of 39 and 70%, respectively). 3. *Medicago sativa*: effects at 50% would be expected between rates 0.247 and 0.741 L/ha (mean effects observed at these rates of 46 and 60%, respectively). Since no endpoints based on phytotoxic effects were calculated, although they are required in line with agreements taken during the Central Zone harmonisation meetings, the zRMS is of the opinion that in the risk assessment ER_{50} of 0.22 L/ha may be considered (i.e. the lowest test rate at which phytotoxic effects at 50% were observed on *Glycine max*). Endpoints for *Lepidium sativum* and *Medicago sativa* would be higher since at 0.247 L/ha (i.e. rate lower than 0.22 L/ha) the effects were <50%.

Reference:	**KCP 10.6.2/02**
Report	AG-E1-500 SC1: Effects on the Vegetative Vigour of Non-Target Terrestrial Plant Species under Greenhouse Conditions, Duffner A., 2020b, S19-22438
Guideline(s):	Yes, OECD 227 (July 2006)
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Materials and methods

Test substance:

Test substance	AG-E1-500 SC1
Batch No.	F4901-A
Active ingredient content	500 g ethofumesate/L (nominal), 529 g ethofumesate/L (actual)
Appearance	Slight beige liquid
Density	1.13 g/mL
Expiry date	01/2021

Test organism:

Test species and origin	Dicotyledonous species:	
	Fagopyrum esculentum (buckwheat)	Bingenheimer
	Glycine max (soybean)	Saatbau Linz
	Helianthus annuus (sunflower)	Bingenheimer
	Lepidium sativum (garden cress)	Wildsameninsel
	Linum usitatissimum (flax)	Templiner Kräuter
	Medicago sativa (lucerne)	Templiner Kräuter
	Solanum lycopersicum (tomato)	Hild
	Virgina radiate (mung bean)	Plötschke
	Monocotyledonous species:	
	Hordeum vulgare (barley)	Partnerbio
	Triticum aestivum (wheat)	Bioland Hof Jeebel
Number of seeds per pot	2 Dicotyledons, 4 Monocotyledons	
Number of pots per treatment	10 Dicotyledons, 5 Monocotyledons	
Planting	Seeds were placed in soil at an appropriate depth and approximately equally spaced.	
Fertiliser	0.2% solution of Hakaphos Blau at 5 to 7-day intervals, four times for trial L1 and twice for trial L3	
Watering	Watering was done regularly to the plant saucer.	

Test soil:

Supplier	EBRD GmbH & Co. KG	
Test	Trial L1	Trial L3
Soil batch	Göbrichen_2019_1	Göbrichen_2020_01
Soil type	Loamy sand	Loamy Sand
Physico-chemical properties Soil Texture: TOC: Organic matter: pH: Conductivity:	 76.7% sand, 20.2% silt, 3.1% clay 0.17% 0.29% 7.97 77.6 µS/cm	 73.7% sand, 23.7% silt, 2.7% clay 0.26% 0.45% 7.66 106.1 µS/cm

Test conditions:

Trial	L1	L3
Test design	Eight dicotyledonous and two monocotyledonous species were sown in pots and the pots were sprayed with AG-E1-500 SC1 at five application rates per plant species in two sets of trials (L1 and L3). In Trial L3 the plant species *Lepidium sativum, Medicago sativa, Linum usitatissimum, Hordeum vulgare* and *Triticum aestivum* were exposed to lower test item rates than in Trial L1 in order to achieve the study objectives.	
Test item concentration	0, 0.22 0.48, 1.06, 2.34, 5.15, 11.34 L product/ha	0, 0.009, 0.027, 0.082, 0.247, 0.741, 2.22, 6.67, 20.0 L product/ha
Spray volume	200 L/ha	200 L/ha
Plant species treated	*Glycine max* 0.22, 0.48, 1.06, 2.34 and 5.15 L product/ha ~~0.22 – 5.15 L product/ha~~ *Helianthus annuus, Fagopyrum esculentum, Solanum lycopersicum, Vigna radiata* 0.48, 1.06, 2.34, 5.15 and 11.34 L product/ha ~~0.48 – 11.34 L product/ha~~	*Medicago sativa* 0.009, 0.027, 0.082, 0.247 and 0.741 L product/ha ~~0.009 – 0.741 L product/ha~~ *Lepidium sativum, Linum usitatissimum* 0.027, 0.082, 0.247, 0.741 and 2.22 L product/ha ~~0.027 – 2.22 L product/ha~~ *Hordeum vulgare, Triticum aestivum* 0.247, 0.741, 2.22, 6.67 and 20.0 L product/ha ~~0.247 – 20.0 L product/ha~~
Control	Tap water	
Application method	Plants were treated when they had reached growth stage BBCH 12-14. There were two application times for trial L1 because *Linum usitatissimum and Medicago sativa* reached the required growth stage later than the other plants. Test substance was diluted to appropriate concentrations using tap water as close to the time of application as possible. Application was by laboratory track-sprayer.	
Application timing	Plants at BBCH 12-14	
Test duration	21 days after treatment	
Observations	Mortality and phytotoxicity on days 7, 14 and 21 Growth stage, shoot height and shoot dry weight were assessed at test end	
Test vessels	15cm diameter plastic pots containing approx. 1.5 kg soil	
Test location	Greenhouse in Neulingen-Göbrichen, Germany	
Temperature	15.07 – 28.36 ºC mean 22.00ºC	18.50 – 33.48 ºC* mean 23.93ºC
Relative humidity	49.21 – 75.79 % mean 57.04%	23.12 – 79.50 % mean 57.12%
Light intensity	310 – 340 µmol/m^2/s	360 – 390 µmol/m^2/s
Photoperiod	16 hours light	
Mortality assessment	Plants were considered dead if completely dried out without any green	

	compartment
Phytotoxicity assessment	Symptoms were graded as follows: Up to 20% = slight symptoms Up to 40% = moderate symptoms Up to 60% = severe symptoms Up to 80% = symptoms on nearly the total plant Up to but not including 100% = moribund plants Symptoms were described as follows: CH = chlorosis LD = leaf deformation NE = necrosis ST = stunted growth SD = stem deformation

*Short term deviation (< 2 hours)

Analytical method:

Method type	HPLC-UV
Equipment	Jasco LG-2080-02 with Jasco autosampler AS-2050 Plus and Jasco Diodenarray-Detektor MD-2010 Plus
Column	YMC-Pack ODS-A 150 x 4.6 mm, 5µm
Column temperature	60ºC
Flow rate	1.3 mL/min
Mobile phase	Acetonitrile/water 66:34 v/v
Wavelength	228 nm
Injection volume	20 µL
Retention time	Approx. 3 min
Sample preparation	Samples were diluted in acetonitrile 1:10, then further diluted in mobile phase 1:100.
Analytical rate verification	Analysis of the test item solution from the highest application rates (11.34 L/ha in Trial L1 and 20.0 L/ha in Trial L3) and the control solution (C).

Calculations

Percentage mortality was calculated from the cumulative number of dead plants in relation to the number of initially applied plants.
Means and standard deviations were calculated for shoot height. Percent inhibition of shoot height per treatment group was calculated by comparison to the control group.
Total shoot weight per replicate was divided by the number of surviving plants per replicate to determine the average shoot weight per plant. Means and standard deviations were calculated per treatment group. Percent inhibition of shoot weight per treatment group was calculated by comparison to the control group.

Statistics

Statistical evaluation was performed using ToxRat professional, version 3.3.0 (Ratte 2018).
Mortality data were analysed with Cochran-Armitage Test with Rao-Scott adjustment ($\alpha = 0.01$).
Shoot weight and height data were analysed with Shapiro-Wilks Test and Leven-Test followed by William's test or Dunett's T-test, Welch's T-test with Bonferroni-Holm adjustment, Jonckheere-Terpstra test or Multiple Median Chi^2-test with Bonferroni-Holm adjustment.
Effect rates were calculated using Probit analysis.

Results and discussions

Validity criteria:

- Seedling emergence in controls is at least 70% (observed: 84 - 96%)
- Control seedlings do not exhibit visible signs of phytotoxicity and normal variation in growth

and morphology (observed: no phytotoxicity and normal growth and morphology variation)
- Mean survival of emerged control seedlings is at least 90% for the duration of the study (observed: 100%)
- Environmental conditions per species are identical and growing media are the same (observed: conditions and growing media per species were the same)

The study was considered to be valid as the validity criteria were met.

Analytical verification of test substance concentrations in control and highest dose test solutions are summarised in the table below.

Table A2.6.1.2-1. Dose verification of test solutions containing AG-E1-500 SC1.

Test solution	Application rate [L product/ha]	Nominal concentration [g a.s./L]*	Measured concentration [g a.s./L]	% recovery
L1[a] control	0	0	<LOD	n/a
L1[a] highest test conc.	11.34	29.994	32.8	109.4
L1[b] control	0	0	<LOD	n/a
L1[b] highest test conc.	11.34	29.994	34.8	116.1
L3 control	0	0	<LOD	n/a
L3 highest test conc.	20.0	52.9	60.2	113.8

LOD = 0.048 µg a.s./mL, n/a not applicable, [a] First application to all plant species except *Linum usitatissimum* and *Medicago sativa*, [b] Second application to *Linum usitatissimum* and *Medicago sativa*; *Based on an application volume of 200 L/ha

Mortality, observations of phytotoxicity, growth stage (BBCH) at test end, shoot height and shoot dry weight results are summarised in the tables below.

Table A2.6.1.2-2. Mortality of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	No. dead plants	% mortality
Fagopyrum esculentum		
Control	0	0
0.48	0	0
1.06	0	0
2.34	0	0
5.15	0	0
11.34	0	0
Glycine max		
Control	0	0
0.22	0	0
0.48	0	0
1.06	0	0
2.34	0	0
5.15	1	5.0
Helianthus annuus		
Control	0	0
0.48	0	0
1.06	0	0
2.34	0	0
5.15	0	0
11.34	0	0
Lepdium sativum		
Control	0	0
0.027	0	0
0.082	0	0
0.247	0	0
0.741	0	0
2.22	0	0
Linum usitatissimum		

Application rate [L product/ha]	**No. dead plants**	**% mortality**
Control	0	0
0.027	0	0
0.082	0	0
0.247	0	0
0.741	0	0
2.22	0	0
Medicago sativa		
Control	0	0
0.009	0	0
0.027	0	0
0.082	0	0
0.247	0	0
0.741	0	0
Solanum lycopersicum		
Control	0	0
0.48	0	0
1.06	0	0
2.34	0	0
5.15	0	0
11.34	0	0
Vigna radiata		
Control	0	0
0.48	0	0
1.06	0	0
2.34	0	0
5.15	0	0
11.34	0	0
Hordeum vulgare		
Control	0	0
0.247	0	0
0.741	0	0
2.22	0	0
6.67	0	0
20.0	1	5.0
Triticum aestivum		
Control	0	0
0.247	0	0
0.741	0	0
2.22	0	0
6.67	0	0
20.0	0	0

n/a not applicable

Table A2.6.1.2-3. Phytotoxicity of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Minimum [%]	Maximum [%]	Mean [%]	Symptoms
Fagopyrum esculentum				
Control	0	0	0	n/a
0.48	10	30	20	CH, NE, LD
1.06	30	50	41	CH, NE, LD, ST
2.34	60	60	60	CH, NE, LD, ST
5.15	70	80	72	CH, NE, LD, ST
11.34	70	80	75	CH, NE, LD, ST
Glycine max				
Control	0	0	0	n/a
0.22	50	50	50	CH, NE, LD, ST
0.48	60	60	60	CH, NE, LD, ST
1.06	60	60	60	CH, NE, LD, ST
2.34	70	70	70	CH, NE, LD, ST
5.15	80	80	80	CH, NE, LD, ST
Helianthus annuus				
Control	0	0	0	n/a
0.48	0	0	0	n/a
1.06	0	0	0	LD
2.34	0	30	8	CH, NE, LD
5.15	0	60	39	LD, NE, ST
11.34	50	70	55	LD, NE, ST
Lepidium sativum				
Control	0	0	0	n/a
0.027	0	0	0	n/a
0.082	0	30	16	NE, ST
0.247	30	60	39	LD, NE, ST
0.741	70	70	70	LD, NE, ST
2.22	80	80	80	LD, NE, ST
Linum usitatissimum				
Control	0	0	0	n/a
0.027	0	0	0	n/a
0.082	0	0	0	n/a
0.247	0	0	0	n/a
0.741	0	0	0	n/a
2.22	0	40	22	NE, LD
Medicago sativa				
Control	0	0	0	n/a
0.009	0	10	3	LD
0.027	0	20	8	LD, NE
0.082	20	60	36	LD, NE, ST
0.247	40	60	46	LD, NE, ST
0.741	50	70	60	LD, NE, ST
Solanum lycopersicum				
Control	0	0	0	n/a
0.48	30	30	30	CH, LD
1.06	50	50	50	NE, CH, LD, SD
2.34	70	70	70	NE, CH, LD, SD
5.15	80	80	80	NE, CH, LD, SD
11.34	80	80	80	NE, CH, LD, SD
Vigna radiata				
Control	0	0	0	n/a
0.48	10	30	26	CH, NE, LD, ST
1.06	40	60	50	CH, NE, LD, ST
2.34	70	70	70	CH, NE, LD, ST
5.15	80	80	80	CH, NE, LD, ST
11.34	80	80	80	CH, NE, LD, ST
Hordeum vulgare				
Control	0	0	0	n/a
0.247	0	0	0	n/a
0.741	10	10	10	ST

Application rate [L product/ha]	Minimum [%]	Maximum [%]	Mean [%]	Symptoms
2.22	20	30	22	ST
6.67	50	50	50	ST, CH, NE
20.0	60	70	62	ST, CH, NE
Triticum aestivum				
Control	0	0	0	n/a
0.247	10	10	10	ST
0.741	30	30	30	ST
2.22	50	50	50	ST
6.67	60	60	60	ST
20.0	60	60	60	ST

n/a not applicable; Phytotoxic symptoms: Chlorosis (CH), Necrosis (NE), Stunted growth (ST), Leaf deformation (LD)

Table A2.6.1.2-4. Growth stage (BBCH) at test end of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Minimum growth stage [BBCH]	Maximum growth stage [BBCH]
Fagopyrum esculentum		
Control	63	63
0.48	63	63
1.06	61	61
2.34	51	51
5.15	51	51
11.34	51	51
Glycine max		
Control	51	51
0.22	21	21
0.48	21	21
1.06	21	21
2.34	14	14
5.15	14	14
Helianthus annuus		
Control	18	18
0.48	18	18
1.06	18	18
2.34	18	18
5.15	18	18
11.34	18	18
Lepdium sativum		
Control	61	61
0.027	61	61
0.082	61	61
0.247	16	16
0.741	14	14
2.22	14	14
Linum usitatissimum		
Control	42	42
0.027	42	42
0.082	42	42
0.247	42	42
0.741	42	42
2.22	42	42
Medicago sativa		
Control	61	61
0.009	61	61
0.027	61	61
0.082	55	55
0.247	55	55
0.741	21	21
Solanum lycopersicum		
Control	19	19
0.48	19	19
1.06	21	21

Application rate [L product/ha]	Minimum growth stage [BBCH]	Maximum growth stage [BBCH]
2.34	14	14
5.15	14	14
11.34	14	14
Vigna radiata		
Control	14	14
0.48	14	14
1.06	13	13
2.34	13	13
5.15	12	12
11.34	12	12
Hordeum vulgare		
Control	22	22
0.247	22	22
0.741	22	22
2.22	22	22
6.67	12	12
20.0	12	12
Triticum aestivum		
Control	23	23
0.247	23	23
0.741	21	21
2.22	13	13
6.67	13	13
20.0	13	13

Table A2.6.1.2-5. Shoot height and shoot dry weight at test end of plants exposed to AG-E1-500 SC1.

Application rate [L product/ha]	Shoot height			Shoot dry weight		
	Mean [cm]	± SD [cm]	% inhibition	Mean [g]	± SD [g]	% inhibition
Fagopyrum esculentum						
Control	101.6	10.0	n/a	2.386	0.313	n/a
0.48	89.7 [a]	8.4	11.7	1.960 [a]	0.360	17.9
1.06	72.8 [a]	10.9	28.3	1.726 [a]	0.420	27.7
2.34	59.1 [a]	6.9	41.8	1.353 [a]	0.304	43.3
5.15	42.7 [a]	6.6	58.0	0.917 [a]	0.375	61.6
11.34	40.2 [a]	4.3	60.4	0.861 [a]	0.182	63.9
Glycine max						
Control	93.3	6.5	n/a	2.658	0.318	n/a
0.22	45.3 [a]	4.1	51.4	1.858 [c]	0.249	30.1
0.48	36.1 [a]	2.5	61.3	1.690 [c]	0.268	36.4
1.06	37.5 [a]	4.3	59.8	1.721 [c]	0.253	35.3
2.34	28.6 [a]	3.3	69.3	1.364 [c]	0.123	48.7
5.15	27.8 [a]	3.0	70.2	1.325 [c]	0.413	50.2
Helianthus annuus						
Control	9.1	1.0	n/a	1.044	0.156	n/a
0.48	8.5	0.8	6.6	0.905	0.113	13.3
1.06	9.5	1.2	-4.4	1.064	0.088	-1.9
2.34	8.8	0.5	3.3	1.038	0.159	0.6
5.15	8.1 [a]	0.6	11.0	0.772 [a]	0.154	26.1
11.34	7.6 [a]	0.7	16.5	0.707 [a]	0.063	32.3
Lepdium sativum						
Control	17.0	3.3	n/a	0.896	0.090	n/a
0.027	13.4 [a]	1.5	21.2	0.751 [a]	0.081	16.2
0.082	13.6 [a]	2.5	20.0	0.665 [a]	0.165	25.8
0.247	12.1 [a]	2.0	28.8	0.571 [a]	0.118	36.3
0.741	9.3 [a]	0.9	45.3	0.335 [a]	0.050	62.6
2.22	7.7 [a]	0.6	54.7	0.169 [a]	0.049	81.1
Linum usitatissimum						
Control	37.2	2.8	n/a	0.342	0.043	n/a
0.027	38.0	3.3	-2.2	0.310	0.048	9.4
0.082	37.3	4.2	-0.3	0.307	0.060	10.2

Application rate [L product/ha]	Shoot height			Shoot dry weight		
	Mean [cm]	± SD [cm]	% inhibition	Mean [g]	± SD [g]	% inhibition
0.247	36.3	2.3	2.4	0.323	0.055	5.6
0.741	36.8	3.4	1.1	0.292	0.043	14.6
2.22	34.5 [a]	3.8	7.3	0.310	0.061	9.4
Medicago sativa						
Control	48.1	7.9	n/a	0.638	0.170	n/a
0.009	44.1	4.5	8.3	0.544	0.130	14.7
0.027	41.6 [a]	6.1	13.5	0.566	0.142	11.3
0.082	42.3 [a]	5.6	12.1	0.463 [a]	0.141	27.4
0.247	35.9 [a]	5.0	25.4	0.404 [a]	0.062	36.7
0.741	27.1 [a]	7.6	43.7	0.216 [a]	0.077	66.1
Solanum lycopersicum						
Control	37.6	3.1	n/a	2.513	0.364	n/a
0.48	32.1 [b]	2.5	14.6	1.654 [a]	0.178	34.2
1.06	29.0 [b]	5.1	22.9	1.338 [a]	0.110	46.8
2.34	25.2 [b]	4.1	33.0	1.257 [a]	0.240	50.0
5.15	18.8 [b]	2.1	50.0	0.942 [a]	0.135	62.5
11.34	16.4 [b]	2.0	56.4	1.053 [a]	0.210	58.1
Vigna radiata						
Control	16.2	1.7	n/a	0.697	0.115	n/a
0.48	18.1	1.9	-11.7	0.598 [b]	0.129	14.2
1.06	17.1	1.6	-5.6	0.339 [b]	0.059	51.4
2.34	15.8	1.5	2.5	0.328 [b]	0.041	52.9
5.15	13.5 [a]	0.8	16.7	0.210 [b]	0.045	69.9
11.34	12.8 [a]	1.9	21.0	0.172 [b]	0.018	75.3
Hordeum vulgare						
Control	41.0	4.2	n/a	0.501	0.113	n/a
0.247	39.0	4.6	4.9	0.500	0.132	0.2
0.741	34.5 [a]	5.2	15.9	0.414	0.083	17.4
2.22	28.5 [a]	6.8	30.5	0.310	0.122	38.1
6.67	18.0 [a]	1.4	56.1	0.133 [b]	0.049	73.5
20.0	17.1 [a]	2.3	58.3	0.105 [b]	0.020	79.0
Triticum aestivum						
Control	31.7	1.4	n/a	0.540	0.125	n/a
0.247	27.5 [a]	2.4	13.2	0.363	0.127	32.8
0.741	21.5 [a]	0.8	32.2	0.199 [d]	0.090	63.1
2.22	18.0 [a]	1.3	43.2	0.081 [d]	0.012	85.0
6.67	19.2 [a]	0.8	39.4	0.086 [d]	0.008	84.1
20.0	18.2 [a]	0.7	42.6	0.074 [d]	0.008	86.3

n/a not applicable, SD: Standard Deviation., Statistically significant: [a] Williams` test, one-sided smaller, $\alpha = 0.05$, [b] Welch's test, one-sided smaller, $\alpha = 0.05$, [c] Jonckheere-Terpstra test one-sided smaller, $\alpha = 0.05$, [d] Multiple Median Chi^2-test with Bonferroni-Holm adjustment one-sided smaller, $\alpha = 0.05$, negative values indicate that there was an enhanced effect compared to the control

Calculated LOER, NOER and ER_{50} values for the different parameters for each plant species are presented in the table below.

Table A2.6.1.2-6. LOER, NOER and ER_{50} for various endpoints for plants exposed to AG-E1-500 SC1.

Endpoint	*Fagopyrum esculentum* (95% confidence limits)	*Glycine max* (95% confidence limits)	*Helianthus annuus* (95% confidence limits)	*Lepidium sativum* (95% confidence limits)	*Linum usitatissimum* (95% confidence limits)	*Medigo sativa* (95% confidence limits)	*Solanum lycopersicum* (95% confidence limits)	*Vigna radiate* (95% confidence limits)	*Hordeum vulgare* (95% confidence limits)	*Triticum aestivum* (95% confidence limits)
Mortality										
LOER	>11.34	>5.15	>11.34	>2.22	>2.22	>0.741	>11.34	>11.34	>20.0	>20.0
NOER	11.34	5.15	11.34	2.22	2.22	0.741	11.34	11.34	20.0	20.0
ER_{50}	>11.34 (ND)	>5.15 (ND)	>11.34 (ND)	>2.22 (ND)	>2.22 (ND)	>0.741 (ND)	>11.34 (ND)	>11.34 (ND)	>20.0 (ND)	>20.0 (ND)
Shoot height										
LOER	0.48 [a]	0.22 [a]	5.15 [a]	0.027 [a]	2.22 [a]	0.027 [a]	0.48 [b]	5.15 [a]	0.741 [a]	0.247 [a]
NOER	ND (<0.48)	ND (<0.22)	2.34	ND (<0.027)	0.741	0.009	ND (<0.48)	2.34	0.247	ND (<0.247)
ER_{50}	s.n.r	s.n.r	>11.34	1.13 (0.13 - 2.13)	>2.22 (ND)	s.n.r	6.39 (4.62 - 8.19)	>11.34 (ND)	7.02 (3.42 - 10.72)	s.n.r
Shoot dry weight										
LOER	0.48 [a]	0.22 [c]	5.15 [a]	0.027 [a]	>2.22	0.082 [a]	0.48 [a]	0.48 [b]	6.67 [b]	0.741 [d]
NOER	<0.48	<0.22	2.34	<0.027	2.22	<0.027	<0.48	<0.48	2.22	0.247
ER_{50}	3.54 (2.16 - 4.95)	s.n.r	>11.34	0.381 (0.30 - 0.49)	>2.22 (ND)	0.37 (0.25 - 0.67)	s.n.r	s.n.r	3.27 (2.20 - 4.95)	0.46 (0.25 - 0.70)

s.n.r statistically not reliable; (ND) – not determinable; LOER determined with: [a] Williams` test, [b] Welch`s test, [c] Jonckheere-Terpstra test, [d] Multiple Median Chi^2-test with Bonferroni-Holm adjustment; all tests one-sided smaller, $\alpha = 0.05$

At the end of the 21-day exposure, no mortality was observed in any species except *Glycine max* and *Hordeum vulgare* with 5% mortality at the highest test substance concentrations. These observed mortalities were not statistically significantly different from the control.

Symptoms of phytotoxicity were observed in all plant species. The observed symptoms were leaf deformation, chlorosis, necrosis and stunted growth. Symptoms on nearly the total plant (up to 80 %) occurred in *Fagopyrum esculentum, Glycine max, Solanum lycopersicum* and *Vigna radiata* down to 5.15 L product/ha and in *Lepidium sativum* down to 2.22 L product/ha.

Test substance effects on the BBCH at test end were observed in all test species except *Helianthus annuus* and *Linum usitatissimum*. For the plant species *Glycine max, Lepidium sativumimum, Hordeum vulgare* and *Triticum aestivum* the number of true leaves were affected down to 0.22, 0.247, 6.67 and 0.741 L product/ha, respectively. The plant species *Fagopyrum esculentum, Lepidium sativum* and *Medicago sativa* already initiated flowers (BBCH growth stage of 61-63) in the control group and up to 1.06, 0.082 and 0.027 L product/ha, respectively, in comparison to the further higher test item rates.

Statistically significantly different effects on shoot height were observed in all test species. *Fagopyrum esculentum, Glycine max, Lepidium sativum, Solanum lycopersicum* and *Triticum aestivum* were statistically significantly different compared to controls at all test substance concentrations. Statistically significantly different effects on shoot height were observed in *Helianthus annuus* at 5.15 and 11.34 L product/ha, *Linum usitatissimum* at 2.22 L product/ha, *Medicago sativa* at 0.027 to 0.741 L product/ha, *Vigna radiata* at 5.15 and 11.34 L product/ha and *Hordeum vulgare* at 0.741 to 20.0 L product/ha. Statistically significantly different effects on shoot dry weight were observed in all test species except *Linum usitatissimum*. *Fagopyrum esculentum, Glycine max, Lepidium sativum, Solanum lycopersicum* and *Vigna radiata* were statistically significantly different compared to controls at all test substance concentrations. Statistically significantly different effects on shoot dry weight were observed in *Helianthus annuus* at 5.15 and

11.34 L product/ha, *Medicago sativa* at 0.082 to 0.741 L product/ha and *Hordeum vulgare* at 6.67 and 20.0 L product/ha.

Conclusion

In a vegetative vigour test with AG-E1-500 SC1 at various application rates on 10 plant species, the lowest ER_{50} for risk assessment was 0.37 L product/ha for effects on shoot dry weight in *Medicago sativum.*

A 2.6.2 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data