FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: CHR/H/ETO 500 SC

Product name(s): BITT 500 SC, BETRON 500 SC, ETONAL 500 SC

Chemical active substance(s):

Ethofumesate, 500 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

Core Assessment

Applicant: Innvigo Sp. z o.o.

Submission date: June 2021

MS Finalisation date: 14/01/2022

Version history

When	What
May 2021	Submission to the Polish Ministry of Agriculture and Rural Development
June 2021	Submission to the evaluation unit
November 2021	zRMS finalised evaluation
January 2022	Final version prepared by zRMS after Commenting period

Table of Contents

9 Ecotoxicology (KCP 10)

Review Comments:

This application was submitted by Innvigo Sp. z o.o. for approval of the formulation CHR/H/ETO 500 SC / BITT 500 SC, BETRON 500 SC, ETONAL 500 SC containing 500 g/L of ethofumesate for use as a herbicide on: sugar beet.

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

Since this document is based on the information provided by the Applicant, all review comments, additions, and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

In the following document, data for active substance ethofumesate was described during its renewal process in 2016. Were reference to active substance data in the current risk assessment has been made, it was based on the data presented by Bayer.
In June 14th, 2018r Kemiron Koncentrat 500 SC product has been renewed in Poland thus according to the art. 59 reg. 1107/2009, data protection for mentioned data expired 30 months from date of first renewal of authorisation of product containing that active substance (in this case December, 14th 2020).
Considering analogous arguments (art. 59 reg 1107/2009) – data protection of studies presented by UPL for renewal of product Bettix Combi 500 SC (renewal of authorisation granted in Poland 14.02.2019 r.) expires August 14th, 2021.
Taking into account that some data was presented by others Notyfiers, Applicant would like to emphasise that unprotected Bayer's endpoints and input parameters accepted during renewal of active substance, should be treated as an equivalent matching data in cases where any of endpoints might be protected.

9.1 Critical GAP and overall conclusions

PPP product name: product code:	CHR/H/ETO	Formulation type:	
Active substance 1:	ethofumesate	Conc. of as 1:	
Active substance 2:	-	Conc. of as 2:	
Active substance 3:	-	Conc. of as 3:	
Safener:	-	Conc. of safener:	
Synergist:	-	Conc. of synergist:	
Applicant:	Innvigo Sp. z o.o.	Professional use:	
Zone(s):	Central (d)	Non professional use:	
Verified by MS:	Yes ~~no~~		
Field of use:	Herbicide		

Table 9.1-1 Table of critical GAPs

1	2	3	4	5	6	7	8	9	15	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**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 (f)	ZRMs Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants

Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL,CZ	Sugar beet *Beta vulgaris* subsp. *vulgaris* var. *altissima* (BEAVA)	F	Dicotylenous weeds	Spray, medium sprayer	Spring BBCH ~~11~~ 12 -18	a) 2 b) 2	5	a) 1.0 l/ha b) 2.0 l/ha	a) 0.5 kg a.s./ha b) 1.0 kg a.s./ha	200 - 300		For BBCH change please refer to efficacy section							
~~**Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)**~~																				
2	PL, CZ	Sugar beet *Beta vulgaris* subsp. *vulgaris* var. *altissima* (BEAVA)	F	Dicotylenous weeds	Spray, medium sprayer	Spring BBCH ~~11~~ 12 - 18	a) 3 b) 3	5	a) 0,6 l/ha b) 1,8 l/ha	a) 0,3 kg a.s/ha b) 0,9 kg a.s/ha	200 - 300		For BBCH change please refer to efficacy section							
3																				
Minor uses according to Article 51 (zonal uses)																				
4																				
5																				
Minor uses according to Article 51 (interzonal uses)																				
6																				
7																				

Remarks table heading:

(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008

(c) g/kg or g/l

(d) Select relevant

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

(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use.

Remarks columns:

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

* 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

Column 15: zRMS conclusion.

A	Acceptable
R	Acceptable with further restriction
C	To be confirmed by cMS
N	Not acceptable / evaluation not possible
n.r.	Not relevant for section 3

Review Comments:

GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

9.1.1 Overall conclusions

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

CHR/H/ETO 500 SC pose no unacceptable risk to birds and mammals used according to the label.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

CHR/H/ETO 500 SC pose no unacceptable risk to aquatic organisms ~~non-target terrestrial plants~~ according to the label. ~~with appropriate buffer zone.~~ Concerned Member States should decide on applicability of the proposed approach at the product registration.

9.1.1.3 Effects on bees (KCP 10.3.1)

CHR/H/ETO 500 SC pose no unacceptable risk to bees according to the label.
The Applicant provided chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies for CHR/H/ETO 500 SC were evaluated and accepted by zRMS but not taken to consideration in risk assessment since the evaluation was done according to SANCO/10329/2002 rev 2 final.
Concerned Member States must decide on the consideration of data requirements on national level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

CHR/H/ETO 500 SC pose no unacceptable risk to arthropods other than bees according to the label

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

CHR/H/ETO 500 SC pose no unacceptable risk to non-target soil meso- and macrofauna and microbial activity according to the label.

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

Based on the predicted rates of CHR/H/ETO 500 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/ETO 500 SC according to the GAP of the formulation CHR/H/ETO 500 SC achieve the acceptability criteria TER ≥ 5 with applying:

For two applications in sugar beet:
- unsprayed buffer zone of 5 m
- unsprayed buffer zone of 1 m and use of 50% ~~75%~~ drift reduction technology to non-agricultural land ~~reducing nozzles~~
- ~~1 m and use of 90% drift reducing nozzles~~

For three applications in sugar beet mitigation measures are not required.

Concerned Member States should decide on applicability of the mitigation measures at the product registration.

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

Not relevant

9.1.2 Grouping of intended uses for risk assessment

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

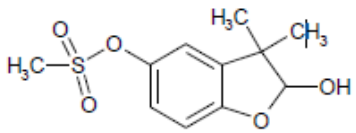
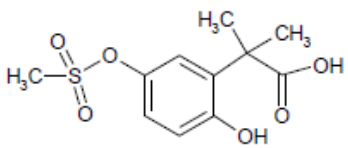
Table 9.1-1: Critical use pattern of CHR/H/ETO 500 SC grouped according to crop, application rate, number of applications, timing criterion

Grouping according to crop, application rate, number of applications, timing criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	Sugar beet BBCH 12-18 2 L [Product]/ha	crop, application rate, number of applications, timing,	crop, application rate, number of applications, timing,

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 CHR/H/ETO 500 SC is indicated in the table.

Table 9.1-2 Metabolites of Ethofumesate

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
NC 8493 ethofumesate-2-hydroxy	258.3		Total Water and Sediment: - Soil: 24.2% molar basis with respect to the parent	PECsoil, PECgw, PECsw
NC 20645	274.3		Total Water and Sediment: 18.8 molar basis with respect to the parent Soil: 1.82% molar basis with respect to the parent	PECgw, PECsw, PECsed

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents. Effects on birds of CHR/H/ETO were not evaluated as part of the EU assessment of ethofumesate. However, the provision of further data on the CHR/H/ETO is not considered essential, because studies from Annex I inclusion can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	Ethofumesate	Acute	**LD_{50} > 2000 ~~1000~~ mg/kg bw per day**	EFSA Journal 2016;14(1):4374
Colinus virginianus	Ethofumesate	Oral 14 d Acute	LD_{50} > 8743 mg/kg bw/d	EFSA Conclusion (2016)
Anas platyrhynchos	Ethofumesate	Oral 14 d Acute	LD_{50} > 3552 mg/kg bw/d	EFSA Conclusion (2016)
Colinus virginianus	Ethofumesate	Oral 14 d Acute	LD_{50} > 2000 mg/kg bw	EFSA Conclusion (2016)
		Acute	$LD_{50\ extrapolated}$ = 3776*	
Colinus virginianus	Ethofumesate	Long- term	**NOEL = 265.0 mg/kg bw per day**	EFSA Journal 2016;14(1):4374

* LD_{50} extrapolated according to the EFSA Guidance Document on Birds and Mammals (EFSA/2009/1438), 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.

Bold values were used for the risk assessment

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).

Birds and mammals risk assessment were performed using worst case scenario application in sugar beets 2 x 500 g a.s./ha per season which is the risk envelope for other uses of CHR/H/ETO 500 SC according to the GAP presented in table 9.1.-1.

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 tables.

Table 9.2-2: Screening ~~First-tier~~ assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/H/ETO 500 SC in sugar beets ~~winter/spring cereals.~~

Intended use		**Sugar beets**			
Active substance/product		Ethofumesate			
Application rate (g/ha)		2 x 500 g a.s/ha			
Acute toxicity (mg/kg bw)		2000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}***	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small omnivorous bird	158.8	1.7 ~~1.5~~	135 ~~119.1~~	14.8 ~~16.8~~
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}**
Screening step	Small omnivorous bird	64.8	0.90	29.16 ~~19~~	9.08 ~~6.9~~

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.

* MAF calculated on the basis of two applications and a 5 day spray interval assuming a default DT_{50} of 10 days

9.2.2.2 Higher-tier risk assessment

Since for ethofumesate acute TER is above 10 and TER long term is above 5, no higher-tier risk assessment is required.

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 CHR/H/ETO 500 SC 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 sorp-

tive 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 mg/L, Ethofumesate belongs to the group of less sorptive substances. To achieve a con-cise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on sugar beets two applications would cover three applications ~~cereals winter:~~

Effective application rate (g/ha)	=	1000		
Acute toxicity (mg/kg bw)	=	2000	quotient	= 0.5
Reprod. toxicity (mg/kg bw/d)	=	265	quotient	= 3.77

Hazard quotient for Puddle scenario for Ethofumesate are below trigger value 50, so no specific calculations of exposure and TER are necessary.

9.2.2.4 Effects of secondary poisoning

The log Pow of Ethofumesate is below 3 and thus do not exceeds the trigger value of 3. 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).A risk assessment for effects due to secondary poisoning is not required.

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

In conclusion, the acute, ~~short term~~ risk and long term to birds from the proposed uses of ethofumesate was found acceptable.

Review comments:

The acute and long-term risk assessment for birds was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints.
For acute risk assessment the Applicant used the lowest available endpoint LD50 > 2000 mg/kg bw instead of accepted at the EU level extrapolated LD_{50} = 3776* endpoint. Since this endpoint represent worst case scenario is acceptable by zRMS for Risk Assessment purpose. No formulation study was required.
Overall, acceptable acute and reproductive risk to birds may be concluded for application of CHR/H/ETO 500 SC to sugar beet according to the intended uses.

CHR/H/ETO 500 SC presents no unacceptable risk to birds resulting from exposure via drinking water. Since the log Pow value of ethofumesate is below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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 and its relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

However, the provision of further data on the CHR/H/ETO 500 SC is not considered essential, because the selection of studies and endpoints for the risk assessment is in line with / deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Rat	Ethofumesate	Acute oral	LD_{50} > 5000 mg/kg bw per day	EFSA Journal 2016;14(1):4374
Rat	Ethofumesate	Long term	NOAEL = 60.9 mg/kg bw per day	EFSA Journal 2016;14(1):4374

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 Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on the sugar beet ~~cereals winter crop~~.

Birds and mammals risk assessment were performed using worst case scenario application in sugar beets 2 x 500 g a.s./ha per season which is the risk envelope for other uses of CHR/H/ETO 500 SC according to the GAP presented in table 9.1.-1.

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

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

Table 9.3-2: Screening and First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/H/ETO 500 SC in sugar beets

Intended use	Sugar beets				
Active substance/product	Ethofumesate				
Application rate (g/ha)	2 x 500 g				
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**
Screening step	Small herbivorous mammal	118.4	1.7 ~~1.5~~	100.64 ~~88.8~~	49.68 ~~56.3~~

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}**
Screening step	Small herbivorous mammal	48.3	0.9	21.76	**2.80**
Sugar beet BBCH 10 - 19	Small insectivorous mammal "shrew" ground dwelling invertebrates without interception 100% ground arthropods	4.2	0.9 -	1.89 -	32.2
Sugar beet BBCH 10-39	Large herbivorous mammal “lagomorph” Non-grass herbs 100% crop leaves	14.3	0.9 -	6.43 -	9.5
Sugar beet BBCH 10-39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	0.9 -	3.51 -	17.3

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.

* MAF calculated on the basis of two applications and a 5 day spray interval assuming a default DT_{50} of 10 days

9.3.2.2 Higher-tier risk assessment

No further risk refinement is required.

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, Ethofumesate belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use on sugar beets two applications would cover three applications. ~~cereals winter~~

Effective application rate (g/ha)	=	1000		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.9
Reprod. toxicity (mg/kg bw/d)	=	60.9	quotient =	16.4

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Ethofumesate is below 3 and thus do not exceeds the trigger value of 3. 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).A risk assessment for effects due to secondary poisoning is not required required.

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

In conclusion, the acute, ~~short term~~ risk and long term to mammals from the proposed uses of ethofumesate was found acceptable.

Review comments:

The acute and long-term risk assessment for mammals was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.
Overall, acceptable acute and reproductive risk to mammals may be concluded for application of CHR/H/ETO 500 SC to sugar beet according to the intended uses.

CHR/H/ETO 500 SC presents no unacceptable risk to mammals resulting from exposure via drinking water. Since the log Pow value of ethofumesate is below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

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

N/A

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 RAR and related documents.

Effects on aquatic organisms of CHR/H/ETO 500 SC 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. Justifications are provided below.

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 mg/L	Reference
Cyprinus carpio	Ethofumesate	96h (static)	LC50= 10.92 mg a.s./L	EFSA Journal 2016;14(1):4374
Oncorhynchus mykiss	Ethofumesate	Acute, 96h (semi static)	LC50= 11.91 mg as/L	EFSA Journal 2016;14(1):4374
Lepomis macochirus	Ethofumesate	Acute, 96h (semi-static)	LC50=21.2 mg as/L	EFSA Journal 2016;14(1):4374
Cyprinodon variegatus	Ethofumesate	Acute, 96h (static)	LC50=25.0 mg as/L	EFSA Journal 2016;14(1):4374
Danio rerio	Ethofumesate	Chronic (flow-through), FFLC	NOEC= 0.156 mg a.s/L	EFSA Journal 2016;14(1):4374
Pimpehales promela	Ethofumesate	Chronic (flow-through) FFLC	NOEC= 4.17 mg a.s/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Ethofumesate	48h (static)	EC50= 13.52 mg a.s/L	EFSA Journal 2016;14(1):4374
Americamysis bahia	Ethofumesate	96h (static)	EC50= 5.4 mg a.s/L	EFSA Journal 2016;14(1):4374
Crassostrea virginica	Ethofumesate	96h (flow-through)	EC50= 1.7 mg as/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Metabolite NC 8493	48h (semi-static)	EC50 > 10 mg/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Metabolite NC 8493	48h (static)	EC50 > 100 mg/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Metabolite NC 20645	48h (semi-static)	EC50 > 10 mg/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Metabolite NC 20645	48h (static)	EC50> 100 mg/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Ethofumesate	21 d (semi static)	NOEC= 0.32 mg as/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Ethofumesate	21 d (semi-static)	NOEC=0.25 mg as/L	EFSA Journal 2016;14(1):4374
Daphnia magna	Ethofumesate	21 d (semi-static)	NOEC= 1.06 mg as/L	EFSA Journal 2016;14(1):4374
Chironomus riparius	Ethofumesate	28 d (static)	NOEC= 3.82 mg as/L	EFSA Journal 2016;14(1):4374
Chironomus riparius	Ethofumesate	28 d (static)	NOEC= 5.33 mg as/L	EFSA Journal 2016;14(1):4374
Chironomus riparius	Ethofumesate	28 d (static)	NOEC=14.05 mg as/L	EFSA Journal 2016;14(1):4374
Pseudokirchneriella subcapitata	Ethofumesate	72h (static)	ErC50=16.3 mg as/L EyC50= 9.68 mg as/L NOEC= 5.91 mg as/L	EFSA Journal 2016;14(1):4374
Anabaena flos-aquae	Ethofumesate	**96h (static)**	ErC50 > 20 mg as/L EbC50> 20 mg as/L NOEC= 20 mg as/L	EFSA Journal 2016;14(1):4374
Skeletonema costatum	Ethofumesate	72h (static)	ErC50 >20 mg as/L EbC50= 15.5 mg as/L	EFSA Journal 2016;14(1):4374
Pseudokirchneriella subcapitata	Metabolite NC 8493	72h (static)	ErC50= 20.7 mg/L EyC50= 0.865 mg/L	EFSA Journal 2016;14(1):4374

Species	Substance	Exposure System	Results mg/L	Reference
			NOEC= 0.367 mg/L	
Desmodesmus subspicatus	Metabolite NC 8493	72h (static)	ErC50= 4.83 mg/L EyC50=1.87 mg/L NOEC=1.33 mg/L	EFSA Journal 2016;14(1):4374
Desmodesmus subcapitatus	Metabolite NC 20645	72h (static)	ErC50= 52.4 mg/L EyC50= 8.83 mg/L NOEC= 1.25 mg/L	EFSA Journal 2016;14(1):4374
Pseudokirchneriella subcapitata	Metabolite NC 20645	72h (static)	ErC50 > 10 mg/L EyC50 > 10 mg/L NOEC= 10 mg/L	EFSA Journal 2016;14(1):4374
Lemna minor	Ethofumesate	14 d (semi-static)	ErC50 > 52.8 mg as/L EbC50= 50.4 mg as/L NOEC= 4.3 mg/L	EFSA Journal 2016;14(1):4374
Lemna minor	Ethofumesate	7 d (semi-static)	ErC50 > 42 mg as/L EbC50 = 35 mg as/L	EFSA Journal 2016;14(1):4374
Myriophyllum spicatum	Ethofumesate	14 d (static)	ErC50= 0.479 mg as/L EyC50= 0.25 mg as/L NOEC= 0.036 mg as/L	EFSA Journal 2016;14(1):4374
Higher-tier studies (micro- or mesocosm studies)				
No further tests submitted				

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/H/ETO 500 SC

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	CHR/H/ETO 500 SC	96h	No study available -	-
Daphnia magna	CHR/H/ETO 500 SC	48h	EC_{50} > 191.7 mg formulation /L Corresponding to 95.85 mg ethofumesate/L	E. Malada, Study code: W-33-20, 2020
Pseudokirchneriella subcapitata	CHR/H/ETO 500 SC	72h	EyC_{50} = 23.16 (mg formulation /L) corresponding to 11.58 mg ethofumesate/L ErC_{50} = 45.33 mg formulation /L corresponding to 22.66 mg ethofumesate/L	E. Malada, Study code: W-34-20, 2020
Anabaena flos-aquae	CHR/H/ETO 500 SC	72h	ErC_{50} = >300 (mg formulation /L) corresponding to 150 mg ethofumesate/L $EyC_{50=}$ >300 (mg formulation /L) corresponding to 150 mg ethofumesate/L	M. Czarnecka, Study code: W-36-20, 2020

Species	Substance	Exposure System	Results	Reference
Lemna gibba	CHR/H/ETO 500 SC	7 d	ErC_{50} = 86.61 mg formulation/L corresponding to 43.30 mg ethofumesate/L EyC50 = 24.52 mg formulation /L corresponding to 12.26 mg ethofumesate/L	M. Czarnecka, Study code: W-35-20, 2020
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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

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).
The relevant global maximum FOCUS Step 1, 2 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.

~~Risk assessment have been provided for worst case use for data gap (pH>7) in winter and spring cereals, which cover a risk envelopment for all uses of data gap.~~

Table 9.5-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ethofumesate for each organism group based on FOCUS Steps 1, 2 , 3 calculations for the use of CHR/H/ETO 500 SC in sugar beet

Group		**Fish acute**	**Fish pro-longed**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Test species		***Cyprinus carpio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***	***P.Subcapitata***	***Chironomus riparius***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	EbC_{50}	ErC50	ErC50
(µg/L)		10 920	156	1700	250	16 300	3 820	479
AF		100	10	100	10	10	10	10
RAC (µg/L)		109.2	15.6	17	25	1 630	382	47.9
Exposure	**PEC $_{gl-max}$(µg/L)**							
Step 1	297.22	2.72179	19.05256	17.48353	11.88880	0.18234	0.77806	6.2050
Step 2								
	46.05	0.42170	2.95192	2.70882	1.84200	0.02825	0.12055	0.9614
Step 3								
D3	2.278	0.0208608	0.14603	0.134000	0.091120	0.00140	0.005963	0.0476
D4	0.5293	0.0048471	0.03393	0.031135	0.021172	0.00032	0.001386	0.0111
D4	1.949	0.0178480	0.12494	0.114647	0.077960	0.00120	0.005102	0.0407
R1	0.3601	0.0032976	0.02308	0.021182	0.014404	0.00022	0.000943	0.0075
R1	4.828	0.0442125	0.30949	0.284000	0.193120	0.00296	0.012639	0.1008
R3	12.78	0.1170330	0.81923	0.751765	0.511200	0.00784	0.033455	0.2668

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

Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite NC 8493 of ethofumesate for each organism group based on FOCUS Steps 1~~, 2~~ calculations for the use of CHR/H/ETO 500 SC in sugar beets

NC 8493									
Group		Fish acute	Fish pro-longed	**Inver-teb. acute**	**Inver-teb. acute**	**Inverteb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Aquat-ic plants**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daphnia magna***	***Daph-nia magna***	***Daphnia magna***	***Desmodeus subspi-catyus***	***Chirono-mus ripar-ius***	***Lemna Gibba***
End-point (µg/L)		- -	- -	EC_{50} 10 000	- -	-	ErC50 4 830	-	- -
AF		100	10	100	10	-	10	-	-
RAC (µg/L)		-	-	100	-	-	483	-	-
Expo-sure	**PEC $_{gl-max}$ (µg/L)**								
Step 1									
PEC/RAC	72.58			0.7258			0.1503		

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite NC 20645 of Ethofumesate for each organism group based on FOCUS Steps 1~~–2~~ calculations for the use of CHR/H/ETO 500 SC in sugar beet.

NC 20645									
Group		**Fish acute**	**Fish pro-longed**	**Inver-teb. acute**	**Inver-teb. acute**	**Inver-teb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Aquati c plants**
Test species		***Oncorhyn-chus mykiss***	***Oncorhyn-chus mykiss***	***Daph-nia magna***	***America-mysis bahia***	***Daphnia magna***	***Scenedes-mus sub-spica-tus***	***Chirono-mus ripar-ius***	***Lemna Gibba***
End-point (µg/L)		- -	- -	EC_{50} 10 000	- -	- -	ErC50 10 000	-	- -
AF		100	-	100	-	10	10	-	-
RAC (µg/L)		-	-	100	-	-	1000	-	-
Expo-sure	**PEC $_{gl-max}$ (µg/L)**								

NC 20645									
Group		**Fish acute**	**Fish pro-longed**	**Inver-teb. acute**	**Inver-teb. acute**	**Inver-teb. pro-longed**	**Algae**	**Sed. dwell. prolonged**	**Aquatic plants**
Step 1									
PEC/RAC	76.76			0.7676			0.07676		

9.5.2.1 Risk assessment for formulation to aquatic organisms

Table 9.5-4: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of CHR/H/ETO 500 SC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use of CHR/H/ETO 500 SC in sugar beets**

Intended use	Sugar beet ~~cereals winter~~
Formulation	CHR/H/ETO 500 SC
Application rate (g[prod]/ha)	1 x 1132
Entry into surface water via spraydrift (Drift alculator from SWASH)	
Buffer zone (m)	**PECsw [µg prod/L**
1	6.0131
Entry into surface water via spraydrift (Drift alculator from SWASH)	
Buffer zone (m)	**RAC/PEC ratio** **Daphnia magna =EC50 191 700 µg/L** **RAC=1 917 (AF=100)**
1	0.003137
Buffer zone (m)	**RAC/PEC ratio** **Pseudokirchmeirella subcapitata =ErC50 45 330 µg/L** **RAC= 4 533 (AF=10)**
1	0.001327
Buffer zone (m)	**RAC/PEC ratio** **Anabaena flos-aque =ErC50 300 000 µg/L** **RAC= 30 000 (AF=10)**
1	0.0002
Buffer zone (m)	**RAC/PEC ratio** **Lemna Gibba =ErC50= 86 610 µg/L** **RAC=8 661 (AF=10)**
1	0.0006943

9.5.3 Overall conclusions

Based on the predicted rates of CHR/H/ETO 500 SC in aquatic system, the TER values describing the risk for aquatic organisms following exposure to CHR/H/ETO 500 SC according to the GAP of the formulation CHR/H/ETO 500 SC achieve the acceptability criteria without applying buffer zone.

Review comments:

The risk assessment for active substance and metabolites are accepted by zRMS.

For ethofumesate and all relevant metabolites, calculated PEC/RAC ratios indicate an acceptable risk for the intended uses in sugar beet without the need for mitigation measures.
For the most sensitive group of organisms (fish chronic) acceptable risk for active substance ethofumesate was provided based on the FOCUS Step 3 assessment without need for risk mitigation measures.

CHR/H/ETO 500 SC is a product containing one active ingredient so the risk assessment could be considered based on the active substance.

For the risk assessment for the formulated product, acceptable risk to aquatic organisms is demonstrated for all group of organisms. The product is not more toxic than predicted from toxicity of the active substance.

Concerned Member States should decide on applicability of the proposed approach at the product registration.

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 and its relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on bees of CHR/H/ETO 500 SC were not evaluated as part of the EU assessment of ethofumesate. New data submitted with this application are listed 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. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Ethofumesate	Acute Oral	LD_{50} > 50 µg a.s/bee	EFSA Journal 2016;14(1):4374
Apis mellifera	Ethofumesate	Acute Contact	LD_{50} >100 µg a.s/bee	EFSA Journal 2016;14(1):4374
Apis mellifera	CHR/H/ETO 500 SC	Oral	LD_{50} > 200 µg test item/bee	E. Kulec-Płoszczyca, Study code: B-67-20, 2020
Apis mellifera	CHR/H/ETO 500 SC	Contact	LD_{50} >200 µg test item/bee	E. Kulec-Płoszczyca, Study code: B-68-20,

Species	Substance	Exposure System	Results	Reference
				2020
Apis mellifera	CHR/H/ETO 500 SC	Larval Toxicity	LD_{50} > 100 µg test item./larva	U. Orzechowska, Study code: 0038/0008/E, 2020
Apis mellifera	CHR/H/ETO 500 SC	Chronic Oral	LD_{50} > 80.508 µg/bee/day	U. Orzechowska, Study code: 0038/0010/E, 2020
Higher-tier studies (tunnel test, field studies)				
Not required				

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).

Risk assessment have been provided for worst case situation (sugar beets, maximum single application rate of 500 g a.s/ha and 1 x 1132 g formulation/ha. ~~20 g/ha), which covered a risk envelopment for all uses of data gap.~~

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 CHR/H/ETO 500 SC in sugar beets~~/spring~~

Intended use	Sugar beets		
Active substance	Ethofumesate		
Application rate (g/ha)	2 x 500		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	50	500	10
Contact toxicity	100		5
Product	CHR/H/ETO 500 SC		
Application rate (g/ha)	2 x 1132 g		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	200	1132	5.66
Contact toxicity	200		5.66

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

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

Not relevant.

Review Comments:

Since acceptable acute risk have been concluded for bees exposed to CHR/H/ETO 500 SC at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of CHR/H/ETO 500 SC.

9.6.3 Effects on bumble bees

Not available

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for bumblebees is not required.

9.6.4 Effects on solitary bees

Not available

Review Comments:
According to SANCO/10329/2002 rev 2 final, the risk assessment for solitary bees is not required.

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that CHR/H/ETO 500 SC applied at the maximum use rate in sugar beet ~~cereals winter~~ poses low risk to bees.

Review Comments:

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final.
The risk assessment performed for active substance tribenuron-methyl and the formulated product CHR/H/ETO 500 SC is agreed by the zRMS.. All hazard quotients calculated are lower than 50, indicating that the acute oral and contact risk to bees is acceptable following the use according to the proposed use pattern of CHR/H/ETO 500 SC.

The Applicant provided chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies for CHR/H/ETO 500 SC were evaluated and accepted by zRMS but not taken to consideration in risk assessment since the evaluation was done according to SANCO/10329/2002 rev 2 final.
Concerned Member States must decide on the consideration of data requirements on national level.

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 carried out with ethofumesate and its relevant metabolites. Full details of these studies are provided in the respective EU RAR and related documents.

Effects on non-target arthropods of CHR/H/ETO 500 SC 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 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	CHR/H/ETO 500 SC	Tier I, glass plates 2D	LR_{50} = 1.7 Lproduct /ha, which is equal to 889.1 ~~1924.4~~ g a.s/ha $NOER_{mortality}$ =0.85 L product/ha	E. Kulec-Płoszczyca, Study code: B-65-20, 2020
Aphidius rhopalosiphi (adults)	CHR/H/ETO 500 SC	Tier I, glass plates 2D	LR_{50} = 1.7 L product /ha, which is equal to 889.1 ~~1924.4~~ g a.s/ha NOERmortality = ≥ 1.7 L product/ha	E. Kulec-Płoszczyca, Study code: B-66-20, 2020
Field or semi-field tests				
Not required				

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.

~~Risk assessment have been provided for worst case situation (sugar beets, 2 × 566 1 x 40 g prod/ha), which covered a risk envelopment for all uses of data gap.~~
Risk assessment was performed with the consideration of crop type, application rate and number of uses.

Table 9.7-2a: First- ~~and higher-tier~~ assessment of the in-field risk for non-target arthropods due to the use of CHR/H/ETO 500 SC in Sugar beets ~~cereals~~

Intended use	Sugar beets		
Active substance/product	CHR/H/ETO 500 SC		
Application rate (g a.s /ha)	2 × 566		
MAF	1.7		
Test species Tier I ~~II~~	**LR_{50} (lab.) (g a.s /ha)**	**$PER_{in\text{-}field}$ (g a.s /ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	889.1 ~~1924.4~~	962.2 ~~1924.4~~	1.08
Aphidius rhopalosiphi	889.1 ~~1924.4~~		1.08

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in bold breach the relevant trigger.

Table 9.7-3b: First- assessment of the in-field risk for non-target arthropods due to the use of CHR/H/ETO 500 SC in Sugar beets

Intended use	Sugar beets		
Active substance/product	CHR/H/ETO 500 SC		
Application rate (g a.s /ha)	3 × 339		
MAF	2.3		
Test species Tier I ~~II~~	**LR_{50} (lab.) (g a.s /ha)**	**$PER_{in\text{-}field}$ (g a.s /ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	889.1	779.7	0.9
Aphidius rhopalosiphi	889.1		0.9

9.7.2.1 Risk assessment for off-field exposure

Table 9.7-4a: First- ~~and higher~~ tier assessment of the off-field risk for non-target arthropods due to the use of CHR/H/ETO 500 SC in Sugar beets ~~cereals~~

Intended use	Sugar beets				
Active substance/product	CHR/H/ETO 500 SC				
Application rate (g a.s/ha)	2 x 556 ~~2 ×1132~~				
MAF	1.7 ~~1.0~~				
vdf	10 (TIER I)				
Test species Tier I ~~II~~	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	889.1 ~~1924.4~~	2.77	2.61 ~~5.33~~	10 ~~5~~	0.03 ~~0.01385~~
Aphidius rhopalosiphi	889.1 ~~1924.4~~		2.61 ~~5.33~~		0.03 ~~0.01385~~

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.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table 9.7-5b: First tier assessment of the off-field risk for non-target arthropods due to the use of CHR/H/ETO 500 SC in Sugar beets

Intended use	Sugar beets				
Active substance/product	CHR/H/ETO 500 SC				
Application rate (g a.s/ha)	3 x 339				
MAF	2.3				
vdf	10 (TIER I)				
Test species Tier I ~~II~~	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	889.1	2.3	1.8	10	0.02
Aphidius rhopalosiphi	889.1		1.8		0.02

9.7.2.2 Additional higher-tier risk assessment

Not relevant.

9.7.2.3 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

All hazard quotients (HQ) are considerably less than 2, indicating that CHR/H/ETO 500 SC applied at the maximum use rate in Sugar beets ~~cereals winter~~ poses no risk to non-target arthropods. No risk mitigation needed.

Review comments:

The evaluation of the risk assessment for non-target arthropods was performed in accordance with the recommendations of the guidance document ESCORT 2.
At TIER 1 in off-field assessment CF should be 10 not 5, the risk assessment was updated. Appropriate MAF was updated.

The HQ for recommended species: *Typhlodromus pyri* and *Aphidius rhopalosiphi* is below the ESCORT 2 trigger value of 2, indicating acceptable in-field and off-field risk to non-target arthropods already at tier I. On this basis acceptable risk for in-field and off-field habitats is concluded with no need of mitigation measures.

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 RAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/H/ETO 500 SC 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. Justifications are provided below.

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 foetida	NC 8493	Chronic (56d) Test item incorporated into the soil / 10% peat	NOEC = 100 mg/kg dry soil	EFSA Journal 2016;14(1):4374
Eisenia foetida	NC 20645	Chronic (56d) Test item incorporated into the soil / 5% peat	NOEC = 100 mg/kg dry soil	EFSA Journal 2016;14(1):4374
Falsomia candida	NC 8493	Test item incorporated into the soil / 5% peat	NOEC= 556 mg/kg dry soil	EFSA Journal 2016;14(1):4374
Falsomia candida	NC 20645	Test item incorporated into the soil / 5% peat	NOEC = 100 mg/kg dry soil	EFSA Journal 2016;14(1):4374
H. aculeifer	NC 8493	Test item incorporated into the soil / 5% peat	NOEC = 309 mg/kg dry soil	EFSA Journal 2016;14(1):4374
Eisenia Andrei	CHR/H/ETO 500 SC	Chronic (56 days)	$NOEC_{reproduction}$ = 100 mg test item/kg soil EC_{10}= 81.814 mg product kg soil	A. Gierbuszeska, Study code: G-26-20, 2020
Folsomia candida	CHR/H/ETO 500 SC	Chronic (28 days)	$NOEC_{reproduction} \geq 56$ mg/kg dry soil $EC_{10reproduction=}$ 63.80 mg/kg dry soil	A. Wróbel, Study code: G-27-20, 2020
H. aculeifer	CHR/H/ETO 500 SC	Chronic (14 days)	NOEC ≥ 1000 mg formulation/kg dry soil EC_{10} > 1000 mg formulation/kg dry soil	P. Pieczka,Study code: G-28-20, 2020
Field studies				
Not required				
Litter bag test				
Not required				

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

Review comments:

As log Pow of ethofumesate (2.7) is >2, correction of the formulation endpoints derived for *Eisenia Andrei, F. candida and H. aculeifer* were updated accordingly.

The Applicant presented new studies with the formulated product. Following conclusions were made:

Results of the study with *Eisenia Andrei* (A. Gierbuszeska, Study code: G-26-20 , 2020)

The EC_{10}= 81.814 (mg formulation/kg dry weight of the artificial soil) value from the reproduction study of the formulation to Eisenia Andrei was provided in Table 9.8-1 as being lower than the NOEC and thus more relevant for the risk assessment.

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).

Risk assessment have been provided for worst case situation (sugar beets, maximum application rate 1 x 500 g a.s/ha ~~1x 20 g/ha),~~ ~~which covered a risk envelopment for all uses of data gap.~~

9.8.2.1 First-tier risk assessment

The relevant PECsoil 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, the risk envelope approach was used for PECs assessment. The multiple application was taken into consideration (2 x 500 g a.s./ha). This application rate covers the application of 3 x 300 g a.s./ha with the same interception of 20%.

~~multi-annual accumulation in soil does not need to be considered for ethofumesate.~~

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 CHR/H/ETO 500 SC in sugar beets

Intended use	Sugar beets ~~Cereals winter~~		
Acute effects on earthworms			
Product/active substance/metabolite	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Ethofumesate	**Not required**		
NC 8493			
NC 20645			
CHR/H/ETO 500 SC			
Chronic effects on earthworms			
Product/active substance/metabolite	**NOEC/ EC_{10} (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Ethofumesate	-	1.3181	-
NC 8493	50	0.0013	38461
NC 20645	-	Not relevant in soil	-

CHR/H/ETO 500 SC	40.91 ~~50~~	1.3181	31.04 ~~37.9~~
Chronic effects on other soil macro- and mesofauna *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Ethofumesate	-	1.3181	
NC 8493	278	0.0013	213846
NC 20645	-	Not relevant in soil	-
CHR/H/ETO 500 SC	28	1.208 ~~1.3181~~	23.17 ~~21.2~~
Chronic effects on other soil macro- and mesofauna *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)***	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Ethofumesate	-	0.0213	-
NC 8493	154.5	0.0013	118 846
NC 20645	-	Not relevant in soil	-
CHR/H/ETO 500 SC	500	1.208 ~~1.3181~~	413.91 ~~379.3~~

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

Review comments:

A higher tier assessment is not required based on the low risk indicated in the chronic assessment on earthworms, collembolan, and soil mite.

9.8.3 Overall conclusions

The ~~acute and~~ long term risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for CHR/H/ETO 500 SC in a first-tier risk assessment.

Review comments:

The risk assessment for earthworms and other soil macro-organisms exposed to ethofumesate relevant metabolite NC 8493 and to formulation CHR/H/ETO 500 SC was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002) was accepted by the zRMS.

No toxicity data were available for the active substance and therefore the risk assessment was performed using the data for the formulation CHR/H/ETO 500 SC. The risk assessment for earthworms and other soil macro-organisms was also presented for relevant soil metabolites. The relevant PECsoil for risk assessments is taken from Section 8 (Environmental Fate), for details please, refer to Section 8.

TERlt values calculated for all considered compounds and CHR/H/ETO 500 SC were above the respective trigger indicating acceptable long-term risk to earthworms and other soil macro-organisms. No fur-

ther evaluation is deemed necessary.

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 RAR and related documents.
Effects on soil microorganisms of CHR/H/ETO 500 SC 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. Justifications are provided below. Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Ethofumesate	-	-	EFSA Journal 2016;14(1):4374
N-mineralisation	NC 8493	28 days	- 1.4% effect at day 28 at 1.20 mg/kg soil dw - 15.2% effect at day 28 at 12 mg/kg soil dw	EFSA Journal 2016;14(1):4374
N-mineralisation	NC 20645	28 day	6.9% effect at day 28 at 1.38 mg/kg soil dw 6.7% effect at day 28 at 13.8 mg/kg soil dw	EFSA Journal 2016;14(1):4374
N-mineralisation	CHR/H/ETO 500 SC	56 days	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils..	A. Gierbuszewska, Study code: G-29-20, 2020

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).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CHR/H/ETO 500 SC in sugar beets

Intended use	Sugar beets		
N-mineralisation			
Ethofumesate	-	1.3181	YES
NC 8493	- 1.4% effect at day 28 at 1.20 mg/kg soil dw - 15.2% effect at day 28 at 12 mg/kg soil dw	0.0013	YES
NC 20645	6.9% effect at day 28 at 1.38 mg/kg soil dw 6.7% effect at day 28 at 13.8 mg/kg soil dw	Not relevant in soil	YES
CHR/H/ETO 500 SC	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.	1.3181	YES
C-mineralisation			
Not required			

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/H/ETO 500 SC and its active substance Ethofumesate in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed after 56 days or more of exposure, indicating that the proposed use of CHR/H/ETO 500 SC poses an acceptable risk to soil microorganisms.

Review comments:

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) and was generally accepted by the zRMS.
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), for details please, refer to Section 8.
Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that CHR/H/ETO 500 SC had no significant impact on soil microorganisms when applied at test item concentrations up to 11.15 mg a.s./kg of dry weight soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

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 RAR and related documents.

Effects on non-target terrestrial plants of CHR/H/ETO 500 SC 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.

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

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

Species	Substance	Exposure System	Results	Reference
Pea Pisum sativum	CHR/H/ETO 500 SC	21 d Seedling emergence	ER50= 1207.5 g prod/ha	ETHOFUMESATE (CHR/H/ETO 500 SC) Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test; P. Pieczka; Study code: G-32-20, 2020
Carrot Daucus carota	CHR/H/ETO 500 SC	21 d Seedling emergence	ER50 = 628.6 g prod/ha	
Flax Linum usitatissimum	CHR/H/ETO 500 SC	21 d Seedling emergence	ER50= 94.3 g prod /ha	
Red clover Trifolium pratense	CHR/H/ETO 500 SC	21 d Seedling emergence	ER50= 1229.3 g prod/ha	
Onion Allium cepa	CHR/H/ETO 500 SC	21 d Seedling emergence	ER50 > 2000 g prod/ha	
Corn Zea mays	CHR/H/ETO 500 SC	21 d Seedling vigour	ER50= 657.2 g prod/ha	
Pea Pisum sativum	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER50 > 2000 g prod/ha	ETHOFUMESATE (CHR/H/ETO 500 SC) Terrestrial Plant Test: Vegetative
Carrot Daucus carota	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER50 > 2000 g prod/ha	

Species	Substance	Exposure System	Results	Reference
Flax Linum usitatissimum	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER50 > 2000 g prod/ha	Vigour Test; A. Wróbel; Study code: G-30-20, 2020
Red clover Trifolium pratense	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER50 = 942.8 g prod/ha	
Onion Allium cepa	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER50 > 2000 g prod/ha	
Corn Zea mays	CHR/H/ETO 500 SC	21 d Vegetative vigour	ER 50> 2000 g prod/ha	

m: monocotyledonous; d: dicotyledonous

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.

Risk assessment was performed with the consideration of maximum single application and drift rate.

~~Risk assessment have been provided for worst case situation (sugar beets, 2 x 1132 g/ha), which covered a risk envelopment for all uses of data gap.~~

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CHR/H/ETO 500 SC in sugar beets

Intended use		Sugar beets
Active substance/product		CHR/H/ETO 500 SC
Application rate (g formulation/ha)		~~2 x~~ 1 x 1132
~~**MAF**~~		~~1.7~~

Test species	ER_{50} (g formulation /ha)	~~Drift rate~~	Drift rate	$PER_{off\text{-}field}$ (g formulation /ha)	TER criterion: TER ≥ 5	~~$PER_{off\text{-}field}$ (g/ha)~~	~~TER criterion: TER ≥ 5~~
Pea Pisum sativum	1207.5	~~0.0238~~	0.0277	31.35	38.51	~~73.13~~	~~16.5~~
Carrot Daucus carota	628.6	~~0.0238~~	0.0277	31.35	20.05	~~73.13~~	~~8.59~~

Intended use		Sugar beets
Active substance/product		CHR/H/ETO 500 SC
Application rate (g formulation/ha)		~~2 x~~ 1 x 1132
~~**MAF**~~		~~1.7~~

Test species	**ER_{50} (g formulation /ha)**	~~**Drift rate**~~	**Drift rate**	**$PER_{off\text{-}field}$ (g formulation /ha)**	**TER criterion: TER ≥ 5**	~~**$PER_{off\text{-}field}$ (g/ha)**~~	~~**TER criterion: TER ≥ 5**~~
Flax Linum usitatissimum	94.3	~~0.0238~~	0.0277	31.35	**3.00**	~~73.13~~	~~**1.28**~~
Red clover Trifolium pratense	1229.3	~~0.0238~~	0.0277	31.35	39.20	~~73.13~~	~~16.81~~
Onion Allium cepa	2000	~~0.0238~~	0.0277	31.35	64.00	~~73.13~~	~~27.35~~
Corn Zea mays	657.2	~~0.0238~~	0.0277	31.35	21.00	~~73.13~~	~~8.99~~
Pea Pisum sativum	2000	~~0.0238~~	0.0277	31.35	63.79	~~73.13~~	~~27.35~~
Carrot Daucus carota	2000	~~0.0238~~	0.0277	31.35	63.79	~~73.13~~	~~27.35~~
Flax Linum usitatissimum	2000	~~0.0238~~	0.0277	31.35	63.79	~~73.13~~	~~27.35~~
Red clover Trifolium pratense	942.8	~~0.0238~~	0.0277	31.35	30.07	~~73.13~~	~~12.89~~
Onion Allium cepa	2000	~~0.0238~~	0.0277	31.35	63.79	~~73.13~~	~~27.35~~
Corn Zea mays	2000	~~0.0238~~	0.0277	31.35	63.79	~~73.13~~	~~27.35~~

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

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 9.10-3a: Risk assessment for non-target terrestrial plants due to the use of CHR/H/ETO 500 SC in sugar beets considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Sugar beets			
Active substance/product		CHR/H/ETO 500 SC			
Application rate (g formulation/ha)		1 ×1132 ~~20~~			
MAF		1			
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)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.77	31.35	15.67	7.83	3.13
5	0.57	6.45	3.22	1.61	0.64
~~1~~	~~2.38~~	~~73.13~~	~~36.57~~	~~18.28~~	~~7.31~~
~~5~~	~~0.47~~	~~9.05~~	~~4.53~~	~~2.26~~	~~0.905~~
Toxicity value		**TER**			
ER_{50} = 94.3 g/ha		**criterion: TER ≥ 5**			
1		3.0	**6.01**	**12.04**	**30.12**
5		**14.62**	-	-	-
~~1~~		~~**1.28**~~	~~**2.58**~~	~~5.16~~	~~12.90~~
~~5~~		~~10.42~~	~~20.82~~	~~41.73~~	~~104.20~~

~~MAF: Multiple application factor;~~ PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

Table 9.10-4b: Risk assessment for non-target terrestrial plants due to the use of CHR/H/ETO 500 SC in sugar beets considering risk mitigation

Intended use		Sugar beets			
Active substance/product		CHR/H/ETO 500 SC			
Application rate (g formulation/ha)		1 ×–678			
MAF		1			
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)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.77	18.7	9.34	-	-
5	0.57	3.86	-	-	-
Toxicity value		**TER**			
ER_{50} = 94.3 g/ha		**criterion: TER ≥ 5**			
1		**5.04**	**10.09**	**-**	**-**
5		**24.43**	**-**	**-**	**-**

9.10.3 Overall conclusions

Based on the predicted rates of CHR/H/ETO 500 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to CHR/H/ETO 500 SC according to the GAP of the formulation CHR/H/ETO 500 SC achieve the acceptability criteria TER ≥ 5 with applying:

For two applications in sugar beet:

- 5 m buffer zone or,
- 1 m and use of 50% drift reducing nozzles to non-agricultural land
- ~~1 m and use of 75% drift reducing nozzles~~
- ~~1 m and use of 90% drift reducing nozzles~~

Review comments:

Risk assessment performed by the Applicant for non-target terrestrial plants was updated and accepted.

No MAF values should be used by default, until a guidance document is developed." This approach is in line with the "Guidance Document on Terrestrial Ecotoxicology" currently in use which does not require the use of a MAF value in the context of NTTP risk assessment. Thus, it is not deemed necessary to apply a MAF when calculating the PER.

Based on the predicted rates of CHR/H/ETO 500 SC in off-field areas, the TER values describing the risk for non-target plants following exposure to formulation according to the GAP achieve the acceptability criteria TER ≥ 5. Following risk mitigation measures should be applied:

For two applications in sugar beet:

- unsprayed buffer zone of 1 m with 50% drift reducing technology or
- unsprayed buffer zone of 5 m without drift reduction technology to non-agricultural land.

For three applications in sugar beet:

- No mitigation measures are required

Concerned Member States must decide on the applicability of indicated risk mitigation measures at the product authorization.

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

Not relevant

9.12 Monitoring data (KCP 10.8)

Not available

9.13 Classification and Labelling

Having considered studies, the following ecotoxicological classification of CHR/H/ETO 500 SC is proposed:

Classification:

Aquatic Acute 1, H400
Aquatic Chronic 1, H410

In accordance with indication of Regulation 1272/2008, when formulation is classified as H410, hazard statement regarding acute classification (i.e. H400) may be omitted.

Hazard statement:
H410 – Very toxic to aquatic life with long lasting effects.

Pictogram:

GHS09

Signal word:
WARNING

Precautionary statement:
P391: Collect spillage
P501: Dispose of contents/container in accordance with local regulation
EUH401: To avoid risks to man and the environment, comply with the instructions for use

Ethofumesate is not readily biodegradable.
The hazard statement of H410 is based on chronic toxicity data on Myriophyllum spicatum for ethofumesate (NOEC = 0.036 mg a.s. /L); and that the active substance is not document-ed readily biode-gradable.

SP 1: Do not contaminate water with the product or its container (Do not clean application equipment near surface water/Avoid contamination via drains from farmyards and roads).
SPe3: Sugar beet - To protect aquatic organisms respect 1 m non-spray buffer zone to surface water bodies.
SPe3: Sugar beet – To protect non target terrestrial plants respect an unsprayed buffer zone of 1 m with 50% drift reducing technology or an unsprayed buffer zone of 5 m without drift reduction technology to non-agricultural land.

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 10.1.1	**K. Florynski**	2021	**Ethofumesate - TER Calculations for Terrestial Verterbrates** Chemirol GLP No Unpublished	N	Chemirol
KCP 10.1.2	**K. Florynski**	2021	**Ethofumesate - TER Calculations for Terrestial Verterbrates** Chemirol GLP No Unpublished	N	Chemirol
KCP 10.2/01	**E. Malada**	2020	CHR/H/ETO 500 SC Daphnia magna, Acute Immobilisation Test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: W-33-20 GLP Unpublished	N	Chemirol
KCP 10.2/02	**E. Malada**	2020	CHR/H/ETO 500 SC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: W-34-20 GLP Unpublished	N	Chemirol
KCP 10.2/03	**M. Czarnecka**	2020	CHR/H/ETO 500 SC Anabaena flos-aquae UTEX B 1444 Growth inhibition test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-toxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: W-36-20 GLP Unpublished	N	Chemirol

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 10.2/04	**M. Czarnecka**	2020	CHR/H/ETO 500 SC Lemna gibba CPCC 310, Growth inhibition test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-toxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: W-35-20 GLP Unpublished	N	Chemirol
KCP 10.3.1/01	**E. Kulec-Ploszczyca**	2020	CHR/H/ETO 500 SC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-toxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: B-67-20 GLP Unpublished	N	Chemirol
KCP 10.3.1/02	**E. Kulec-Ploszczyca**	2020	CHR/H/ETO 500 SC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-toxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: B-68-20 GLP Unpublished	N	Chemirol
KCP 10.3.1/03	**U. Orzechowska**	2020	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239 STUDY CODE: 0038/0008/E; SORBOLAB Research Laboratory LLC, Zaniemyska 11 Street,61-029 Poznań GLP Yes Published No	N	Chemirol
KCP 10.3.1/04	**U. Orzechowska**	2020	Honey Bee, Chronic Oral Toxicity Test according to OECD 245 STUDY CODE: 0038/0010/E SORBOLAB Research Laboratory LLC, Zaniemyska 11 Street,61-029 Poznań GLP Yes Published No	N	Chemirol
KCP 10.3.2/01	**E. Kulec-Płoszczyca**	2020	A laboratory test for evaluating the effects of CHR/H/ETO 500 SC on the predatory mite, Typhlodromus pyri (Sch.). Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-toxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code B-65-20 GLP	N	Chemirol

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
			Unpublished		
KCP 10.3.2/02	**E. Kulec-Płoszczyca**	2020	A laboratory test for evaluating the effects of CHR/H/ETO 500 SC on the parasitic wasp, Aphidius rhopalosiphi (De Stephani - Perez). Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code B-66-20 GLP Unpublished	N	Chemirol
KCP 10.4/01	**Gierbuszewska A.**	2020	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Earthworm reproduction test (Eisenia Andrei) Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code: G -26-20 GLP Unpublished	N	Chemirol
KCP 10.4/02	**A. Wróbel**	2020	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Collembolan (Folsomia candida) Reproduction Test, STUDY CODE: G-27-20; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Yes Published No	N	Chemirol
KCP 10.4/03	**P. Pieczka**	2020	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil STUDY CODE: G-28-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Yes Published No	N	Chemirol
KCP 10.5/01	**Gierbuszewska A.**	2010	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Soil Microorganisms: Nitrogen Transformation Test Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland Study Code G-29-20 GLP Unpublished	N	Chemirol
KCP	**P. Pieczka**	2020	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Terrestial Plant Test: Seedling Emergence and Seedling Growth	N	Chemirol

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
10.6/01			Test Study code: G-31-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Yes Published No		
KCP 10.6/02	**A. Wróbel**	2020	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Terrestial Plant Test: Vegetative Vigour Test STUDY CODE: G-30-20 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Depart-ment of Eco-tox-icological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland GLP Yes Published No	N	Chemirol

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1/01		1990	THE ACUTE ORAL TOXICITY (LD50) OF ETHOFUMESATE TO THE MALLARD DUCK Bayer CropScience, Report no.: A87610, Edition Number: M-161543-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/02		1990	THE ACUTE ORAL TOXICITY (LD50) OF ETHOFUMESATE TO THE BOBWHITE QUAIL Bayer CropScience, Report no.: A87612, Edition Number: M-161547-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/03		1977	THE ACUTE ORAL TOXICITY (LD50) OF NC 8438 TO THE MALLARD DUCK Bayer CropScience, Report no.: A83331, Report includes Trial Nos.: FPL 245 WL/77937	Y	Bayer CropScience

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
			Edition Number: M-155600-01-1 GLP/GEP: no, unpublished		
KCP 10.1/04		1977	ACUTE ORAL TOXICITY (LD50) OF NC8438 TO THE BOBWHITE QUAIL Bayer CropScience, Report no.: A83330, Report includes Trial Nos.: FPL 245 WL/77934 Edition Number: M-155599-01-1 GLP/GEP: no, unpublished	Y	Bayer CropScience
KCP 10.1/05		1994	TF- Ethofumesate, Report no.: M-468479-01-1, Edition Number: M-468479-01-1 GLP/GEP: no, unpublished	Y	Bayer CropScience
KCP 10.1/06		1991	TECHNICAL ETHOFUMESATE: SUBACUTE DIETARY TOXICITY (LC 50) TO MALLARD DUCK Bayer CropScience, Report no.: A83367, Report includes Trial Nos.: SMS 269/91303 TOX 90539 Edition Number: M-155635-01-1 EPA MRID no.: 41949202 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/07		1990	THE DIETARY TOXICITY (LC50) OF ETHOFUMESATE TO THE MALLARD DUCK Bayer CropScience, Report no.: A87611, Edition Number: M-161545-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/08		1991	TECHNICAL ETHOFUMESATE: SUBACUTE DIETARY TOXICITY (LC 50) TO BOBWHITE QUAIL Bayer CropScience, Report no.: A83369, Report includes Trial Nos.: SMS 268/91302 TOX 90538 Edition Number: M-155637-01-1	Y	Bayer CropScience

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
			EPA MRID no.: 41949201 GLP/GEP: yes, unpublished		
KCP 10.1/09		1990	THE DIETARY TOXICITY (LC50) OF ETHOFUMESATE TO THE BOBWHITE QUAIL Bayer CropScience, Report no.: A87613, Edition Number: M-161549-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/10		1994	Effects on reproduction TF- Ethofumesate, Report no.: M-468481-01-1, Edition Number: M-468481-01-1 Date: 1994-12-01 GLP/GEP: no, unpublished	N	Adama (formerly Feinchemie Schwebda)
KCP 10.1/11		2001	Bobwhite quail dietary reproduction study Ethofumesate Code: AE B049913 00 1D97 0002 Bayer CropScience, Report no.: C013708, Report includes Trial Nos.: 1999.0060 Edition Number: M-205119-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.1/12		2000	Mallard duck dietary reproduction study Ethofumesate Code: AE B049913 00 1D97 002 ., Bayer CropScience, Report no.: C008193, Report includes Trial Nos.: TOX99077 Edition Number: M-197270-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/01		1991	THE ACUTE TOXICITY OF [14C]- ETHOFUMESATE TO BLUEGILL SUNFISH (Lepomis macrochirus) UNDER SEMISTATIC CONDITIONS Bayer CropScience,	Y	Bayer CropScience

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
			Report no.: A83373, Report includes Trial Nos.: 86B Edition Number: M-155641-01-1 EPA MRID no.: 42015501 GLP/GEP: yes, unpublished		
KCP 10.2/02		1990	DETERMINATION OF ACUTE TOXICITY (LC50) TO RAINBOW TROUT (96H, SEMISTATIC) Ethofumesate Bayer CropScience, Report no.: A87614, Report includes Trial Nos.: 141714 Edition Number: M-161551-01-1 EPA MRID no.: 46546301 GLP/GEP: yes, unpublished	Y	Bayer CropScience
Kcp 10.2/03		1990	DETERMINATION OF ACUTE TOXICITY (LC50) TO BLUEGILL SUNFISH (96H, SEMI-STATIC) Ethofumesate Bayer CropScience, Report no.: A87615, Report includes Trial Nos.: 141709 Edition Number: M-161552-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/04		1989	TECHNICAL ETHOFUMESATE - DETERMINATION OF ACUTE TOXICITY (LC50) TO MIRROR CARP (96 HOURS, SEMISTATIC) AND THE ANALYSIS OF ETHOFUMESATE IN WATER SAMPLES Bayer CropScience, Report no.: A83349, Report includes Trial Nos.: 140438 79B Edition Number: M-155618-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/05		1992	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL TO THE SHEEPSHEAD MINNOW (Cyprinodon variegatus) IN A STATIC SYSTEM Bayer CropScience, Report no.: A83384, Edition Number: M-155652-01-1	Y	Bayer CropScience

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
			EPA MRID no.: 42409301 GLP/GEP: yes, unpublished		
KCP 10.2/06		1991	Acute toxicity in rainbow trout (Salmo Gairdneri) test article: Ethofumesate Feinchemie Schebda , Report no.: OFC00004887, Edition Number: M-352116-01-1 GLP/GEP: yes, unpublished	Y	Adama (formerly Feinchemie Schwebda)
KCP 10.2/07		1993	Feinchemie Schebda , Report no.: OFC00004888, Edition Number: M-352126-01-1 GLP/GEP: yes, unpublished	Y	Adama (formerly Feinchemie Schwebda)
KCP 10.2/08		1990	STUDY OF THE PROLONGED TOXICITY TO FISH (Salmo gairdneri) OF ETHOFUMESATE TECHNICAL Bayer CropScience, Report no.: A83355, Report includes Trial Nos.: 78B BE-ET-12-89-02-F1P- 2 Edition Number: M-155624-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/09		1991	Prolonged toxicity test in rainbow trout (Salmo Gairdneri) – Test article: Ethomumesate Feinchemie Schebda , Report no.: OFC00004889, Edition Number: M-352123-01-1 GLP/GEP: yes, unpublished	Y	Adama (formerly Feinchemie Schwebda)
KCP 10.2/10		1993	21-DAY PROLONGED TOXICITY STUDY IN THE RAINBOW TROUT UNDER FLOW-THROUGH CONDITIONS Ethofumesate Bayer CropScience, Report no.: A87616, Edition Number: M-161553-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/11		2013	Ethofumesate technical: Statistical Re-evaluation of the fish early life stage toxicity study with fathead Minnow (Pimephales promelas) by 1991 Bayer CropScience	Y	TaskForce Ethofumesate

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
			Bayer CropScience, Report no.: M-470756-01-1, Edition Number: M-470756-01-1 GLP/GEP: n.a., unpublished		
KCP 10.2/12		1991	ETHOFUMESATE – FATHEAD MINNOW (Pimephales promelas) EARLY LIFE STAGE TOXICITY TEST Bayer CropScience, Report no.: A83372, Edition Number: M-155640-01-1 EPA MRID no.: 42008901 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/13		2013	Zebra fish (Danio rerio), life cycle test, flow through conditions - Ethofumesate Bayer CropScience, Report no.: BAY-035/4-60/A, Edition Number: M-464613-01-1 GLP/GEP: yes, unpublished	Y	TaskForce Ethofumesate
KCP 10.2/14		1991	DETERMINATION OF THE ACCUMULATION AND ELIMINATION OF [14C]- ETHOFUMESATE IN BLUE-GILL SUNFISH (Lepomis macrochirus L.) Bayer CropScience, Report no.: A83371, Report includes Trial Nos.: 83B Edition Number: M-155639-01-1 EPA MRID no.: 41970704 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/15		1992	BIOACCUMULATION TEST IN BLUEGILL SUNFISH 14CEthofumesate Bayer CropScience, Report no.: A87617, Report includes Trial Nos.: 141541 Edition Number: M-161555-01-1 GLP/GEP: yes, unpublished	Y	Bayer CropScience
KCP 10.2/18	Koenig, N.	2013	Acute toxicity of ethofumesate acetic acid to the waterflea Daphnia magna in a static laboratory test system - Limit test Bayer CropScience, Report no.: EBADN008,	N	TaskForce Ethofumesate

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
			Edition Number: M-444843-01-1 GLP/GEP: yes, unpublished		
KCP 10.2/19	Riebschlaeger, T.	2012	Acute toxicity of BCS-BB94377 (tech.) to the waterflea Daphnia magna in a static-renewal laboratory test system - Limit test - Amendment 1 to report Bayer CropScience, Report no.: EBADL039, Edition Number: M-434284-02-1 Date: 2012-06-27 ...Amended: 2013-03-18 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/20	Riebschlaeger, T.	2012	Acute toxicity of BCS-AV65501 (tech.) to the waterflea Daphnia magna in a static-renewal laboratory test system - Limit test - Amendment 1 to report Bayer CropScience, Report no.: EBADL038, Edition Number: M-434289-02-1 Date: 2012-06-27 ...Amended: 2013-03-18 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/21	Thun, S.	1993	Acute toxicity in Daphnia Magna - Test article: Ethofumesate techn. IBR Forschungs GmbH, Walsrode, Germany Feinchemie Schebda , Report no.: 80-91-2312-02-93, Edition Number: M-352128-01-1 GLP/GEP: yes, unpublished	N	Adama (formerly Feinchemie Schweb-da)
KCP 10.2/22	Schupner, J.K.; Stachura, B.J.	1992	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL TO THE MYSID SHRIMP Mysidopsis bahia IN A STATIC SYSTEM Nor-Am Chemical Company, Pikeville, NC, USA Bayer CropScience, Report no.: A83389, Edition Number: M-155657-01-1 EPA MRID no.: 42364502 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.2/22	Adema, D.M.M., de Ruiter, A.	1989	THE CHRONIC TOXICITY OF ETHOFUMESATE TO Daphnia magna TNO;	N	Bayer CropScience

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
			Bayer CropScience, Report no.: A83345, Report includes Trial Nos.: 70B Edition Number: M-155614-01-1 EPA MRID no.: 41554103 GLP/GEP: yes, unpublished		
KCP 10.2/23	Bellmann, W.	1992	21 d Daphnia-reproduction test according to OECD guideline 202, part II - Test article ethofumesate Technischer Ueberwachungsverein, Filderstadt, Germany Feinchemie Schebda , Report no.: OFC00004891, Edition Number: M-352134-01-1 GLP/GEP: yes, unpublished	N	Adama (formerly Feinchemie Schweb-da)
KCP 10.2/23	Douglas, M. T.; James, C.M.; McDonald, I. A.	1990	AN ASSESSMENT OF THE EFFECTS OF ETHOFUMESATE ON THE REPRODUCTION OF Daphnia magna Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87619, Edition Number: M-161558-01-1 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.2/24	Mattock, S.D.	1998	Chronic toxicity to the sediment dwelling organism Chironomus riparius (BBA method) Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report no.: A91783, Report includes Trial Nos.: 194/183 Envir 208B Edition Number: M-168438-01-1 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.2/26	Bruns, E.	2008	Pseudokirchneriella subcapitata growth inhibition test withethofumesate (techn.) Bayer CropScience, Report no.: EBADL004,	N	TaskForce Ethofumesate

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
			Edition Number: M-302092-03-1 Date: 2008-06-04 ...Amended: 2010-02-16 GLP/GEP: yes, unpublished		
KCP 10.2/27	Bruns, E.	2012	Pseudokirchneriella subcapitata growth inhibition test with ethofumesate-NC8493 (AE C508493) Bayer CropScience, Report no.: EBADL032, Edition Number: M-436372-01-1 Date: 2012-08-03 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/28	Bruns, E.	2012	Amendment no.1- Pseudokirchneriella subcapitata growth inhibition test with BCSAV65501 - limit test Bayer CropScience, Report no.: EBADL035, Edition Number: M-437568-02-1 Date: 2012-08-27 ...Amended: 2012-10-08 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/31	Sobczyk, H.	2013	Pseudokirchneriella subcapitata growth inhibition test with BCSCW35117 Bayer CropScience, Report no.: E 323 4457-8, Edition Number: M-459906-01-1 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/31	Banman, C. S.; Daly, R. A.; Lam, C. V.	2009	Toxicity of ethofumesate technical to the blue green algae Anabaena flos-aquae Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL008, Edition Number: M-349150-01-1 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/32	Banman, C. S.; Daly, R. A.; Lam, C. V.	2009	Toxicity of ethofumesate technical to the saltwater diatom Skeletonema costatum Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL009,	N	TaskForce Ethofumesate

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
			Edition Number: M-347965-01-1 GLP/GEP: yes, unpublished		
KCP 10.2/33	Banman, C.S.	2011	Toxicity of ethofumesate technical to the aquatic macrophyte, Myriophyllum spicatum (amended final report) Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL019-1, Edition Number: M-411454-02-1 Date: 2011-07-25 ...Amended: 2013-05-22 GLP/GEP: yes, unpublished	N	TaskForce Ethofumesate
KCP 10.2/35	Scheerbaum, D.	1998	Ethofumesate – Substance technical 98.8 percent w/w - Lemna minor: Semi static phytotoxicity test - Code: AE B049913 00 1D97 0002 Dr. U. Noack-Laboratorium fuer Angewandte Biologie, Sarstedt, Germany Bayer CropScience, Report no.: A91865, Report includes Trial Nos.: ENVIR/211B TLA5699- TLA56991 Edition Number: M-168516-01-1 Date: 1998-05-28 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.2/36	Papadopoulou-Mourkidou, E.; Vassiliou, G.; Vryzas, Z.; Alexoudis, C.; Galanis, K.	2011	Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in northeastern Greece. Journal:Ecotoxicol. Environ. Saf., Volume:74, Issue:2, Pages:174-181, Year:2011, Report no.: M-458635-01-1, Edition Number: M-458635-01-1	N	LIT

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
			Date: 2011-12-31 GLP/GEP: no, published		
KCP 10.2/37	Yurk, J. J.; Ache, B. W.	1992	EFFECT OF ETHOFUMESATE TECHNICAL ON NEW SHELL GROWTH IN THE EASTERN OYSTER (Crassostrea virginica) UNDER FLOW-THROUGH TEST CONDITIONS Environmental Science and Engineering, Inc., Gainsville, FL, USA Bayer CropScience, Report no.: A83386, Report includes Trial Nos.: 507B Edition Number: M-155654-01-1 EPA MRID no.: 42388101 Date: 1992-05-28 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.3/01	Barrett, K.L.	1991	THE ACUTE ORAL AND TOPICAL TOXICITIES OF ETHOFUMESATE TO WORKER HONEYBEES (Apis mellifera L) Schering AG, Berlin, Germany Bayer CropScience, Report no.: A83374, Report includes Trial Nos.: 87B Edition Number: M-155642-01-1 EPA MRID no.: 41970703 Date: 1991-07-09 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.3/02	Cole, J.H.	1990	THE ACUTE CONTACT AND ORAL TOXICITY TO HONEY BEES OF ETHOFUMESATE TECHNICAL Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87621, Edition Number: M-161561-01-1 Date: 1990-02-19 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.3/03	Schmitzer, S.	2011	Effects of ethofumesate tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany	N	TaskForce Ethofumesate

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
			Bayer CropScience, Report no.: 64231035, Edition Number: M-421681-01-1 Date: 2011-12-19 GLP/GEP: yes, unpublished		
KCP 10.3/04	Barrett, K. L.	1991	THE ACUTE ORAL AND TOPICAL TOXICITIES OF ETHOFUMESATE TO WORKER HONEYBEES (Apis mellifera L) Schering AG, Berlin, Germany Bayer CropScience, Report no.: A83374, Report includes Trial Nos.: 87B Edition Number: M-155642-01-1 EPA MRID no.: 41970703 Date: 1991-07-09 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.3/05	Cole, J.H.	1990	THE ACUTE CONTACT AND ORAL TOXICITY TO HONEY BEES OF ETHOFUMESATE TECHNICAL Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87621, Edition Number: M-161561-01-1 Date: 1990-02-19 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.3/06	Schmitzer, S.	2011	Effects of ethofumesate tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the Laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report no.: 64231035, Edition Number: M-421681-01-1 Date: 2011-12-19 GLP/GEP: yes, unpublished	N	Task ForceE-thofumesae
KCP	Barrett, K. L.;	1986	DETERMINATION OF THE TOXICITY OF ETHOFUMESATE IN EARTHWORMS (Eisenia	N	Bayer CropScience

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
10.4/01	Arnold, D. J.		andreii) USING AN ARTIFICIAL SOIL TEST FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report no.: A83286, Report includes Trial Nos.: 61B Edition Number: M-155555-01-1 Date: 1986-02-18 GLP/GEP: no, unpublished		
KCP 10.4/02	Hakin, B.; Rodgers, M. H.; Johnson, A. J.	1991	THE ACUTE TOXICITY (LC50) OF ETHOFUMESATE TO THE EARTHWORM (Eisenia foetida) Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87622, Edition Number: M-161563-01-1 Date: 1991-03-27 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.4/04	Friedrich, S.	2012	AE C508493: Effects on the reproduction of the collembolans Folsomia candida BioChem agrar GmbH, Gerichshain, Germany TF- Ethofumesate, Report no.: 12 10 48 018 S, Edition Number: M-435155-01-1 Date: 2012-04-05 GLP/GEP: yes, unpublished	N	Task Force Ethofumesate
KCP 10.5/01	Aldred, D.	1993	A LABORATORY ASSESSMENT OF THE EFFECTS OF ETHOFUMESATE ON SOIL MICROFLORA RESPIRATION Euro Laboratories Ltd., Buckinghamshire, United Kingdom Bayer CropScience, Report no.: A83392, Report includes Trial Nos.: 96B Edition Number: M-155660-01-1 Date: 1993-06-07 GLP/GEP: yes, unpublished	N	Bayer CropScience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.5/02	Hossack, D. J. N.; Thomas, F. J.; Chanter, D. O.	1991	THE EFFECT OF ETHOFUMESATE ON SOIL MICRO-FLORA RESPIRATION Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87660, Edition Number: M-161631-01-1 Date: 1991-01-03 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.5/03	Voets, J. P.; Angerosa, I. M. O.; Goddeeris, H.; Verstraete, W.	1977	The influence of pyrazon, ethofumesate and metamitron on the soil microbiota Publisher:Magyar Tudoma´nyos Akade´mia., Location:Hungary, Journal:Acta Phytopathologica Academiae Scientiarum Hungaricae, Volume:12, Issue:1-2, Pages:31-39, Year:1977, Report no.: Lit. 2128, Edition Number: M-155551-01-2 Date: 1977-01-01 GLP/GEP: n.a., published	N	
KCP 10.5/04	Vonk, J. W.	1988	EFFECT OF ETHOFUMESATE ON NITROGEN TRANSFORMATIONS IN SOIL TNO Division of Technology for Society, Delft, Netherlands Bayer CropScience, Report no.: A87623, Edition Number: M-161564-01-1 Date: 1988-02-22 GLP/GEP: yes, unpublished	N	Bayer CropScience
KCP 10.5/07	Castillo, M. J.	1979	EFFECTS OF TECHNICAL ETHOFUMESATE ON THE PERFORMANCE OF ACTIVATED SLUDGE PROCESS Union Carbide Corporation, USA Bayer CropScience, Report no.: A83275, Edition Number: M-155544-01-1 Date: 1979-02-01 GLP/GEP: no, unpublished	N	Bayer CropScience

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

Review Comment:
In order to provide sufficient detail, where appropriate, the following studies summaries have been adapted by the zRMS. Details were taken directly from the full studies reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No additional studies were performed.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional studies were performed.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No additional studies were performed.

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

Summarised in Section 6 (Mammalian Toxicology)

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional studies were performed.

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

No additional studies were performed.

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.1.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. In the definitive test the validity criteria were met according to OECD Guideline No. 202. No deviations from the guideline were noted during the study. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations. Following endpoints based on nominal test item concentrations would be used for risk assessment purposes: EC_{50}/48 h is 191.7 mg/L. LOEC/48 h value is 103.3 mg/L. NOEC/48 h value is 47.0 mg/L.

Reference:	KCP 10.2/01
Report	CHR/H/ETO 500 SC Daphnia magna ACUTE IMMOBILIZATION TEST, E. Malada; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W-33-20, 2020
Guideline(s):	According to OECD Guideline No 202/EU test method C.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/ETO 500 SC; batch no. PFORM03, content of ethofumesate is 523.0 g/L; production date: April, 2020, expiry date: April, 2022.
Test Species:	Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna..
Test Design:	Static test (48 h of exposure); 4 replicates per test item concentration and the control; 5 Daphnia magna in each replicate.
Endpoints:	EC50/48 h, LOEC/48h and NOEC/48h.
Test Concentration:	500.0, 227.3, 103.3, 47.0, 21.3, 9.7 mg/L plus the control..
Test Conditions:	Temperature: 19.6 – 20.0°C; pH of the control: 7.31 – 7.55; dissolved oxygen concentration in the control: 7.9 – 8.4 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no

feeding; no aeration; medium: Elendt M7.

Results and discussion:

Immobilisation of Daphnia magna exposed to the test item, CHR/H/ETO 500 SC, was investigated during a 48-hour static test. The test was performed in glass beakers of 150 mL capacity, containing 100 mL of either the test item concentration or the control per replicate. The definitive test was performed with a test item concentrations of 500.0, 227.3, 103.3, 47.0, 21.3 and 9.7 mg/L plus the control.
The Daphnia magna were observed for immobilisation after 24 h and 48 h of exposure. The Daphnia magna were considered immobile if they showed no ability to swim within 15 seconds after gentle swirling of the test vessel.
In the test item concentrations of 47.0, 21.3, 9.7 mg/L and the control no immobilisation of Daphnia magna was observed during exposure. At exposure termination in the test item concentrations of 500, 227.3 and 103.3 mg/L immobilisation of Daphnia magna was 85, 60 and 30%, respectively.
The concentrations of ethofumesate were determined using a validated liquid chromatographic method with DAD detection. Samples of all the test item concentrations and the control were analysed at exposure initiation and at exposure termination.
In samples at exposure initiation the determined concentration of ethofumesate was in the range of 92.8 – 100.3% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly.
In samples at exposure termination the determined concentration of ethofumesate was in the range of 95.1 – 101.8% of the nominal concentration. Therefore, the concentration of ethofumesate was stable under test conditions.
The endpoint values were determined based on nominal test item concentration..

Preliminary non-GLP test

In the first preliminary test the recorded temperature was in a range of 20.9 – 22.5°C. In the second preliminary test the recorded temperature was in a range of 21.8 – 22.4°C. In the first preliminary test the measured pH values were in the ranges of 7.50 – 7.62 at exposure initiation and in the range of 7.46 – 7.57 at exposure termination. The measured dissolved oxygen concentrations at exposure initiation were in the ranges of 8.9 – 9.4 mg/L and were in the range of 8.6 – 8.9 mg/L at exposure termination.

Table 3. pH values and dissolved oxygen concentrations, first preliminary test (non-GLP)

Nominal test item concentration [mg/L]	pH values		Dissolved oxygen concentrations [mg/L]	
	at exposure initiation#	at exposure termination*	at exposure initiation#	at exposure termination*
Control	7.50	7.50	8.9	8.8
0.1	7.59	7.55	9.4	8.9
1.0	7.57	7.52	9.0	8.9
10	7.59	7.57	9.2	8.7
100	7.62	7.46	9.1	8.6

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates

In the second preliminary test measured pH values at exposure initiation were in the ranges of 7.48 – 7.54 and were in the range of 7.69 – 8.16 at exposure termination. The measured dissolved oxygen concentrations at exposure initiation were in the ranges of 8.6 – 8.7 mg/L and were in the range of 8.40 – 7.83 mg/L at exposure termination.

Table 4. pH values and dissolved oxygen concentrations, second preliminary test (non-GLP)

Nominal test item concentration [mg/L]	pH values		Dissolved oxygen concentrations [mg/L]	
	at exposure initiation#	at exposure termination*	at exposure initiation#	at exposure termination*
Control	7.50	8.16	8.6	8.40
62.5	7.48	7.90	8.6	8.17
125	7.50	7.85	8.7	8.29
250	7.50	7.80	8.6	8.20
500	7.53	7.74	8.6	7.98
1000	7.54	7.69	8.7	7.83

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates

In the first preliminary test, in concentrations of 100 mg/L, immobilisation of Daphnia magna was observed after 48 h of exposure. In the control and in the remaining test item concentrations no immobilisation of Daphnia magna was observed during exposure. In the second preliminary test, in concentrations of 1000, 500, 250, 125 and 62.5 mg/L, immobilisation of Daphnia magna was observed after 48 h of exposure. In the control no immobilisation of Daphnia magna was observed during exposure.

Table 5. Immobilisation of *Daphnia magna*, first preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
0.1	20	0	0	0	0	0	0	0	0	0	0
1.0	20	0	0	0	0	0	0	0	0	0	0
10	20	0	0	0	0	0	0	0	0	0	0
100	20	1	0	1	0	1	0	2	0	10	15

Time of exposure: 11.08.2020 – 13.08.2020

Table 6. Immobilisation of Daphnia magna, second preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
62.5	20	1	1	1	0	1	1	1	0	15	15
125	20	2	1	2	1	3	3	3	5	30	70
250	20	3	3	3	5	3	5	4	5	70	85
500	20	3	3	4	3	5	5	4	4	65	90
1000	20	4	5	3	4	5	5	5	5	80	100

Time of exposure: 23.09.2020 – 25.09.2020

Results of chemical determinations

In the first preliminary test, the concentrations of ethofumesate were determined with a validated liquid chromatographic method with DAD detection. Samples of the test item concentration of 10 mg/L and the control were analysed at exposure initiation and at exposure termination. At exposure initiation the determined concentration of ethofumesate in the test item concentration of 10 mg/L was 105.0% of the nominal concentration. At exposure termination the determined concentration of ethofumesate was 104.0% of the nominal concentration. Therefore, the concentration of ethofumesate was stable under test conditions. Based on the chemical analyses results the definitive test was performed as a static test.

Definitive test

In the definitive test, the recorded temperature during exposure was in the range of 19.6 – 20.0°C and constant within 0.4ºC. The measured pH values were in the ranges of 7.28 – 7.36 at exposure initiation and in the range of 7.36 – 7.59 at exposure termination. The measured dissolved oxygen concentrations were in the ranges of 8.3 – 8.4 mg/L at exposure initiation and in the range of 7.9 – 8.2 mg/L at exposure termination.
In the test item concentrations of 47.0, 21.3, 9.7 mg/L and in the control no immobilisation of Daphnia magna was observed during exposure. At exposure termination in the test item concen-

trations of 500, 227.3 and 103.3 mg/L immobilisation of Daphnia magna was 85, 60 and 30%, respectively. In the test item concentrations of 500, 227.3 and 103.3 mg/L after 48h of exposure Daphnia magna were covered with a white precipitate. The immobilisation of Daphnia magna after 24 h and 48 h of exposure.

Table 8. Immobilisation of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
9.7	20	0	0	0	0	0	0	0	0	0	0
21.3	20	0	0	0	0	0	0	0	0	0	0
47.0	20	0	0	0	0	0	0	0	0	0	0
103.3	20	1	1	1	2	1	2	1	2	25	30
227.3	20	1	3	3	1	2	4	3	3	40	60
500	20	3	3	4	3	4	4	4	5	65	85

Time of exposure: 27.10.2020 – 29.10.2020

Results of chemical determinations

The concentrations of ethofumesate were determined using a validated liquid chromatographic method with DAD detection. Samples of all the test item concentrations and the control were analysed at exposure initiation and at exposure termination**.**

Table 9. Concentration and stability of ethofumesate, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of ethofumesate in the test item [mg/L]	Average determined concentration of ethofumesate (n=3) in samples collected [mg/L]			
		at exposure initiation	% of nominal concentration	at exposure termination	% of nominal concentration
Control	---	<LoD	---	<LoD	---
9.7	4.489	4.496	100.2	4.571	101.8
21.3	9.858	9.883	100.3	9.926	100.7
47.0	21.752	21.555	99.1	21.719	99.8
103.3	47.807	45.615	95.4	46.541	97.4
227.3	105.194	97.663	92.8	113.328	107.7
500	231.400	219.379	94.8	220.031	95.1

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
--- no value

In samples at exposure initiation the determined concentration of ethofumesate was in the range of 92.8 – 100.3% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. In samples at exposure termination the determined concentration of ethofumesate was in the range of 95.1 – 107.7% of the nominal concentration. Therefore, the concentration of ethofumesate was stable under test conditions.

Endpoint values

The endpoint values were determined based on the nominal test item concentrations [1]. The endpoint values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analysis. To make calculations and to conduct statistical analysis, the ToxRat Professional commercial software was used (Appendix 1) [7], [SOP/W/68].
The median concentration causing 50% immobilisation of Daphnia magna after 48 h of exposure, i.e. the EC50/48 h value is 191.7 mg/L (95% confidence interval: 146.1 – 259.0). The EC20/48 h value is 97.9 mg/L (95% confidence interval: 63.6 – 129.9). The EC10/48 h value is 68.9 mg/L (95% confidence interval: 38.9 – 96.0). The data on immobilisation of the Daphnia magna at exposure termination were analysed using Step-down Cochran – Armitage Test Procedure, which showed a significant difference between the test item concentrations in the range of 103.3 - 500 mg/L and the control. Therefore, the LOEC/48 h value is 103.3 mg/L and the NOEC/48 h value is 47.0 mg/L.

Table 10. Endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	297.7 (212.5 – 488.3)	191.7 (146.1 – 259.0)
EC_{20}	123.1 (75.0 – 172.5)	97.9 (63.6 – 129.9)
EC_{10}	77.6 (38.0 – 114.7)	68.9 (38.9 – 96.0)
LOEC	103.3	103.3
NOEC	47.0	47.0

Calculations were made according to [7], [SOP/W/68]
(-) - 95% confidence interval
n.d. – not determined

The validity criteria:

The validity criteria were met according to OECD Guideline No 202/test method C.2.
– the percentage of immobilisation of Daphnia magna in the control was 0%
(criterion: not more than 10%),
– the dissolved oxygen concentrations in the test vessels were within the range of
7.9 – 8.4 mg/L (criterion: not less than 3 mg/L).

A 2.2.1.1.2 Study 2

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. In the definitive test the validity criteria were met according to OECD Guideline No. 201. No deviations from the guideline were noted during the study. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations. Following endpoints based on nominal test item concentrations would be used for risk assessment purposes: For algae the E_rC_{50} values based on growth rate are the relevant endpoints for risk assessment purposes as decreed by EFSA in their "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290). However, for the consistency of the report all obtained and accepted endpoints from the study are provided. Since the ErC_{50}/72 h is preferable endpoint it is written in bold. **ErC_{50}= 45.33 mg formulation/L (nom) corresponding to 20.98 mg ethofumesate/L** EyC_{50}= 23.16 mg formulation/L (nom)

Reference:	KCP 10.2/02
Report	CHR/H/ETO 500 SC Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test, E. Malada; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W-34-20, 2020
Guideline(s):	According to OECD Guideline No 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/ETO 500 SC; batch no. PFORM03, content of ethofumesate is 523.0 g/L; production date: April, 2020, expiry date: April, 2022.
Test Species:	The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata (Korshikov) Hindák, Selenastrum capricornutum Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany. .

Test Design:	72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial algal cell density: 1 x 104 cells/mL..
Endpoints:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h
Test Concentration:	100, 50, 25, 12.5, 6.25, 3.13 mg/L plus the control.
Test Conditions:	Temperature: 22.8 – 21.9°C; pH of the control: 7.66 – 8.53; mean light intensity: 6868 – 7005 lux; constant illumination and shaking; medium: AAP.

Results and discussion:

The effect of the test item on the green algal growth was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary test results. The growth inhibition was estimated on the basis of them density of the algae cells determined in the definitive test.

Preliminary non-GLP test

In the preliminary test, the recorded temperature was in the range of 22.0 – 22.6°C, the mean light intensity was in the range of 6922 – 6930 lux. The pH values were in the ranges of 7.21 – 7.49 at exposure initiation and 7.61 – 7.85 at exposure termination.

Table 3. pH values, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	pH values# at exposure initiation	pH values* at exposure termination
Control	7.49	7.85
0.1	7.43	7.77
1	7.27	7.81
10	7.22	7.75
100	7.21	7.61

#- pH measured in samples before splitting up into replicates
*- pH measured in samples of pooled replicates
Time of exposure: 07.08.2020 – 10.08.2020

The average transmittance values were in the range of 67.8 – 100.9% at exposure initiation and in the range of 79.7 – 100.0% at exposure termination when compared with the control.

No growth rate inhibition was observed in the control and the test item concentrations of 1 and 0.1 mg/L. The growth rate inhibition after 72 hours of exposure was 6.53 and 70,12% in the test item concentrations of 10 and 100 mg/L, respectively. No yield inhibition was observed in the control and the test item concentrations of 1 and 0.1 mg/L. The yield inhibition after 72 hours of

exposure was 24.71 and 96.24% in the test item concentrations of 10 and 100 mg/L, respectively.

Table 4. Inhibition of growth rate and yield, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.1	-3.25*	-12.00*
1	-1.53*	-4.00*
10	6.53	24.71
100	70.12	96.24

* inhibition of growth rate and yield are lower than 0.0%, which means that the algae cell density at exposure termination was higher than the algae cell density in the control

Definitive test

The definitive test was performed using the test item concentrations of 100, 50, 25, 12.5, 6.25 and 3.13mg/L plus the control.
The recorded temperature was in the range of 21.9 – 22.8 ºC and constant within 0.9ºC. The mean light intensity was in the range of 6868 – 7005 lux. The pH values measured at exposure initiation were in the range of 7.33 – 7.66 and at exposure termination were in the range of 7.54 – 8.53. In the all test item concentrations no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control. The effect of the test item on the growth rate and yield of Raphidocelis subcapitata is presented in Figure 10 (after 72 hours of exposure). The relationship between the inhibition of growth rate and the nominal test item concentrations at 72 h.

Table 9. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
3.13	-1.4*	-7.2*
6.25	-0.4*	-1.8*
12.5	2.3	11.6
25	15.5	55.3
50	60.5	96.1
100	83.3	99.2

* inhibition of growth rate and yield are lower than 0.0%, which means that the algae cell density at exposure termination was higher than the algae cell density in the control

Results of the analytical study

The concentrations of ethofumesate were chemically determined using a validated liquid chromatographic method with DAD detection. Samples of each treatment were collected at exposure initiation and at exposure termination.

Nominal test item concentration [mg/L]	Nominal concentration of ethofumesate [mg/L]	Average determined concentration of ethofumesate (n=3) in samples collected			
		at exposure initiation [mg/L]	[%] of nominal concentration	at exposure termination [mg/L]	[%] of nominal concentration
Control	---	< LoD	---	< LoD	---
3.13	1.449	1.436	99.1	1.410	97.3
6.25	2.893	2.897	100.1	2.869	99.2
12.5	5.785	5.825	100.7	5.728	99.0
25	11.570	11.681	101.0	11.290	97.6
50	23.140	23.453	101.4	23.081	99.7
100	46.280	44.579	96.3	43.523	94.0

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
--- no value

At exposure initiation, the determined concentrations of ethofumesate were in the range of 96.3 – 101.4% of the nominal concentration. The results confirm that the test item concentrations were prepared correct-

ly. At exposure termination, the determined concentrations of ethofumesate were in the range of 94.0 – 99.7% of the nominal concentration. Therefore, the concentrations of ethofumesate were stable under test conditions.

Endpoint values

The endpoint values were determined on the basis of the determined nominal test item concentrations [1]. The ECx values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analyzes. To conduct statistical analyses, the ToxRat Professional commercial software was used (Appendix 1) [8], [SOP/W/68].

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	37.66 (25.58 – 62.88)	56.32 (52.01 – 61.13)	45.33 (42.97 – 47.81)
E_rC_{20}	7.22 (2.76 – 11.87)	29.68 (25.71 – 33.19)	25.76 (23.52 – 27.81)
E_rC_{10}	3.05 (0.70 – 6.08)	21.24 (17.40 – 24.66)	19.18 (16.95 – 21.21)
LOEC	12.50	12.50	12.50
NOEC	6.25	6.25	6.25

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	16.68 (11.27 – 24.51)	30.49 (25.97 – 35.84)	23.16 (22.53 – 23.82)
E_yC_{20}	3.53 (1.31 – 5.96)	14.96 (10.97 – 18.34)	15.42 (14.68 – 16.09)
E_yC_{10}	1.57 (0.38 – 3.17)	10.31 (6.73 – 13.43)	12.46 (11.66 – 13.19)
LOEC	6.25	6.25	12.50
NOEC	3.13	3.13	6.25

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]

The median test item concentration causing 50% inhibition of the average specific growth rate of Raphidocelis subcapitata, i.e. the ErC50/72 h value is 45.33 mg/L (95% confidence interval: 42.97 – 47.81). The ErC20/72 h value is 25.76 mg/L (95% confidence interval: 23.52 – 27.81) and the ErC10/72 h value is 19.18 mg/L (95% confidence interval: 16.95 – 21.21).
Statistical tests based on the growth rate data were the Shapiro-Wilk's Test on Normal Distribution which confirm normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogenous and the Williams Multiple

Sequential t-test Procedure which showed a significant difference between the test item concentrations in the range of 12.50 – 100 mg/L and the control. Therefore, the LOEC/72 h value is 12.50 mg/L and the NOEC/72 h value is 6.25 mg/L).

The median test item concentration causing 50% yield inhibition of Raphidocelis subcapitata, i.e. the EyC50/72 h value is 23.16 mg/L (95% confidence interval:
22.53 – 23.82). The EyC20/72 value is 15.42 mg/L (95% confidence interval: 14.68 – 16.09) and EyC10/72 h value is 12.46 mg/L (95% confidence interval: 11.66 – 13.19). Statistical tests based on the yield data were the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were heterogenous and Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm which showed a significant difference between the test item concentrations in the range of 12.50 – 100 mg/L and the control. Therefore, the LOEC/72 h value is 12.50 mg/L and the NOEC/72 h value is 6.25 mg/L.

The validity criteria:

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) were met:
– the biomass in the control increased by a factor of 172.5 within the 72-hour test period (criterion: at least a 16-fold growth),
– the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.5% (criterion: it must not exceed 7%).
– the mean coefficient of variation for the section-by-section growth rate in the control culture was 24.9% (criterion: it must not exceed 35%).

A 2.2.1.1.3 Study 3

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. In the experimental part of study, no deviations occurred from the guideline. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations. For algae the E_rC_{50} values based on growth rate are the relevant endpoints for risk assessment purposes as decreed by EFSA in their "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290). However, for the consistency of the report all obtained and accepted endpoints from the study are provided. Since the ErC_{50}/72 h is preferable endpoint it is written in bold. Following endpoints are relevant for risk assessment purposes based on the nominal concentrations: The concentration causing 50% inhibition of the growth rate of *Anabaena flos-aquae*, **ErC_{50}/72 h value is > 300 mg formulation/L** **LOEC/72 h value for growth rate is 120 mg formulation/L.** **NOEC/72 h value for growth rate is 48 mg formulation/L.**

	The concentration causing 50% inhibition of yield of *Anabaena flos-aquae*, i.e. the EyC_{50}/72 h value is > 300 mg formulation/L LOEC/72 h value for yield is 120 mg formulation/L. NOEC/72 h value for yield is 48 mg/L.

Reference:	KCP 10.2/03
Report	CHR/H/ETO 500 SC Anabaena flos-aquae, Czarnecka M. UTEX B 1444 Growth inhibition test, Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W-36-20, 2020
Guideline(s):	According to OECD Guideline No 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/ETO 500 SC; batch no. PFORM03, content of ethofumesate is 523.0 g/L; production date: April, 2020, expiry date: April, 2022..
Test Species:	The freshwater cyanobacteria, Anabaena flos-aquae (Lyng.) Bréb UTEX B 1444 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Organisms Toxicology. The culture was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA.
Test Design:	72 hours of exposure; three replicates per each test item concentration; six replicates per the control; initial cyanobacterial cell density: 1×10^4 cells/mL.
Endpoints:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.
Test Concentration:	300, 120, 48, 19.2, 7.68 mg/L plus the control
Test Conditions:	Temperature: 22.2 – 22.8°C; pH of the control: 7.50 – 7.69; mean light intensity: 3565 - 3668 lux; constant illumination and shaking; medium: AAP.

Results and discussion:

The effect of the test item on the cyanobacterial growth was assessed. The range of the test item concentrations used in the definitive test were determined on the basis of the preliminary test result. The growth inhibition was estimated on the basis of the density of the cyanobacteria cells determined in the definitive test.

Preliminary non-GLP test

In the preliminary test, the recorded temperature was in the range of 22.8 – 23.1°C, the mean light intensity was in the range of 3400 – 3458 lux. The pH values were in the ranges of 7.59 – 7.71 at exposure initiation and 7.24 – 7.32 at exposure termination.

Table 3. pH values, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	pH values# at exposure initiation	pH values* at exposure termination
Control	7.71	7.32
1	7.65	7.24
10	7.66	7.25
100	7.59	7.25

#- pH measured in samples before splitting up into replicates
*- pH measured in samples of pooled replicates

The growth rate inhibition after 72 hours of exposure was 14.85% in the test item concentration of 100 mg/L, -2.44% in the test item concentration of 10 mg/L, and -1.16% in the test item concentration of 1 mg/L. The yield inhibition after 72 hours of exposure was 43.24% in the test item concentration of 100 mg/L, -10.86% in the test item concentration of 10 mg/L, and -6.35% in the test item concentration of 1 mg/L.

Table 4. Inhibition of growth rate and yield, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
1	-1.16*	-6.35*
10	-2.44*	-10.86*
100	14.85	43.24

* - calculated inhibition values are lower than 0%, what means that the cyanobacterial cell density at exposure termination is higher than the cyanobacterial cell density in the control

Definitive test

The recorded temperature was in the range of 22.2 – 22.8ºC with a variation of up to 0.6ºC (Figure 8). The mean light intensity was in the range of 3565 - 3668 lux. The pH values measured at exposure initiation were in the range of 7.50 – 7.72 and at exposure termination in the range of 7.54 – 7.95 (Table 5). Morphology observations of cyanobacteria cells were performed at exposure termination. In all test item concentrations, no differences in shape, size and colour of cyanobacterial cells were reported as compared to the cyanobacteria cells in the control.

Table 9. Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure	
	growth rate	yield
Control	0.0	0.0
7.68	-0.4*	-1.1*
19.2	-4.3*	-14.9
48	-3.4*	-12.7*
120	6.7	20.2
300	18.6	46.4

* - calculated inhibition values are lower than 0%, what means that the cyanobacterial cell density at exposure termination is higher than the cyanobacterial cell density in the control

Results of the analytical study

The concentrations of ethofumesate were chemically determined using the validated high performance liquid chromatographic method with DAD detection. Samples of each treatment were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of ethofumesate were in the range of 96.2 – 104.4% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of ethofumesate were in the range of 91.1 – 108.3% of the nominal concentration.

Table 10. Concentration and stability of ethofumesate, definitive test

Nominal test item concentration [mg/L]	Nominal concentration of ethofumesate [mg/L]	Average determined concentration of ethofumesate (n=3) in samples collected [mg/L]			
		at exposure initiation	[%] of nominal concentration	at exposure termination	[%] of nominal concentration
Control	0.000	< LoD	–	< LoD	–
7.68	3.554	3.709	104.4	3.488	98.1
19.2	8.886	9.080	102.2	8.719	98.1
48	22.214	22.416	100.9	21.051	94.8
120	55.536	55.868	100.6	50.579	91.1
300	138.840	133.523	96.2	150.318	108.3

LoQ = 0.2 mg/L
LoD = 0.06 mg/L
– no value

Endpoint values

The EC_x values were determined based on nominal concentrations of the test item as the analytical measurements confirmed the correct test item application and stability in the test conditions. The calculations and statistical analysis were performed with a commercial software ToxRat Professional. The E_rC_x and E_yC_x values in tables below. Median concentration causing 50% of yield inhibition of *Anabaena flos-aquae* culture E_yC_{50}/72h is 0.442 mg/L with 95% confidence limits of (0.326 – 0.608). The E_yC_{20}/72 h is 0.035 mg/L with 95% confidence limits of (0.019 – 0.056) and the E_yC_{10}/72 h is 0.009 mg/L with 95% confidence limits of (0.004 – 0.018). The statistical tests performed with data on yield were: Shapiro-Wilk's Test on normal distribution confirmed normal distribution of data, Levene's Test on variance homogeneity showed that variances are homogeneous, Williams Multiple Sequential t-test Procedure showed a significant difference between test concentration 0.015 mg/L compared with the control. Therefore, based on statistical analysis results the lowest concentration of the test item causing the yield inhibition effect LOEC/72 h is equal or lower than 0.015 mg/L. The estimated value of the test item concentration not causing any effect on yield NOEC/72 h is lower than 0.015 mg/L. Median concentration causing 50% inhibition of average specific growth rate of *Anabaena flos-aquae* culture E_rC_{50}/72 h is 4.749 mg/L with 95% confidence limits of (3.465 – 7.159). The E_rC_{20}/72 h is 0.373 mg/L with 95% confidence limits of (0.252 – 0.506). The E_rC_{10}/72 h is 0.099 mg/L with 95% confidence limits of (0.052 – 0.157). The statistical tests performed with data on growth rate were: Shapiro-Wilk's Test on normal distribution confirmed normal distribution of data, Levene's Test on variance homogeneity showed that variances are homogeneous, Williams Multiple Sequential t-test Procedure showed a significant difference between test concentration 0.015 mg/L compared with the control. Therefore, based on statistical analysis results the lowest concentration of the test item causing the growth rate inhibition effect LOEC/72 h is equal or lower than 0.015 mg/L. The estimated value of the test item concentration not causing any effect on growth rate NOEC/72 h is lower than 0.015 mg/L.

Table 11. Growth rate endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	>300	n.d.	>300
E_rC_{20}	127.65 (82.70-213.05)	n.d.	>300
E_rC_{10}	63.94 (35.36-97.95)	n.d.	117.42 (82.99-179.95)
LOEC	300	>300	120
NOEC	120	≥300	48

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]
n.d. – not determined

Table 12. Yield endpoint values based on the nominal test item concentrations, definitive test

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	252.95 (214.01-290.28)	n.d.	>300
E_yC_{20}	163.90 (106.18-198.16)	n.d.	125.10 (92.56-151.45)
E_yC_{10}	130.64 (71.32-167.53)	n.d.	75.45 (45.82-99.97)
LOEC	300	>300	120
NOEC	120	≥300	48

(-) – 95% confidence interval
Calculations were made according to [8], [SOP/W/68]
n.d. – not determined

The validity criteria:

In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) were met:
- the biomass in the control increased by a factor of 24.4 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 3.0% (criterion: it must not exceed 10%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 22.5% (criterion: it must not exceed 35%).

A 2.2.1.1.4 Study 3

Comments of zRMS:	CHR/H/ETO 500 SC *Lemna gibba* Growth inhibition test was conducted to OECD guideline 221 and according to the principles of GLP. No deviations were noted during the study. In the definitive test all the validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason end-points are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2/04
Report	CHR/H/ETO 500 SC Lemna gibba CPCC 310, Growth inhibition test, Czarnecka M, ,Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: W-35-20, 2020
Guideline(s):	according to the OECD Guideline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/ETO 500 SC; batch no. PFORM03, content of ethofumesate is 523.0 g/L; production date: April, 2020, expiry date: April, 2022..
Test Species:	Freshwater aquatic plant Lemna gibba L. specification CPCC 310, cultured in the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Organisms Toxicology, stock G3 from Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada..
Test Design:	Static system (7 days of exposure); three replicates for each test item concentration and six replicates for the control..
Endpoints:	ErC50, ErC20, ErC10, EyC50, EyC20, EyC10, LOEC and NOEC, based on frond number and dry weight.
Test Concentration:	320, 100, 31.25, 9.77, 3.05, 0.95 mg/L plus control
Test Conditions:	Temperature: 22.5 – 22.9ºC; pH of the control: 7.50 – 8.56; light intensity: 7570 – 7760 lux; constant illumination; test vessels: glass beakers with a capacity of 600 mL containing 400 mL of each treatment; initial frond number: 9, i.e. 3 plants per 3 fronds; medium: 20X AAP..

Results and discussion:

The effect of the test item on growth of Lemna gibba was estimated. The test item concentrations used in the definitive test and the test design were determined on the basis of the preliminary non-GLP test results.

Preliminary non-GLP test

In the preliminary test, the recorded temperature was in the range of 23.2 – 24.3ºC, whereas the mean light intensity was in the range of 7458 – 7560 lux. The pH values measured in all test item concentrations and the control were in the ranges of 7.54 – 7.67 at exposure initiation and 8.91 – 9.10 at exposure termination.

Table 3. pH values – preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Exposure initiation#	Exposure termination*
Control	7.54	8.99
1.0	7.57	9.04
10	7.64	8.91
100	7.67	9.10

#- pH measured in the samples before the splitting up into replicates
*- pH measured in the samples of pooled replicates

Number of fronds distinctly visible in each test vessel was counted and recorded as well as changes in plant development were observed on days 2, 4 and after 7 days of exposure. At exposure termination, in the test item concentration of 1.0 mg/L, no growth rate inhibition and yield inhibition based on the frond number was observed. The growth rate inhibition based on the frond number was 8.8% in the test item concentration of 10 mg/L and 49.6% in the test item concentration of 100 mg/L when compared to the control. The yield inhibition based on the frond number was 18.4% in the test item concentration of 10 mg/L and 72.2% in the test item concentration of 100 mg/L when compared to the control.

Table 4. Inhibition of growth rate and yield – preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Based on frond number		Based on dry weight	
	% Inhibition of growth rate	% Inhibition of yield	% Inhibition of growth rate	% Inhibition of yield
Control	0.0	0.0	0.0	0.0
1.0	-0.3*	0.0	3.3	8.6
10	8.8	18.4	8.2	19.5
100	49.6	72.2	31.6	58.7

*inhibition is lower than 0% what means that growth rate based on frond number in the test item concentration at exposure termination was higher than in the control
Time of exposure: 17.08 – 24.08.2020

The growth rate inhibition based on the dry weight was 3.3% in the test item concentration of 1.0 mg/L,

8.2% in the test item concentration of 10 mg/L, and 31.6% in the test item concentration of 100 mg/L when compared to the control. The yield inhibition based on the dry weight was 8.6% in the test item concentration of 1.0 mg/L, 19.5% in the test item concentration of 10 mg/L and 58.7% in the test item concentration of 100 mg/L when compared to the control.

Results of the chemical determinations

In the preliminary test, the concentration of ethofumesate was determined using the validated liquid chromatographic method with DAD detection [SOP/C/557]. Samples of the test item concentration of 10 mg/L and the control were analyzed at exposure initiation, after 2, 3 days of exposure and at exposure termination. At exposure initiation, the determined concentration of ethofumesate was 98.4% of the nominal concentration. The results confirm correct preparation of the test item concentration.
After 2 days of exposure, the determined concentration of ethofumesate was 94.3% of the nominal concentration. After 3 days of exposure, the determined concentration of ethofumesate was 95.2% of the nominal concentration, whereas at exposure termination, the determined concentration of ethofumesate was 100.7% of the nominal concentration. Therefore, concentration of ethofumesate was stable under test conditions. Based on the chemical determinations results, the definitive test was performed in a static design.

Definitive test

In the definitive test, Lemna gibba of initial nine fronds were exposed to the test item concentrations of 320, 100, 31.25, 9.77, 3.05, 0.95 mg/L (with a separation factor of 3.2) plus the control for 7 days under static test design. During exposure, the temperature was in the range of 22.5 – 22.9ºC with a variation of up to 0.4°C, whereas the average light intensity was in the range of 7570 – 7760 lux. The pH values measured in all test item concentrations and the control were in the range of 7.50 – 7.58 at exposure initiation and in the range of 8.56 – 8.82 in all test item concentrations and the control at exposure termination.

Table 6. pH values – definitive test

Nominal test item concentration [mg/L]	Exposure initiation#	Exposure termination*
Control	7.50	8.56
0.95	7.55	8.75
3.05	7.56	8.72
9.77	7.56	8.66
31.25	7.56	8.73
100	7.56	8.73
320	7.58	8.82

#- pH of the samples measured before division into replicates
*- pH of the samples of pooled replicates

The number of fronds distinctly visible in each test vessel was counted and recorded as well as changes in plant development on days 2, 4 and after 7 days of exposure. The morphology of plants was observed in the test item concentration and the control on days 2, 4 and at exposure termination. The morphological effects were compared with appearance of colonies in the control. After 2 and 4 days of exposure, in the test item concentrations of 0.95, 3.05, and 9.77 mg/L no distinctive changes from the normal development of plants in the control were observed. In

the test item concentrations of 31.25, 100 and 320 mg/L slightly bending down of fronds were observed.

Table 9. Results of observations on day 2 and 4 – definitive test

Nominal test item concentration [mg/L]	Observations
Control	normal size, color, shape of fronds, normal appearance of roots
0.95	no changes
3.05	no changes
9.77	no changes
31.25	slightly bending down of fronds
100	slightly bending down of fronds
320	slightly bending down of fronds

At exposure termination, in the test item concentrations of 0.95, 3.05 and 9.77 mg/L, no distinctive changes from the normal development of plants in the control were observed. In the test item concentration of 31.25 mg/L slightly bending down of fronds were observed. In the test item concentrations of 100 and 320 mg/L bending down of fronds were observed.

Nominal test item concentration [mg/L]	Observations
Control	normal size, color, shape of fronds, normal appearance of roots
0.95	no changes
3.05	no changes
9.77	no changes
31.25	slightly bending down of fronds
100	bending down of fronds
320	bending down of fronds

Results of the analytical study

The concentrations of ethofumesate were determined using the validated high performance liquid chromatographic method with DAD detection. Samples of each test item concentration and the control collected at exposure initiation and at exposure termination were chemically determined. At exposure initiation, the determined concentrations of ethofumesate were in the range of 95.6 – 105.7% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of ethofumesate were in the range of 82.6 – 110.8% of the nominal concentration. Therefore, the concentrations of ethofumesate were stable under test conditions.

Table 12. Concentration and stability of ethofumesate – definitive test

Nominal test item concentration [mg/L]	Nominal concentration of ethofumesate	Average determined concentration of ethofumesate (n=3) in samples collected			
		at exposure initiation [mg/L]	[%] of nominal concentration	at exposure termination [mg/L]	[%] of nominal concentration
Control	–	< LoD	–	< LoD	–
0.95	0.440	0.458	104.1	0.483	109.8
3.05	1.412	1.492	105.7	1.467	103.9
9.77	4.522	4.647	102.8	5.010	110.8
31.25	14.925	14.458	96.9	14.855	99.5
100	46.280	46.267	100.0	42.583	92.0
320	148.096	141.562	95.6	122.310	82.6

LOQ = 0.2 mg/L
LOD = 0.06 mg/L

Endpoint values

The endpoint values were determined on the basis of the nominal test item concentrations. To conduct statistical analyses, the ToxRat Professional commercial software was used.The median concentration causing 50% inhibition of the mean specific growth rate of Lemna gibba determined on the basis of the frond number ErC50/7 d value is 86.61 mg/L (95% confidence interval: 65.14 – 119.11). The ErC20/7 d value is 13.50 mg/L (95% confidence interval: 7.88 – 19.76) and the ErC10/7 d value is 5.11 mg/L (95% confidence interval: 2.34 – 8.61).
The growth rate data based on the frond number were analyzed using Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogeneous, and the Williams Multiple Sequential t-test Procedure which showed significant differences between the nominal test item concentrations in the range of 9.77 – 320 mgL and the control. The lowest concentration of the test item causing an effect on growth rate, i.e. the LOEC/7 d value is 9.77 mg/L and the highest concentration of the test item not causing any effect on growth rate, i.e. the NOEC/7 d value is 3.05 mg/L.

Table 13. Growth rate endpoint values based on nominal test item concentration [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_rC_{10}	10.83 (3.79 – 19.48)	8.91 (5.03 – 13.32)	5.11 (2.34 – 8.61)	4.52 (2.10 – 7.69)
E_rC_{20}	24.52 (11.97 – 37.85)	19.73 (13.17 – 26.56)	13.50 (7.88 – 19.76)	18.20 (11.39 – 25.91)
E_rC_{50}	117.05 (83.14 – 175.15)	90.18 (72.56 – 113.73)	86.61 (65.14 – 119.11)	261.95 (184.93 – 414.61)
LOEC	31.25	31.25	9.77	≤0.95
NOEC	9.77	9.77	3.05	<0.95

Calculations according to [8], [SOP/W/68]
(-) - 95% confidence interval

Table 14. Yield endpoint values based on nominal test item concentration [mg/L] – definitive test

Endpoint value [mg/L]	Frond number			Dry weight
	0-2 d	0-4 d	0-7 d	0-7 d
E_yC_{10}	6.75 (2.28 – 12.45)	4.29 (1.99 – 7.04)	1.67 (0.54 – 3.29)	<0.95
E_yC_{20}	15.02 (7.00 – 23.88)	9.15 (5.24 – 13.36)	4.20 (1.86 – 7.06)	2.48 (1.01 – 4.48)
E_yC_{50}	69.35 (48.01 – 103.02)	39.01 (29.16 – 52.42)	24.52 (16.36 – 37.00)	28.25 (18.58 – 43.96)
LOEC	31.25	3.05	3.05	≤0.95
NOEC	9.77	0.95	0.95	<0.95

Calculations according to [8], [SOP/W/68]
(-) - 95% confidence interval

The median concentration causing 50% inhibition of yield of Lemna gibba determined on the basis of the frond number EyC50/7 d value is 24.52 mg/L (95% confidence interval: 16.36 – 37.00). The EyC20/7 d is 4.20 mg/L (95% confidence interval 1.86 – 7.06) and the EyC10/7 d value is 1.67 mg/L (95% confidence interval: 0.54 – 3.29). The yield data based on the frond number were analyzed using Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogeneous, and the Williams Multiple Sequential t-test Procedure which showed significant differences between the nominal test item concentrations in the range of 3.05 – 320 mgL and the control. The lowest test item concentration causing a yield inhibition effect, i.e. the LOEC/7 d value is 3.05 mg/L. The highest test item concentration at which no yield inhibition effects are observed, i.e. the NOEC/7 d value is 0.95 mg/L.The median concentration causing 50% inhibition of the mean specific growth rate of Lemna gibba determined on the basis of the dry weight ErC50/7 d value is 261.95 mg/L (95% confidence interval

184.93 – 414.61). The ErC20/7 d value is 18.20 mg/L (95% confidence interval 11.39 – 25.91) and the ErC10/7 d value is 4.52 mg/L (95% confidence interval: 2.10 – 7.69).
The growth rate data based on the dry weight were analyzed using Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogeneous, and the Williams Multiple Sequential t-test Procedure which showed significant differences between all nominal test item concentrations and the control. The lowest concentration of the test item causing an effect on growth rate, i.e. the LOEC/7 d value is equal to or lower than 0.95 mg/L and the highest concentration of the test item not causing any effect on growth rate, i.e. the NOEC/7 d value is lower than 0.95 mg/L. The median concentration causing 50% inhibition of yield of Lemna gibba determined on the basis of the dry weight EyC50/7 d value is 28.25 mg/L (95% confidence interval: 18.58 – 43.96). The EyC20/7 d value is 2.48 mg/L (95% confidence interval: 1.01 – 4.48) and the EyC10/7 d value is lower than 0.95 mg/L.The yield data based on the dry weight were analyzed using the Shapiro-Wilk's Test on Normal Distribution which confirmed normal distribution of the data, the Levene's Test on Variance Homogeneity (with Residuals) which showed that the variances were homogeneous, and the Williams Multiple Sequential t-test Procedure which showed significant differences between all nominal test item concentrations and the control. The lowest test item concentration causing a yield inhibition effect, i.e. the LOEC/7 d value is equal to or lower than 0.95 mg/L. The highest test item concentration at which no yield inhibition effects are observed, i.e. the NOEC/7 d value is lower than 0.95 mg/L.

The validity criteria:

In the definitive test, the following validity criteria specified in the OECD Guideline No. 221 were met:
- the doubling time of frond number in the control was 2.3 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 8.4),
- the average specific growth rate in the control between day 0 and day 7 was 0.302 d^{-1} (minimum requirement: higher than 0.275 d^{-1}).

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

No additional studies were performed.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional studies were performed.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met according to OECD Guideline No. 213. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: LD_{50}/48 h oral > 200.0 µg formulation/honeybee.

Reference:	KCP 10.3.1.1/01
Report	CHR/H/ETO 500 SC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test, E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B-67-20, 2020
Guideline(s):	according to the OECD Guideline No 213 and corresponding method C.16.)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item: CHR/H/ETO 500 SC content: 523.0 g/L of ethofumesate, batch no.: PF0RM03 production date: 04.2020 expiry date: 04.2022

Test Species: the honeybee, Apis mellifera L., strain: carnica
– age: approximately 3 weeks
– source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,

Test Design: – **the test item:**
- exposure duration: 48 hours
- number of doses: 5 doses and a control
- number of replicates: 3 replicates
- number of bees: 10 bees/replicate
- **the reference item:**
- exposure duration: 24 hours
- number of doses: 3 doses
- number of replicates: 3 replicates
- number of bees: 10 bees/replicate

Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the oral LD50/24 h of the reference item (dimethoate).

Test Concentration: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Test Conditions: – temperature:

23.5 - 27.0°C
– relative air humidity:
55 - 61%

Preliminary non-GLP test range-finding test

Mortality results obtained in the preliminary non-GLP test are presented in Tables 1 and 2. Mortality of the control group after 48 hours of exposure was 0.0%. After 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee were 0.0%.

Table 3. Behavioural effects – preliminary non-GLP range-finding test

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h
		Number of bees showing adverse behaviour*/ number of living bees		
0.0 (control)	I	0/10	0/10	0/10
8.0	I	0/10	0/10	0/10
40.0	I	0/10	0/10	0/10
200.0	I	0/10	0/10	0/10

*: sub-lethal toxic effects were:

a- uncoordinated movements
b- increased activity
c- intensive cleaning
d- paralysis

Definitive test

After 4 hours of exposure, mortality of the control group and the groups treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee was 0.0% After 24 hours of exposure, mortality of the control group was 3.3% and for the treated groups' mortality percentages at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were (-3.5), 0.0, 0.0, (-3.5) and 0.0%, respectively. The negative values indicate that the mortality in the group treated with the test item was lower than in the control group.

Table 4. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	**0.0**
12.5	30	0	0	0	0	**0.0**
25.0	30	0	0	0	0	**0.0**
50.0	30	0	0	0	0	**0.0**
100.0	30	0	0	0	0	**0.0**
200.0	30	0	0	0	0	**0.0**

Table 5. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%][a]	
0.0 (control)	30	1	0	0	1	**3.3**	–	**> 200.0**
12.5	30	0	0	0	0	**0.0**	**-3.5***	
25.0	30	0	0	1	1	**3.3**	**0.0**	
50.0	30	0	1	0	1	**3.3**	**0.0**	
100.0	30	0	0	0	0	**0.0**	**-3.5***	
200.0	30	1	0	0	1	**3.3**	**0.0**	

[a]: mortality corrected according formula of Abbott's [6]

After 48 hours of exposure, mortality of the control group was 6.7%. For the treated groups' mortality percentages (corrected using the formula of Abbott [6]) at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 0.0, 0.0, (-3.6), 3.6 and 7.1%, respectively. The median lethal doses LD50/24 h and LD50/48 h are higher than the highest test item dose No abnormal behavioural effects were observed during the test.

Table 6. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%]ª	
0.0 (control)	30	0	0	2	2	6.7	-	> 200.0
12.5	30	0	1	1	2	6.7	0.0	
25.0	30	1	0	1	2	6.7	0.0	
50.0	30	0	1	0	1	3.3	-3.6	
100.0	30	0	1	2	3	10.0	3.6	
200.0	30	1	3	0	4	13.3	7.1	

ª: mortality corrected according formula of Abbott's [6]
*: the negative values indicate that the mortality in the group treated with the test item was lower than in the control group

The definitive test was conducted between 08 – 10.09.2020.

The reduction during 24 and 48 ranged from -8.05 to 29.55% as compared to the control. The negative value indicates higher sucrose solution consumption in group treated with the test item compared to the control group

Endpoint values

The LD50 (median lethal dose) oral is a statistically derived single dose of a test or reference item that can cause death in 50 per cent of biological test systems when administered by the oral route. The LD50 is expressed in µg of the test item per bee or in µg of the active ingredient contained in the reference item per bee. It was calculated with the log-probit method using ToxRat Proffesional software, version 3.3.0. Mortality: a honeybee is considered dead if it is completely immobile.

The validity criteria:

The following validity criteria were met during the test:
- the mortality for the control was 6.7% at the end of the experiment (criterion: it must not exceed 10%).
- the LD50/24 h of the reference item (dimethoate) was 0.19 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. Deviation from the method was observed. Anaesthesia recommended by the OECD Guideline No. 214 / EU Method C.17. (with carbon dioxide or nitrogen for application of the test item) was replaced with mechanical immobilization. However, since no unintentional bee injuries appeared, and all validity criteria are met change of the method did not have any impact on the study result. In the definitive test all the validity criteria were met according to OECD Guideline No. 214. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: LD_{50}/48 h contact > 200.0 µg formulation/honeybee.

Reference:	KCP 10.3.1.1/02
Report	CHR/H/ETO 500 SC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test, E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B-68-20, 2020
Guideline(s):	according to the OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	CHR/H/ETO 500 SC content: 523.0 g/L of ethofumesate batch no.: PF0RM03 production date: 04.2020 expiry date: 04.2022
Test Species:	the honeybee, Apis mellifera L., strain: carnica – age: approximately 3 weeks – source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna.
Test Design:	**– the test item:** - exposure duration: 48 hours - number of doses: 5 doses and one control - number of replicates: 3 replicates - number of bees: 10 bees/replicate - **the reference item:** - exposure duration: 24 hours - number of doses: 3 doses - number of replicates: 3 replicates

- number of bees: 10 bees/replicate

Endpoints: – honeybee mortality after 24 and 48 hours of the exposure,
– the contact LD50/24 h of the reference item (dimethoate).

Test Concentration: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Test Conditions: – temperature:
23.5 - 27.0°C
– relative air humidity:
54.0 - 61.0%
16 hours light : 8 hours dark

Preliminary non-GLP test

Mortality results obtained in the preliminary experiment are presented in Tables 1 and 2. Mortality of the control groups after 48 hours of exposure was 0.0%. After 24 and 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee were 0.0%. No abnormal behavioural effects were observed during the test

Table 3. Behavioural effects – preliminary range-finding test

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h
		Number of bees showing adverse behaviour*/ number of living bees		
0.0 (control)	I	0/10	0/10	0/10
8.0	I	0/10	0/10	0/10
40.0	I	0/10	0/10	0/10
200.0	I	0/10	0/10	0/10

*: sub-lethal toxic effects were:

a- uncoordinated movements
b- increased activity
c- intensive cleaning
d- paralysis

Definitive test

Mortality of the control group after 4 hours of the test was 0.0%. After 24 and 48 hours the percentages of mortality of the bees in control group was 3.3%. After 4 hours of exposure, the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0%. After 24 and 48 hours of exposure, the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0, 0.0, 0.0, 0.0 3.3%, respectively.

Table 4. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	**0.0**
12.5	30	0	0	0	0	**0.0**
25.0	30	0	0	0	0	**0.0**
50.0	30	0	0	0	0	**0.0**
100.0	30	0	0	0	0	**0.0**
200.0	30	0	0	0	0	**0.0**

Table 5. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	1	0	0	1	**3.3**	–	**> 200.0**
12.5	30	0	0	0	0	**0.0**	-3.5*	
25.0	30	0	0	0	0	**0.0**	-3.5*	
50.0	30	0	0	0	0	**0.0**	-3.5*	
100.0	30	0	0	0	0	**0.0**	-3.5*	
200.0	30	0	0	1	1	**3.3**	**0.0**	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

Table 6. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	1	0	0	1	3.3	–	> 200.0
12.5	30	0	0	0	0	0.0	-3.5*	
25.0	30	0	0	0	0	0.0	-3.5*	
50.0	30	0	0	0	0	0.0	-3.5*	
100.0	30	0	0	0	0	0.0	-3.5*	
200.0	30	0	0	1	1	3.3	0.0	

[a]: mortality corrected according to the Abbott formula

*: the negative value indicates that the mortality in the group treated with the test item was lower than in the control group

The preliminary non-GLP test was conducted between 08 – 10.09.2020.

The median lethal doses (LD50/24 h and LD50/48 h contact) are higher than 200.0 µg/honeybee. During the definitive test no abnormal behavioural effects were. The median lethal dose of dimethoate (LD50/24 h) determined with the log-probit method is 0.21 µg a.i./bee (95% confidence limits: 0.19 – 0.25 µg a.i./bee).

Table 9. Honeybee mortality and the LD_{50} after 24 hours of exposure – dimethoate

Dose [µg a.i./bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%] Corr[a]	
0.0 (control)	30	1	0	0	1	3.3	–	0.21** (0.19 – 0.25)
0.1	30	0	0	1	1	3.3	0.0	
0.2	30	5	4	4	13	43.3	41.4	
0.4	30	9	10	10	29	96.7	96.6	

[a]: mortality corrected according formula of Abbott's [9]

**: contact LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software)

Endpoint values

The LD50 (median lethal dose) contact, is a statistically derived single dose of a substance that can cause death in 50 per cent of biological test system when administered by contact route. The LD50 is expressed in µg test item per bee or in µg of the active ingredient contained in the reference item per bee. It was

calculated with the log-probit method.
Mortality: a honeybee is dead if it is completely immobile.

The validity criteria:

The following validity criteria were met during the test:
- the mortality for the control was 3.3% after 48 h (criterion: it must not exceed 10.0%),
- the LD50/24 h of the reference item (dimethoate) was 0.21 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	Comments of zRMS: The study would be evaluated according to most recent OECD guideline 239 Honey bee (Apis mellifera) larval toxicity test and according to the principles of GLP. All the validity criteria were met. LD_{50} was not determined statistically. In course of the experiment, the test item has shown no apitoxic effect in mortality of following developmental stages of bees after 22 days of the test. At the end of the study, due to mathematical reasons, the concentration and the dose causing 50% mortality of the population in the test (LC_{50} and LD_{50} values) were not determined, however NOEC and NOED values were determined at 22 day. Based on the results analysis, LD_{50} value was defined as >100 µg/larva which is agreed by zRMS. The study is accepted and reliable with following endpoint: LD_{50}= > 100 µg test item./larva NOED= ≥ 100 µg test item./larva

Reference:	KCP 10.3.1.2/01
Report	Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239, U. Orzechowska; SORBOLAB Research Laboratory LLC, Zaniemyska 11 Street, 61-029 Poznań; STUDY CODE: 0038/0008/E, 2020
Guideline(s):	OECD GD 239
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:

Name: Etofumesate 500 SC
Test item description: white liquid
Batch No.: PFORM03
Name of active ingredient: ethofumasate
Content of active ingredient: 500 g/L
CAS of active ingredient: 26225-79-6

	Production date: 04.2020 Expiration date: 04.2022
Test Species:	Apis mellifera L.)
Test Design:	stability test: tested concentrations and control in one replicate range-finding, definitive, reference test: tested concentrations and control in one replicate; 36 larvae per replicate
Endpoints:	- honeybee mortality after 10 days of exposure - LC50 and LD50 after 10 days of exposure - NOEC,
Test Concentration:	stability test: control, 0.05 g/L corresponding to 0.65 mg/kg of food; 50 g/L corresponding to 650 mg/kg of food range-finding test: control; 0.65 mg/kg; 6.5 mg/kg; 65 mg/kg; 650 mg/kg of food definitive test: control; 16.64 mg/kg; 41.6 mg/kg; 104 mg/kg; 260 mg/kg; 650 mg/kg of food reference test: control; fenoxycarb 0.32 mg/kg of food
Test Conditions:	stability test: average temperature 5.310°C (minimal temperature 4.7°C; maximal temperature 7.2°C); darkness range-finding test: average temperature 33.986°C (minimal temperature 32.2°C; maximal temperature 35.2°C); average humidity: 81.527% (minimal humidity 45.0%; maximal humidity 98.8%); darkness definitive test and reference test: average temperature 34.118°C (minimal temperature 32.6°C; maximal temperature 35.8°C); average humidity: 83.769% (minimal humidity 53.5%; maximal humidity 99.6%); darkness

Results and discussion:

Test item stability in storage conditions was confirmed based on results solutions chemical analysis at the test beginning and after 24, 48 and 72 hours for all tested concentrations and control. Test item concentration determination results during stability test are shown bellow. Test item concentrations were differed by no more than ±20% from initial concentrations. Based on the results obtained in the test, test item was found to be stable in storage conditions.

Date of analysis	Labeling of the sample by the Ecotoxicology laboratory	Labeling of the sample by the Physicochemistry and Analytics laboratory	Determination of active substance Ethofumesate concentration [mg/L]	Dilution	Active substance Ethofumesate concentration after including dilution [mg/L]	Test item concentration [mg/L]	Average test item concentration [mg/L]	Average test item concentration [g/L]
02.06.2020	Kontrola	69/2020 1	0.00000	1	0.00000	0.00000	0.00	0.00000
		69/2020 2	0.00000	1	0.00000	0.00000		
	0.05 g/l	70/2020 1	2.12949	10	21.29490	47.53326	47.43	0.04743
		70/2020 2	2.12021	10	21.20210	47.32612		
	50 g/l	71/2020 1	1.90023	10000	19002.30000	42415.84821	42829.35	42.82935
		71/2020 2	1.93728	10000	19372.80000	43242.85714		
03.06.2020	Kontrola dzień 1	78/2020 1	0.00000	1	0.00000	0.00000	0.00	0.00000
		78/2020 2	0.00000	1	0.00000	0.00000		
	0.05 g/l dzień 1	79/2020 1	2.09275	10	20.92750	46.71317	46.51	0.04651
		79/2020 2	2.07476	10	20.74760	46.31161		
	50 g/l dzień 1	80/2020 1	1.97322	10000	19732.20000	44045.08929	43570.54	43.57054
		80/2020 2	1.93070	10000	19307.00000	43095.98214		
04.06.2020	kontrola dzień 2	88/2020 1	0.00000	1	0.00000	0.00000	0.00	0.00000
		88/2020 2	0.00000	1	0.00000	0.00000		
	0.05 g/l dzień 2	89/2020 1	2.10916	10	21.09160	47.07946	47.25	0.04725
		89/2020 2	2.12437	10	21.24370	47.41897		
	50 g/l dzień 2	90/2020 1	1.94801	10000	19480.10000	43482.36607	43426.45	43.42645
		90/2020 2	1.94300	10000	19430.00000	43370.53571		
05.06.2020	kontrola dzień 3	97/2020 1	0.00000	1	0.00000	0.00000	0.00	0.00000
		97/2020 2	0.00000	1	0.00000	0.00000		
	0.05 g/l dzień 3	98/2020 1	2.08649	10	20.86490	46.57344	46.35	0.04635
		98/2020 2	2.06681	10	20.66810	46.13415		
	50 g/l dzień 3	99/2020 1	2.08108	10000	20810.80000	46452.67857	46985.27	46.98527
		99/2020 2	2.12880	10000	21288.00000	47517.85714		

Methods of preparation of food

Larval diets were adjusted depending on the developmental stage (all solutions were prepared in weight percentage):

- Food A: 50% fresh royal jelly + 50% aqueous solution containing 2% yeast extract/ 12% glucose/ 12% fructose
- Food B: 50% fresh royal jelly + 50% aqueous solution containing 3% yeast extract / 15% glucose / 15% fructose
- Food C: 50% fresh royal jelly + 50% aqueous solution containing 4% yeast extract / 18% glucose / 18% fructose.

Following the above, prepared food should have density around 1.1 mg/µL (20 µL of food corresponds to 22 mg of food).

Before administration, food was warmed to 35°C. It was provided using automatic pipette, with caution to avoid touching a larva or drowning it in food liquid.

From the emergence phase (D15 - D22) as food was used:

- 50% aqueous solution of sucrose
- pine pollen.

Range-finding test

During the range-finding test, test item was added to the food during 4-day exposition. The observations of mortality and behavioral changes were recorded daily during 22 days of the test. Basing on the range-finding test, number and range of concentrations for definitive test were determined. Parallel, test with use of two reference items was performed – dimethoate and fenoxycarb, to determine preferable substance for reference test, carried out parallel to the definitive test.

Test design

Range-finding test was performed for four concentrations of test item and control. Following concentrations of test item were used: 0.65 mg/kg; 6.5 mg/kg; 65 mg/kg and 650 mg/kg of food, which correspond to dose: 0.1 µg/larva; 1 µg/larva; 10 µg/larva and 100 µg/larva. Each concentration and control were prepared in one replicate, 36 larvae per replicate (12 larvae from 3 colonies).

Test conditions

Measurement of physicochemical conditions during range-finding test:

- for larval stage (day 1-8): average temperature 34.079°C (minimal temperature 32.2°C; maximal temperature 34.3°C); average relative humidity 95.071% (minimal humidity 61.9%; maximal humidity

98.8%)
- for pupal stage (day 8-15): average temperature 34.057°C (minimal temperature 32.8°C; maximal temperature 34.1°C); average relative humidity 77.159% (minimal humidity 45.8%; maximal humidity 81.9%)
- for imago stage (day 15-22): average temperature 33.820°C (minimal temperature 32.3°C; maximal temperature 35.2°C); average relative humidity 72.323% (minimal humidity 45.0%; maximal humidity 80.3%).
The study was conducted in darkness.

Results and conclusions
During range-finding test, no statistically significant larval mortality was observed in all tested concentrations. The NOEC value was determined for the following tested concentration. Based on the results, it was decided to perform definitive test using concentrations with spacing factor equal 2.5; starting from the concentration of 650 mg/kg of food.Analysis of range-finding test results was conducted using program for statistical analysis ToxRat Professional.

Definitive test
During the definitive test, test item was administrated in food during 4-day exposition. The observations of mortality and behavioral changes were recorded daily during 22 days of the test. Parallel to definitive test, reference test was performed using fenoxycarb as reference item.

Test design
In the definitive test, were used following concentration: 16.64 mg/kg; 41.6 mg/kg; 104 mg/kg; 260 mg/kg and 650 mg/kg of food. Each concentration and control were prepared in one replicate, 36 larvae per replicate (12 larvae from 3 colonies) on single breeding plate.

Test conditions
Measurement of physicochemical conditions during definitive test:
- larval stage (day 1-8): average temperature 34.088°C (minimal temperature 33.6°C; maximal temperature 34.3°C); average relative humidity 95.287% (minimal humidity 53.5%; maximal humidity 99.6%)
- pupal stage (day 8-15): average temperature 34.142°C (minimal temperature 32.6°C; maximal temperature 35.2°C); average relative humidity 80.574% (minimal humidity 72.5%; maximal humidity 96.1%)
- imago stage (day 15-22): average temperature 34.125°C (minimal temperature 33.0°C; maximal temperature 35.8°C); average relative humidity 75.446% (minimal humidity 60.8%; maximal humidity 85.9%).
The study was conducted in darkness.

Observations
During the test, following measurements and observations were performed:
- larval mortality from day 4 to day 8 and on day 15, observations recorded during feeding periods; immobile or non-reacting larva was noted as dead; on day 15, larvae, which have not transformed into pupae were recorded as dead; during the feeding, dead individuals were removed for sanitary reasons
- on day 22 mortality of adults and pupae – amount of emerged or non-emerged
- on day 22 emerged adults, alive or dead
- at the end of the test was determined percentage of emerged adults (by comparing the number of bees emerged on day 22 to the number of larvae on day 3); pupal mortality (calculated in percentage by comparing the number of pupae failed to emerge, including those without emergence on day 22 and dead pupae remove during pupa stage from day 8 to day 22 to the number pf bees entering pre-pupa stage on day 8); the larval mortality (percentage calculated by comparing the number of bees died during larvae stage - from day 3 to day 8 - to the number of larvae on day 3) - on day 8, presence of uneaten food
- temperature and humidity during definitive test was recorded continuously using temperature and humidity recorder
- other observations (for larvae, pupae and adults: appearance, size, behavior, morphological differences).

Signs of intoxication and mortality for following stages of bee development

During definitive test, no statistically significant final mortality was observed in all tested concentrations. Larvae shown slight signs of intoxication, represented by stunted development, i.e. inhibited food intake, smaller size, decreased mobility. Similar signs of intoxication were recorded in case of pupae. Mortality of following stages of bee development and signs of intoxication during definitive test are presented below

Table 8. Mortality of following developmental stages - definitive test

Concentration [mg/kg of food]	Mortality of larvae										Mortality of pupae			Emergence of imago	Total mortality at the end of the test [%]	Total survival at the end of the test [%]
	day 4		day 5		day 6		day 7		day 8		day 15		day 22	day 22		
	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	No. of emerged imago		
Control	0	stunted development – 1	0	none	0	none	0	none	0*)	stunted development – 1	4	stunted development – 2	5	31	13.9	86.1
16.64	0	none	0	none	0	none	0	stunted development – 1	0	stunted development – 2	2	stunted development – 4	4	32	11.1	88.9
41.6	0	none	0	stunted development – 1	0	none	0	stunted development – 1	1	stunted development – 3	7	stunted development – 1	9	26	27.8	72.2
104	0	stunted development – 3	0	stunted development – 5	0	stunted development – 5	1	stunted development – 3	3	stunted development – 6	2	stunted development – 4	2	31	13.9	86.1
260	0	none	0	stunted development – 2	0	stunted development – 2	0	stunted development – 4	0	stunted development – 8	8	stunted development – 6	11	25	30.6	69.4
650	0	stunted development – 1	0	stunted development – 2	0	stunted development – 2	0	stunted development – 3	1	stunted development – 5	3	stunted development - 3	5	30	16.7	83.3

*) larval mortality in control on days 3-8 was 0.0%

Amount of uneaten food

On day 8 of the test, the presence of food uneaten by larvae was recorded. The amount of food left was minor, resulted mainly from retarded development of larvae.

Concentration [mg/kg of food]	Presence of uneaten food
Control	NO
16.64	NO
41.6	NO
104	YES
260	NO
650	YES

Chemical analysis

For chemical analysis, the aqueous solutions of the lowest and the highest tested concentration were given at the beginning of the definitive test. The concentration of test item Etofumesate 500 SC in deionized water was determined. Determination was performed in accordance with Standard Experimental Procedure SPB-FA/161. Analytical method was validated in the study under the code 0038/0007/FA based on the Standard Experimental Procedure SPB-FA/11 and guideline SANCO/3029/99 rev. 4. During the validation of the analytical method, the following parameters were determined: selectivity, linearity, accuracy, precision, limit of detection and limit of quantification.

Parameter	Required criterion	Obtained result		
Selectivity	no interfering peaks of other substances, which surface is exceeding 3% active substance area, are present at the place of peak of active substance module in test item solution in the conditions of analysis, active substance time of retention in module solution and in solution of tested sample are comparable (no difference higher than 2%) UV spectrum of standard and test item were comparable.	no interfering peaks of other substances, which surface is exceeding 3% active substance area, are present at the place of peak of active substance module in test item solution in the conditions of analysis, active substance time of retention in module solution and in solution of tested sample is comparable (below 2%) UV spectrum of standard and test item were comparable.		
Linearity	r ≥ 0.99	r = 0.999 Calibration curve: 1.74226 mg/L – 27.87612 mg/L		
Accuracy [%]	70-110	level I	87.6	90
		level II	92.2	
Precision [% RSDr]	≤ 20	level I	0.29	
		level II	0.61	
Limit of detection [mg/L]	-	0.229664		
Limit of quantification [mg/L]	-	0.075789		

Reagents and materials
- acetonitrile p. HPLC, Avantor, batch no. 1927601865
- methanol p. HPLC, Honeywell, batch no. J357C
- ethofumesate Standard, IPO Warsaw, batch no. 6C/20
- deionized water
- ultrapure water
- analytical balance Radwag XA 82_220.4Y.A
- high pressure liquid chromatography analyzer Shimadzu Nexera X2 series LC-30 with PDA detector
- volumetric flasks class A
- automatic pipettes: Acura Manual 826 XS, Transferpette 1 mL
- deionizator Solpure 78
- water purification system Millipore Synergy UV
- ultrasonic water bath Sonic-10
- syringes and syringic filters 0,22 µm

Chromatographic conditions
Column Kinetex F5 2.6 µm 100 Å 100 x 4.6 mm
Detection 225 nm
Introduced volume 10 µL
Column thermostat temperature 30ºC
Mobile phase ultrapure water (40%): acetonitrile (60%)

Mobile phase flow 1.0 mL/min

Preparation of samples for test
Test item solutions were analyzed directly, or after prior dilution using deionized water to the calibration curve level.

Results of the test item content determination
Test item concentrations in analyzed solution were obtained from chromatographic set control program and recalculated using formula:

$$C_{SA} = n \cdot C_{ozn}$$

where:
CSA active substance concentration in sample [mg/L]
Cozn active substance concentration obtained from calibration curve [mg/L]
n sample dilution (in case of undiluted samples n=1)
Based on determined active substance concentration, concentration of the test item was calculated in the deionized water solution using formula:

$$C_{BM} = \frac{C_{SA} * 100\%}{44{,}8\%}$$

where:
CBM test item concentration in deionized water [mg/L]
CSA active substance concentration in the sample [mg/L]
44,8 active substance concentration in test item [% (w/w)]
(Calculated basing on nominal concentration of active substance given by the Sponsor and test item density; density determined in the study under the code 0038/0007/FA).
Final result of test item concentration was given as an arithmetic mean from all the replicates and was given in mg/L with an accuracy of 0.01 mg/L.

Date of analysis	Labeling of the sample by the Ecotoxicology laboratory	Labeling of the sample by the Physicochemistry and Analytics laboratory	Determination of active substance Ethofumesate concentration [mg/L]	Dilution	Active substance Ethofumesate concentration after including dilution [mg/L]	Test item concentration [mg/L]	Average test item concentration [mg/L]	Average test item concentration [g/L]
12.06.2020	Kontrola	151/2020 1	0.00000	1	0.00000	0.00000	0.00	0.00000
		151/2020 2	0.00000	1	0.00000	0.00000		
	50 g/l	152/2020 1	2.00787	10000	20078.70000	44818.52679	44464.40	44.46440
		152/2020 2	1.97614	10000	19761.40000	44110.26786		
	1.28 g/l	153/2020 1	5.23810	100	523.81000	1169.21875	1167.51	1.16751
		153/2020 2	5.22282	100	522.28200	1165.80804		

Reference test
Experiment with the reference item (fenoxycarb) was conducted in parallel with the definitive test. The aim of the study was to determine reference item influence on the honey bees development (Apis mellifera L.) used for test. The concentration causing 80% mortality of the population in imago stage after 22 days of the test was determined.

Test design
One concentration of reference item was used: 0,32 mg/kg of food and control. Concentration and control were prepared in one replicate, 36 larvae per replicate (12 larvae from 3 colonies). Test conditions were the same as in definitive test.

Results of reference test and final conclusions
Observations of mortality of honey bee in reference test were conducted parallel to the observations of intoxication of test item. As a result of 4-day exposition to reference item, adults emergence rate at 22 day was 5.6%. The reference item in the course of the present study showed apitoxic effects on the honey bee (Apis mellifera, L.). The results obtained are in accordance with the requirements of the OECD GD 239 Guideline (required ≤20%) and confirm the correct reaction of the test system. Statistical analysis was

performed using statistical software ToxRat Professional.

Concentration [mg/kg of food]	Mortality of larvae										Mortality of pupae			Emergence of imago	Total mortality at the end of the test [%]	Total survival at the end of the test [%]
	day 4		day 5		day 6		day 7		day 8		day 15		day 22	day 22		
	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	Sings of intoxication	No. of dead	No. of emerged imago		
Control	0	stunted development – 1	0	none	0	none	0	none	0	stunted development – 1	4	stunted development – 2	5	31	13.9	86.1
0.32	0	stunted development – 2	0	stunted development – 3	0	stunted development – 4	1	stunted development – 4	1	stunted development – 5	11	stunted development – 6	33	2	94.4	5.6

Validity criteria
The test met the validity criteria of the experiment listed in OECD GD 239 Guideline:
- cumulative larval mortality in control in days 3-8 was 0.0% (required: ≤15%),
- the adults emergence rate in control at day 22 was 86.1% (required: ≥70%)
- the adults emergence rate in reference test at day 22 was 5.6% (required: ≤20%)

Final results
In course of the experiment, the test item has shown no apitoxic effect in mortality of following developmental stages of bees after 22 days of the test. At the end of the study, due to mathematical reasons, the concentration and the dose causing 50% mortality of the population in the test (LC50 and LD50 values) were not determined, however NOEC and NOED values were determined at 22 day.

Parameter	Concentration [mg/kg of food]	Parameter	Dose [µg/larva]
LC_{10}	695.399 (n.d. – n.d.)*	LD_{10}	106.984 (n.d. – n.d.)*
LC_{20}	n.d. (n.d. – n.d.)*	LD_{20}	n.d. (n.d. – n.d.)*
LC_{50}	n.d.** (n.d. – n.d.)*	LD_{50}	n.d.*** (n.d. – n.d.)*
NOEC	≥650.000	NOED	≥100.000

* upper and lower confidence limits (95%)
** **based on the results analysis, value was defined as >650 mg/kg of food**
*** **based on the results analysis, value was defined as >100 µg/larva**
n.d. not determined

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

Comments of zRMS:	The study was performed according to OECD guideline 245 chronic oral toxicity test (10 day feeding test) and according to the principles of GLP. *During the range-finding test, periodic, humidity fluctuations of short duration were recorded as an effect of feedings and observations record, which did not affect the condition of research system.* *During the definitive test, slight increase of temperature above 35°C was noted (required: 33±2°C). The average air humidity was 78.616% (required: 50-70%). The changes were minor, which did not affect the condition of the research system; the study met validity criteria*

	All the validity criteria were met during the test. The study is considered acceptable with following endpoints: NOEC value was determined as >2500 mg formulation/kg; NOED value was determined as >80.508 µg formulation/bee/day (nominal dose 100 µg/bee/day); LC_{50} value was determined as >2500 mg/kg and LD_{50} value was determined as >80.508 µg formulation/bee/day (nominal dose 100 µg/bee/day).

Reference:	KCP 10.3.1.3/01
Report	Honey Bee, Chronic Oral Toxicity Test according to OECD 245, U. Orzechowska; SORBOLAB Research Laboratory LLC, Zaniemyska 11 Street, 61-029 Poznań, Study code: 0038/0010/E, 2020
Guideline(s):	OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:

Name: Etofumesate 500 SC
Test item description: white liquid
Batch No.: PFORM03
Name of active substance: ethofumasate
Content of active substance: 500 g/L
CAS of active substance: 26225-79-6
Production date: 04.2020
Expiration date: 04.2022

Test Species: Apis mellifera L.

Test Design:

stability test:
tested concentrations and control in one replicate
range-finding test:
tested concentrations and control in three replicates, 10 bees per replicate
definitive test:
tested concentration and control in five replicates, 10 bees per replicate
reference test:
tested concentration in three replicates, control in five, 10 bees per replicate

Test Concentration:

stability test:
control; 2.5 mg/kg; 2500 mg/kg of food
range-finding test:
control; 2.5 mg/kg; 25 mg/kg; 250 mg/kg; 2500 mg/kg of food

definitive test (limit test):

	control; 2500 mg/kg of food reference test: control; dimethoate: 0.5 mg/kg of food
Test Conditions:	stability test: storage conditions: average temperature 5.247°C (minimal temperature 4.9°C; maximal temperature 5.9°C); darkness test conditions: average temperature 34.748°C (minimal temperature 34.1°C; maximal temperature 35.1°C); average humidity: 67.464% (minimal humidity 62.1%; maximal humidity 71.0%); darkness range-finding test: average temperature 34.399°C (minimal temperature 33.4°C; maximal temperature 34.5°C); average humidity: 70.292% (minimal humidity 32.7%; maximal humidity 78.2%); darkness definitive test and reference test: average temperature 34.5815°C (minimal temperature 33.6°C; maximal temperature 35.5°C); average humidity: 78.616% (minimal humidity 57.1%; maximal humidity 87.6%); darkness

Results and discussion:

Range-finding test

The range-finding test was performed to determine number and range of concentrations for definitive test.

Test design

The range-finding test was performed for four concentrations of test item and control. Following concentrations of test item were used: 2.5 mg/kg; 25 mg/kg; 250 mg/kg and 2500 mg/kg of food. Each concentration and control were prepared in three replicate, 10 bees per replicate. Feeders were changed daily, every 24 ± 2 hours. Food was prepared daily

Test conditions

- Test duration: 10 days
- Test vessels: cages 20 x 20 x 20 cm
- Lighting: darkness
- Temperature: average temperature 34.399°C (minimal temperature 33.4°C; maximal temperature 34.5°C); humidity: average humidity 70.292% (minimal humidity 32.7%; maximal humidity 78.2%). Temperature and humidity chart during the range-finding test is shown in Chart 3.

Observations and measurements

Mortality of bees in each cage as well as signs of intoxication were recorded every 24 ±2 h, 24 h after first food administration. A honeybee was considered dead which did not react to being touched by a small brush, abdomen, legs, antennas. Signs of the toxic effect of the test item were evaluated according to following categories:

- m (moribund) - bees cannot walk, only very weak movements of legs and antennas, weak responses to stimulation, e.g. light, breeze, bees can recover, but usually die
- a (affected) - bees are standing, trying to walk, there are signs of reduced coordination, over activity, aggressiveness, increased self-cleaning behaviors, rotations, chills)
- c (cramps)
- abdominal or full body cramps
- ap (apathy) - bees show small or delayed responses to stimulations, e.g. light, air; the bees are sitting motionless on the unit)
- v (vomiting).

Results

In the experiment no statistically significant mortality of bees was observed in all tested concentration of

food in comparison to the control. No signs of intoxication were recorded.
.

Statistical analysis
Based on obtained data in range-finding test, statistical analysis was performed in accordance with OECD 245 Guideline using ToxRat Professional statistical software. The values NOEC ≥ 2500 mg/kg and LOEC > 2500 mg/kg of food were determined.

Conclusions
The test item have shown no apitoxic effect in the tested concentrations range.
On this basis, it was decided to perform definitive test as a limit test using one terminal concentration – 2500 mg/kg of food.

Definitive test
Study design
The definitive test was performed for one concentration of test item - 2500 mg/kg of food and control. Each concentration and control were prepared in five replicate, 10 bees per replicate.
Feeders were changed daily, every 24 ± 2 hours. Food was prepared every 4 days.
Test conditions
- Test duration: 10 days
- Test vessels: cages 20 x 20 x 20 cm
- Lighting: darkness
- Temperature: average temperature 34.5815°C (minimal temperature 33.6°C; maximal temperature 35.5°C); humidity: average humidity 78.616% (minimal humidity 57.1%; maximal humidity 87.6%).

Observations and measurements
Mortality of bees in each cage as well as signs of intoxication were recorded every 24h±2 h, 24 h after first food administration. As dead, bee that has not reacted to the touch of abdomen, leg or antennas by fine brush. Signs of the toxic effect of the test item were evaluated according to following categories:
- m (moribund) - dying (bees cannot walk, only very weak movements of legs and antennas, weak responses to stimulation. e.g. light, breeze, bees can recover, but usually die)
- a (affected) - bees are standing, trying to walk, there are signs of reduced coordination, over activity, aggressiveness, increased self-cleaning behaviors, rotations, chills)
- c (cramps) - abdominal or full body cramps
- ap (apathy) - bees show small or delayed responses to stimulations, e.g. light. air; the bees are sitting motionless on the unit
- v (vomiting).

Concentration of test item [mg/kg of food]	*r	day 1		day 2		day 3		day 4		day 5		day 6		day 7		day 8		day 9		day 10		Mortality at the end of test [%]	Statistical significance **
		Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*		
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	6.0	na.
	2	0		0		0		0		0		0		0		0		0		0			
	3	0		0		0		0		1		1		1		1		1		1			
	4	0		0		0		0		0		1		1		1		2		2			
	5	0		0		0		0		0		0		0		0		0		0			
2500	1	0	none	0	none	0	none	0	none	0	none	0	1ap	0	1ap	0	none	0	none	0	none	4.0	-
	2	0		0		0		0		0		1		1		1		1		1			
	3	0		0		0		0		0		0		0		1		1		1			
	4	0		0		0		0		0		0		0		0		0		0			
	5	0		0		0		0		0		0		0		0		0		0			

*r replicate
na. not applicable
- statistical insignificant
* abbreviations explained in point 4.2.4.3.
** statistical calculations using Fisher Test using ToxRat Professional software

During the experiment. the amount of food consumed by bees was registered daily by weighting the feeders before and after administration of food. The consumed amount of food was corrected by the evaporated solution. For this purpose, an additional feeder (previously weighed) filled with food was placed in an empty cage under the test conditions (three replicates were used). During daily change of feeders, the

additional feeder was weighted again and replaced with new one.

Statistical analysis
Based on the obtained results from the definitive test, statistical analysis was performed according to OECD 245 Guideline. Due to the fact of conducting the definitive as limit test, no concentration (LC50 value) nor dose (LDD50 value) causing 50% mortality of the population after 10 days. Using ToxRat Professional statistical software, using Fisher's Test, no statistically significant differences for bee mortality relative to the control were shown. Statistical significance at 5% level ($p \leq 0.05$).

Chemical analysis
The test solutions were prepared every four days. The test solution at the beginning of the solution validity period were subjected to chemical analysis. For determination, analytical method was validated during study under the Study Code 0038/0009/FA based on the Standard Experimental Procedure SPB-FA/11 and guideline SANCO/3029/99 rev. 4. During the validation of the analytical method, the following parameters were determined: selectivity, linearity, accuracy, precision, limit of detection and limit of quantification.

Parameter	Required criterion	Obtained result		
Selectivity	no interfering peaks of other substances. which surface is exceeding 3% active substance area are present at the place of peak of active substance module in test item solution in the conditions of analysis, active substance time of retention in module solution and in solution of tested sample are comparable (no difference higher than 2%)	no interfering peaks of other substances. which surface is exceeding 3% active substance area are present at the place of peak of active substance module in test item solution in the conditions of analysis, active substance time of retention in module solution and in solution of tested sample is comparable (below 2%)		
Linearity	$r \geq 0.99$	r = 0.999 calibration curve: 2.60188 mg/L – 50.23321 mg/L		
Accuracy [%]	70-110	level I	105.1	104
		level II	101.9	
Precision [% RSDr]	≤ 20	level I	4.10	
		level II	0.99	
Limit of detection [mg/kg]	-	0.51078		
Limit of quantification [mg/kg]	-	0.16856		

The method consisted of active substance - ethofumesate determination, next recalculation of determined concentration to test item content in 50% sucrose solution. Determination was conducted using liquid chromatography method with UV-DAD detection in accordance with Standard Experimental Procedure SPB-FA/177 using calibration curve plotted during analytical method validation. Stability of calibration curve was checked before each analysis in accordance with SPT-FA/80.

Preparation of samples for chromatographic analysis
The test item samples in 50% sucrose solution were analyzed in the range of calibration curve after presumptive dilution with 50% sucrose solution. About 5 g of sample with accuracy to 0,01 mg was weighted into the test tube. Next, 0.5 g of sodium chloride and 2 mL of ethyl acetate was added. Whole was shaken on the tube roller for 45 minutes. After the end of extraction, 1mL of ethyl acetate was transferred into Eppendorf tube and then completely evaporated in the sample concentrator. Next, 1 mL of methanol was added to the tube with residue, whole was mixed, filtered through syringe filter into chromatographic vial and subjected to the analysis.

Reagents and materials
- acetonitrile, HPLC p., POCH, 0811/05/20
- methanol, HPLC p., Honeywell, batch no. J357C
- ethyl acetate, Chempur, batch no. 19/07/01; Avantor, batch no. 0850/10/18
- sodium chloride, Chempur, batch no. 18/08/58
- ethofumesate standard, IPO Warsaw, batch no. 6C/20
- deionized water
- ultrapure water
- 50% sucrose solution
- analytical balance Radwag XA 82_220.4Y.A
- precise balance Ohaus PA2102CM/1
- roller shaker RM10V-W
- sample concentrator Stuart SMP3
- vaccum pump Silent19 type PL 2/4
- high pressure liquid chromatography analyzer Shimadzu Nexera X2 series LC-30 with PDA detector
- automatic pipettes: Acura manual 826 XS, Transferpette S 5 mL, Transferpette 1 mL
- deionizator Solpure 78
- water purification system Millipore Synergy UV
- ultrasonic water bath Sonic-10
- timer ISOLAB
- syringes and syringe filters 0,22 µm

Chromatographic conditions
Column: Kinetex C18, 5 □m, 100 Å, 4.6x150 mm
Detection: 275 nm
Introduced volume: 10 µL
Column thermostat temperature: 30ºC
Mobile phase: ultrapure water : acetonitrile (40 : 60)
Mobile phase flow: 1.5 mL/min

Results
Active substance concentration in analyzed solutions was obtained with chromatographic set control program and recalculated using formula:

$$C_{mg/kg} = \frac{C_{ozn} * 2}{m_e}$$

where:
Cmg/kg ethofumesate concentration in sucrose solution [mg/kg]
Cozn active substance concentration obtained from calibration curve [mg/L]
me sample weight taken for extraction [g]
Next, obtained result was recalculated by presumptive dilution using the formula:

$$C_{SA} = \frac{C_{mg/kg} * m_r}{m_p}$$

where:
CSA active substance concentration in undiluted sample [mg/kg]
Cmg/kg ethofumesate concentration in sucrose solution [mg/kg]
mr sample weight after dilution with 50% sucrose solution (in case of no dilution mr=1) [g]
mp undiluted sample weight taken for dilution (in case of no dilution mp=1) [g]
Based on the calculated active substance concentration, test item concentration in 50% sucrose solution was calculated using the formula:

$$C_{BM} = \frac{C_{SA} * 100\%}{C}$$

where:
CBM test item concentration in 50% sucrose solution [mg/kg]
CSA active substance concentration in undiluted sample [mg/kg]
C active substance concentration in test item [% (m/m)]
(C – calculated based on nominal active substance concentration given by the Sponsor and test item density; density determined in accordance with SPB- FA/02 Procedure).
Final results of test item concentration determination were given as arithmetic mean of all replicates in mg/kg with accuracy to 0.01 mg/kg.

Date of analysis	Sample labeling by Ecotoxicology laboratory	Sample labeling by Physicochemistry and Analytics laboratory	Determination of active substance ethofumesate concentration [mg/L]	Weight taken for extration [g]	Ethofumesate concentration [mg/kg]	Sample weight taken for dilution [g]	Sample weight after dilution [g]	Ethofumesate concentration after including dilution [mg/kg]	Test item concentration [mg/kg]	Average test item concentration [mg/kg]
24.07.2020	Kontrola	404/2020 1	0.00000	5.02237	0.00000	na.	na.	0.00000	0.00000	0.00
		404/2020 2	0.00000	5.03178	0.00000	na.	na.	0.00000	0.00000	
	2500 mg/kg	405/2020 1	28.57104	5.03531	11.34827	0.21220	21.44518	1146.86988	2559.97742	2499.53
		405/2020 2	26.46203	5.01859	10.54560	0.20345	21.08098	1092.70905	2439.08269	
28.07.2020	Kontrola	443/2020 1	0.00000	5.18596	0.00000	na.	na.	0.00000	0.00000	0.00
		443/2020 2	0.00000	5.11128	0.00000	na.	na.	0.00000	0.00000	
	2500 mg/kg dzień 4	444/2020 1	17.56545	5.03218	6.98125	0.20760	27.83378	936.00453	2089.29582	2132.86
		444/2020 2	17.57314	5.04428	6.96755	0.19985	27.96696	975.03743	2176.42283	
31.07.2020	Kontrola	454/2020 1	0.00000	5.05581	0.00000	na.	na.	0.00000	0.00000	0.00
		454/2020 2	0.00000	5.11225	0.00000	na.	na.	0.00000	0.00000	
	2500 mg/kg dzień 7	455/2020 1	36.93191	5.15182	14.33742	0.16344	11.09500	973.28501	2172.51119	2175.07
		455/2020 2	36.87754	5.14896	14.32427	0.16311	11.10885	975.57561	2177.62414	

na. not applicable

Reference test
The test with the reference item - dimethoate was conducted in parallel with the definitive test. The aim of the study was to determine the sensitivity of the honey bee (Apis mellifera L.) used for test. The concentration causing mortality of 50% of the population in the test after 10 days was determined.

Study design
One concentration of reference item was used: 0.5 mg/kg of food and control. The test was performed in triplicate for concentration and in five replicate for control, 10 bees each in each replicate. The test conditions and observations were the same as in definitive test.

Results of reference test
During the test, tested concentration of reference item 0.5 mg/kg of food caused mortality 73.3% of population. The reference item in the course of the study showed apitoxic effects on the honey bee (Apis mellifera, L.). The results obtained are in accordance with the requirements of the OECD Guideline 245 (required: ≥ 50%) and confirm the correct response of the test system.

Concentration of test item [mg/kg of food]	*r	day 1		day 2		day 3		day 4		day 5		day 6		day 7		day 8		day 9		day 10		Mortality at the end of test [%]	Statistical significance **
		Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*		
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	6.0	na.
	2	0		0		0		0		0		0		0		0		0		0			
	3	0		0		0		0		1		1		1		1		1		1			
	4	0		0		0		0		0		1		1		1		2		2			
	5	0		0		0		0		0		0		0		0		0		0			
0.5	1	0	none	0	none	0	1ap	0	1ap	0	1ap	0	1ap	0	1ap	2	2ap	3	2ap	4	2ap	73.3	+
	2	0		0		0		0		0		2		5		8		8		9			
	3	0		0		0		2		3		4		4		5		8		9			

*r replicate
na. not applicable
+ statistical significant
* abbreviations explained in point 4.2.4.3.
** statistical calculations using Fisher Test using ToxRat Professional software

Validity criteria
The test met the validity criteria of the experiment:
- bee mortality in control after 10 days was 6.0% (required: ≤15%)
- bee mortality in the reference test after 10 days was 73.3% (required: ≥50%)

Final results and conclusions
In the course of the test, test item have shown no apitoxic effect on bee mortality after 10 days of test. The test item is nontoxic in the concentration of 2500 mg/kg of food. Due to the fact of conducting the definitive test for terminal concentration (limit test), concentration causing mortality of 50% of the population in the study (LC50 value) and dose of test item per bee causing mortality of 50% of the population after 10 days (LDD50 value) were not determined on the basis of statistical analysis, as well as NOEC and NOEDD values. On the basis of data analysis, NOEC value was determined as >2500 mg/kg; NOEDD value was determined as >80.508 µg/bee/day (nominal dose 100 µg/bee/day); LC_{50} value was determined as >2500 mg/kg and LDD_{50} value was determined as >80.508 µg/bee/day (nominal dose 100 µg/bee/day).

Honey bee mortality at the end of the study			
Test item concentration	**Mortality [pcs.]**	**Mortality [%]**	**Statistical significance in comparison to the control***
Control	3	6.0	not applicable
2500 mg/kg of food (which corresponds to 1000 µg/bee)	2	4.0	-

- statistically insignificant
* for statistical calculations was used Fisher's Test using ToxRat Professional Software

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional studies were performed.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional studies were performed.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional studies were performed.

A 2.3.1.7 KCP 10.3.1.6 Non target arthropods studies

A 2.3.1.7.1 Study 1

Comments of zRMS:	The study follows the guideline specified by Blümel et al. (2000) and according to the principles of GLP. The study was conducted according to the guidelines developed by the IOBC, BART and EPPO Joint initiative, SOP/B/23 and other procedures related to the study and the Study Plan. However, in the experimental part of the study the following deviations from the mentioned documents occurred: – According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen is used. However, in the experiment additional food in the form of the two-spotted spider mite (*T. urticae*) eggs, was used. Another food source prevents the mites from escaping from discs. It is noted that effects of CHR/H/ETO 500 SC on reproduction were not evaluated, however according to ESCORT guidance: *''The way in which the HQ value is derived makes it unnecessary to include sub-lethal or reproductive endpoints at the first tier of risk assessment. This is particularly important as sub-lethal assessments for non-target arthropods to date have been associated with significant technical difficulties e.g. extremely variable fecundity''* As the study represents Tier I evaluation, effects on reproduction is deemed not necessary and the derived LR_{50} is sufficient for the risk assessment. The study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} > 1.7 L formulation/ha corresponding to 889.1 g ethofumesate/ha $NOER_{mortality}$ = 0.85 L formulation/ha

Reference:	KCP 10.3.1.6/01
Report	A laboratory test for evaluating the effects of CHR/H/ETO 500 SC on the predatory mite, Typhlodromus pyri (Sch.), E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B-65-20, 2020
Guideline(s):	according to the ESCORT 1 (Barrett K. L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	No
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	name: CHR/H/ETO 500 EC active substance: 523.0 g/L of ethofumesate batch number: PF0RM03 production date: 04.2020 expiry date: 04.2022
Test Species:	the predatory mite, Typhlodromus pyri (Sch.) (Acari: Phytoseiidae) – age: 24-hour-old protonymphs – source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies; the culture was obtained from commercial breeder
Test Design:	5 study groups: - a control group (0.0 L/ha) - CHR/H/ETO 500 SC at the rate of 0.43 L/ha - CHR/H/ETO 500 SC at the rate of 0.85 L/ha - CHR/H/ETO 500 SC at the rate of 1.7 L/ha - Bi 58 Top 400 EC at the rate of 9.0 mL/ha number of replicates: 3 replicates/group number of mites in each replicate: 20
Endpoints:	cumulative juvenile mortality 7 days after treatment,
Test Conditions:	– temperature: 23.5 – 25.0ºC – relative air humidity: 63 – 76% – photoperiod: 16 hours light : 8 hours dark – light intensity: 669 lux

Results and discussion:

Mortality *of Typhlodromus pyri*

In the preliminary test, mortality of the control group after 7 days of exposure was 2.5%. After 7 days of exposure to CHR/H/ETO 500 EC at the rates of 0.27, 0.68 and 1.7 L/ha, the *T. pyri*, corrected [1] mortality percentages were equal to 12.8, 18.0 and 35.9%, respectively.

Table 2. Mortality of *T. pyri* after 7 days of exposure – preliminary non GLP test

Study group [rate] [L/ha]	Number of tested mites [no.]	Mortality				
		Number of dead & escaped mites [no.]		Total dead & escaped		
		Replicates				
		I	II	[no.]	[%]	Corr [a] [%]
CHR/H/ETO 500 EC						
Control	40	0	1	1	2.5	–
0.27	40	3 + 2[e]	1	6	15.0	**12.8**
0.68	40	3	2 + 3[e]	8	20.0	**18.0**
1.7	40	5 + 3[e]	6 + 1[e]	15	37.5	**35.9**

[a]: mortality corrected according to the Abbott's formula [12]
x[e]: number of escaped mites
The preliminary non-GLP test was performed between 27.08 – 03.09.2020.

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to CHR/H/ETO 500 EC at the rates of 0.43, 0.85 and 1.7 L/ha, the *T. pyri*, mortality percentages were equal to 6.7, 3.3 and 21.7%, respectively.There were no statistically significant differences in mortality between the groups treated with the test item at the rates of 0.43 and 0.85 L/ha in comparison to the control group (Step-down Cochran-Armitage test procedure, $p > 0.01$). There was statistically significant difference in mortality between group treated with the test item at rate of 1.7 L/ha in comparison to the control group (Step-down Cochran-Armitage test procedure, $p < 0.01$). On the basis of the obtained results the LR50 value is higher than 1.7 L/ha. The NOERmortality value is equal to 0.85 L/ha. After 7 days of exposure to Bi 58 Top 400 EC at the rate of 9.0 mL/ha, the mortality was 86.7%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate. Based on the results it can be stated that CHR/H/ETO 500 EC/ha has an adverse effect on mortality of the tested organisms at the rate of 1.7 L/ha.

Table 3. Mortality of *T. pyri* after 7 days of exposure – definitive test

Study group [rate] [L/ha]	Number of tested mites [no.]	Mortality				
		Number of dead & escaped mites [no.]			Total dead & escaped	
		Replicates				
		I	II	III	[no.]	[%]
CHR/H/ETO 500 EC						
Control	60	0	0	0	0	**0.0**
0.43	60	2	1	1	4	**6.7**
0.85	60	1	1^e	0	2	**3.3**
1.7+	60	5	2	$5 + 1^e$	13	**21.7**
LR_{50}		**> 1.7 [L/ha]**				
$NOER_{mortality}$		**0.85 [L/ha]**				
[mL/ha]	**Bi 58 Top 400 EC**					
9.0	60	$17 + 1^e$	18	16	52	**86.7**

$^+$: statistically significant difference [10], [SOP/B/67]
x^e: number of escaped mites

The definitive test was performed between 25.09 – 02.10.2020.

Escaped individuals *of Typhlodromus pyri*
In the control group there were no escaped mites observed. The percentages of escaped mites at the rates of 0.43, 0.85 and 1.7 L/ha were 0.0, 1.7 and 1.7%, respectively. From the obtained results, the LR50 and NOER values for escape could not be determined. In regards to the mites that escaped during the study, no statistically significant difference was found at all the tested rates.

TEST VALIDITY CRITERIA
The following validity criteria were met during the study:
- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
- corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 86.7% on day 7 of exposure (criterion: from 50 to 100%).

A 2.3.1.7.2 Study 2

Comments of zRMS:	The study follows the guideline specified by Mead Briggs M.A. et al. (2000) and according to the principles of GLP. In the definitive test all the validity criteria were met. It is noted that effects on reproduction were not investigated, however according to ESCORT guidance: ‘*’The way in which the HQ value is derived makes it unnecessary to include sub-lethal or reproductive endpoints at the first tier of risk assessment. This is particularly important as sub-lethal assessments for non-target arthropods to date have been associated with significant technical difficulties e.g. extremely variable fecundity*’’ As the study represents Tier I evaluation of effects on reproduction is deemed not necessary and the derived LR_{50} is sufficient for the risk assessment. The study is considered acceptable with following endpoints relevant for the risk assessment: LR_{50} > 1.7 L formulation/ha corresponding to 889.1 g ethofumesate/ha $NOER_{mortality}$ = ≥ 1.7 L formulation/ha

Reference:	KCP 10.3.1.6/02
Report	A laboratory test for evaluating the effects of CHR/H/ETO 500 SC on the parasitic wasp, Aphidius rhopalosiphi (De Stephani – Perez, E. Kulec-Płoszczyca; Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: B-66-20, 2020
Guideline(s):	according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	name: CHR/H/ETO 500 EC active substance: 523.0 g/L of ethofumesate batch number: PF0RM03 production date: 04.2020 expiry date: 04.2022
Test Species:	the parasitic wasp, Aphidius rhopalosiphi (De Stephani-Perez); Hymenoptera: Braconidae, Aphiidinae – age: adult wasps (24 - 48 hours after emerging from mummies)

– source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies; the culture was obtained from commercial breeder

Test Design:
5 test groups:
– a control group (0.0 L/ha)
– CHR/H/ETO 500 SC at the rate of 0.43 L/ha
– CHR/H/ETO 500 SC at the rate of 0.85 L/ha
– CHR/H/ETO 500 SC at the rate of 1.7 L/ha
– Bi 58 Top 400 EC at the rate of 0.12 mL/ha (0.48 g a.i./ha)
number of replicates: 4 replicates/group
number of wasps: 10 wasps/replicate
.

Endpoints:
– wasp mortality after 48 hours of exposure
– LR50 and the NOERmortality

Test Conditions:
temperature: 19 – 20°C
– relative air humidity: 63 – 65%
– photoperiod: 16 hours light: 8 hours dark
– light intensity: mortality assessment: 2902 lx

Results and discussion:

Mortality of A. rhopalosiphi
In the non-GLP preliminary test mortality in the control group was 0.0%. After 48 hours of the exposure to CHR/H/ETO 500 SC at the rates of 0.27, 0.68 and 1.7 L/ha, the percentages of A. rhopalosiphi, mortality were 0.0%.

Table 2. Mortality of *A. rhopalosiphi* after 48 hours – preliminary non-GLP test

Study group [L/ha]	Tested wasps [no.]	Mortality			
		Dead wasps [no.]		Total	
		Replicates			
		I	II	[no.]	[%]
Control	20	0	0	0	0
CHR/H/ETO 500 SC					
0.27	20	0	0	0	0.0
0.68	20	0	0	0	0.0
1.7	20	0	0	0	0.0

The preliminary test was performed between 18 – 20.08.2020.

In the definitive test, mortality of the control group, after 48 hours, was 0.0%. After 48 hours of the exposure to CHR/H/ETO 500 SC at the rates of 0.43, 0.85 and 1.7 L/ha, the percentages of mortality of A. rhopalosiphi, were 0.0% On the basis of the obtained mortality results, the LR50 value is higher than 1.7 L/ha and NOERmortality is higher than or equal to 1.7 L/ha.

Study group [application rate] [L/ha]	Tested wasps [no.]	Mortality					
		Dead wasps [no.]				Total	
		Replicates					
		I	II	III	IV	[no.]	[%]
Control	**40**	0	0	0	0	**0**	**0**
CHR/H/ETO 500 SC							
0.43	**40**	0	0	0	0	**0**	**0**
0.85	**40**	0	0	0	0	**0**	**0**
1.7	**40**	0	0	0	0	**0**	**0**
LR_{50} [L/ha]	**> 1.7**						
$NOER_{mortality}$ [L/ha]	**≥ 1.7**						

The definitive test was performed between 17 – 19.09.2020.

Mortality of the wasps exposed to Bi 58 Top 400 EC at the rate of 0.12 mL/ha, after 24 hours, was 82.5%. Therefore, the validity criterion specified in the Method description was met. The results showed that the test organisms were sensitive to dimethoate

TEST VALIDITY CRITERIA
The following validity criteria were met during the study:
– after 48 hours mortality of the control group was 0.0% (criterion: a maximum of 13.0%),
– after 24 hours mortality of the group treated with the reference item at the rate of 0.12 mL/ha was 82.5% (criterion: from 75 to 100%).

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviations occurred during the study. In the definitive test all the validity criteria were met according to OECD Guideline No. 222. No deviations from OECD Guideline occurred. Overall, the study is considered acceptable.

Reference:	KCP 10.4.1/01
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Earthworm reproduction test (Eisenia andrei), A. Gierbuszewska; Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-26-20, 2020
Guideline(s):	According to the OECD Guideline No. 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch no.: PF0RM03
Test Species:	the earthworm, Eisenia andrei obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology
Test Design:	test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate
Endpoints:	EC10, EC20, EC50, NOEC, LOEC (reproduction) LC50, NOEC, LOEC (survival)
Test Concentration:	control, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, and 320.0 mg/kg dry weight of the artificial soil
Test Conditions:	temperature: 19.4 – 22.0°C;

pH at the beginning of the experiment: 5.89 – 5.97;
pH at the end of the experiment: 5.50 – 5.59;
soil moisture content at the beginning of the experiment: 23.3 – 25.1% (46.2 – 49.8% of the maximum water holding capacity);
soil moisture content at the end of the experiment: 21.6 – 23.1% (43.0 – 46.0% of the maximum water holding capacity);
light-dark cycle: 16h : 8h;
light intensity at the beginning of the experiment: 544 – 674 lux
light intensity at the end of the experiment: 586 – 632 lux

Results and discussion:

Mortality of the adult earthworms

After 4 weeks of the experiment, at the control group mortality of adult earthworms was equal to 2.5%. At concentrations ranging from 1.8 to 320.0 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0% and 5%. The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 320 mg of the test item/kg dry weight of artificial soil (148.106 mg of ethofumesate/kg dry weight of artificial soil).

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of ethofumesate / kg dry weight of artificial soil]
EC_{10}	81.814 (45.320 – 109.841)	37.866 (20.976 – 50.838)
EC_{20}	125.793 (87.192 – 154.860)	58.221 (40.355 – 71.674)
EC_{50}	286.465 (237.917 – 382.864)	132.585 (110.116 – 177.202)
NOEC (reproduction)	100	46.283
LOEC (reproduction)	180	83.310
LC_{50}	> 320	> 148.106
NOEC (survival)	≥ 320	≥ 148.106
LOEC (survival)	> 320	> 148.106

Observations of the earthworms

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After 4 weeks of the experiment, at the concentrations between 1.8 and 320.0 mg of the test item/kg dry weight of the artificial soil, the changes in appearance and behaviour of the adult earthworms were not observed

Table 6. Results of the observations of the adult earthworms (*Eisenia andrei*) for changes in behavior and in morphology.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Changes in behaviour and in morphology
0.0 (control)	1	10	8 nc 2 d
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
	5	10	10 nc
	6	10	10 nc
	7	10	10 nc
	8	10	10 nc
1.8	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	9 nc 1 d
3.2	1	10	10 nc
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc
5.6	1	10	10 nc
	2	10	8 nc 2 d
	3	10	10 nc
	4	10	10 nc
10.0	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
18.0	1	10	10 nc
	2	10	10 nc
	3	10	9 nc 1 d
	4	10	10 nc
32.0	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
56.0	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
100.0	1	10	10 nc
	2	10	10 nc
	3	10	10 nc
	4	10	10 nc
180.0	1	10	9 nc 1 d
	2	10	10 nc
	3	10	10 nc
	4	10	9 nc 1 d
320.0	1	10	9 nc 1 d
	2	10	9 nc 1 d
	3	10	10 nc
	4	10	10 nc

nc – no changes, d – dead earthworm

Body weights of the living adult earthworms

After 4 weeks of the exposure period of the test item at the concentrations ranging from 1.8 to 20.0 mg/kg dry weight of artificial soil, the body weight increase was between -18.1 and 35.2%. As for the control group, the body weight increase was equal to 32.6%.

Impact of the test item on reproduction of the earthworms

After the application of the test item at the concentrations ranging from 1.8 to 320.0 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 61.0 and 156.8 per replicate. The mean number of juveniles in the control group was equal to 138.5 per replicate.

After 8 weeks of the experiment, it was concluded that ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 180.0 to 320.0 mg/kg dry weight of artificial soil. The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 81.814 mg/kg dry weight of the artificial soil (37.866 mg of ethofumesate / kg dry weight of artificial soil). The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 125.793 mg/kg dry weight of the artificial soil (58.221 mg of ethofumesate / kg dry weight of artificial soil). The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is equal to 286.465 mg/kg dry weight of the artificial soil 132.585 mg of ethofumesate / kg dry weight of artificial soil). The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal to 100 mg/kg dry weight of the artificial soil (46.283 mg of ethofumesate / kg dry weight of artificial soil). The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is equal to 180 mg/kg dry weight of the artificial soil (83.310 mg of ethofumesate / kg dry weight of artificial soil).

Observations of the juveniles of earthworms

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After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles after 8 weeks of the experiment [no.]	Changes in behaviour and in morphology
0.0 (control)	1	128	nc
	2	102	nc
	3	119	nc
	4	162	nc
	5	144	nc
	6	164	nc
	7	121	nc
	8	168	nc
1.8	1	177	nc
	2	159	nc
	3	156	nc
	4	135	nc
3.2	1	135	nc
	2	129	nc
	3	106	nc
	4	171	nc
5.6	1	148	nc
	2	125	nc
	3	142	nc
	4	123	nc
10.0	1	137	nc
	2	154	nc
	3	132	nc
	4	149	nc
18.0	1	99	nc
	2	127	nc
	3	117	nc
	4	161	nc
32.0	1	131	nc
	2	126	nc
	3	133	nc
	4	122	nc
56.0	1	149	nc
	2	151	nc
	3	170	nc
	4	93	nc
100.0	1	148	nc
	2	114	nc
	3	86	nc
	4	112	nc
180.0	1	96	nc
	2	77	nc
	3	97	nc
	4	124	nc
320.0	1	56	nc
	2	49	nc
	3	90	nc
	4	49	nc

Results of the reference test

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According to the OECD Guideline No. 222, the LOEC should be between 1 – 5 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Concentration [mg/kg dry soil]	Replicate	Number of juveniles [no.]	Mean ±SD	Comparison to the control [%]	CV* [%]
0.0 (control with acetone)	1 2 3 4 5 6 7 8	147 143 127 136 134 149 174 123	141.6 ± 16.0	-	11.3
0.0 (control)	1 2 3 4 5 6 7 8	138 166 160 154 163 172 144 127	153.0 ± 15.4	108.0	10.1
1.0	1 2 3 4	148 151 136 121	139.0 ± 13.6	98.1	9.8
1.5	1 2 3 4	135 120 146 139	135.0 ± 11.0	95.3	8.1
2.25	1 2 3 4	102 109 123 132	116.5+ ± 13.5	82.3	11.6
3.37	1 2 3 4	88 130 91 60	92.3+ ± 28.8	65.1	31.2
5.0	1 2 3 4	39 31 18 27	28.8+ ± 8.7	20.3	30.4
NOEC (mg/kg dry weight of the artificial soil)		1.50			
LOEC (mg/kg dry weight of the artificial soil)		2.25			

* - coefficient of variation
+- statistically significant differences comparison of treatments with "control A" by the Williams Multiple Sequential t- test Procedure (alpha = 0.05, one-sided smaller)

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the controls:
- each replicate produced from 102 to 168 juveniles (138.5 mean) at the end of the experiment (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 17.8% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 2.5% (criterion: ≤ 10%).

A 2.4.1.2 Earthworms - field studies

No additional studies were performed.

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 232 and according to the principles of GLP. Following deviations to the guideline were noted: - culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016), - at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016), - physiological or pathological symptoms or distinct changes in behavior were not described Since In the definitive test all the validity criteria were met according to OECD Guideline No. 232: the deviations did not affect the results of the study. The study is reliable and suitable for the risk assessment with following endpoints: Endpoint values - the impact of the test item on reproduction of collembolans (*Folsomia candida*). EC_{10}= 63.8 (mg formulation/kg dry weight of the artificial soil) EC_{50}= 142.3 (mg formulation/kg dry weight of the artificial soil) NOEC= 56.0 (mg formulation/kg dry weight of the artificial soil) The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below. LC_{10} = > 1000 (mg formulation/kg dry weight of the artificial soil) LC_{50}= >1000 (mg formulation/kg dry weight of the artificial soil) NOEC= > 1000 (mg formulation/kg dry weight of the artificial soil)

Reference:	KCP 10.4.2/01
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Collembolan (Folsomia candida) Reproduction Test, A. Wróbel; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-27-20, 2020
Guideline(s):	according to the OECD Guideline No. 232 (2016)
Deviations:	Yes ~~No~~

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch no. PFORM03
Test Species:	the collembolan, Folsomia candida obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Toxicology. The collembolans used in the study were 9 – 11 days old.
Test Design:	Exposure time: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Test Concentration:	a control, 5.6, 10, 18, 32; 56; 100; 180; 320; 560; 1000 mg of the test item/kg of dry weight of the artificial soil
Test Conditions:	temperature: 18.0 – 20.0°C; pH at the beginning of the test: 5.68 – 5.87; pH at the end of the test: 5.60 – 5.65; soil moisture content at the beginning of the test: 14.6 – 15.7% (41.4 – 44.5% of the maximum water holding capacity); soil moisture content at the end of the test: 14.1 – 15.0% (40.2 – 42.6% of the maximum water holding capacity); lighting: 16 h light and 8h dark; light intensity at the beginning of the experiment: 555.8 – 577.3 lux; light intensity at the end of the experiment: 555.7 – 622.5 lux

Results and discussion:

Mortality

Table 6. Endpoint values - the impact of the test item on the mortality of adult collembolans *(Folsomia candida)*.

Endpoint	Value [mg test item /kg dry weight of the artificial soil]	Value [mg of ethofumesate/kg dry weight of the artificial soil]
LC_{10}	>1000	> 462.8
LC_{20}	> 1000	> 462.8
LC_{50}	> 1000	> 462.8
NOEC	≥ 1000	≥ 462.8

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At the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil, the mortality of adults was between 10.0 and 12.5%. As for the control group, it was equal to 10.0%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is above 1000 mg/kg dry weight of the artificial soil (462.8 mg of ethofumesate/kg dry weight of the artificial).

Impact on reproduction

Table 8. Endpoint values - the impact of the test item on reproduction of collembolans *(Folsomia candida)*.

Endpoint	Value [mg test item /kg dry weight of the artificial soil]	Value [mg of ethofumesate/kg dry weight of the artificial soil]
EC_{10}	63.8 (57.1 – 69.9)	29.5 (26.4 – 32.3)
EC_{20}	87.8 (81.5 – 93.5)	40.7 (37.7 – 43.3)
EC_{50}	142.3 (137.2 – 147.4)	65.8 (63.5 – 68.2)
NOEC	56.0	25.9

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 320 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 30 and 1219 per replicate. After the exposure of collembolans to the test item at the concentrations 560 and 1000 mg/kg dry weight of the artificial soil no juveniles was observed. As for the control group, the number of juveniles was equal to 1041 per replicate.

The obtained results led to the following conclusions:
- The concentration of Etofumesate 500 SC (CHR/H/ETO 500 SC) causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 63.8 mg/kg dry weight of the artificial soil (i.e. 29.5 mg of ethofumesate/kg dry weight of the artificial).
- The concentration of Etofumesate 500 SC (CHR/H/ETO 500 SC) causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 87.8 mg/kg dry weight of the artificial soil (i.e. 40.7 mg of ethofumesate /kg dry weight of the artificial).
- The concentration of Etofumesate 500 SC (CHR/H/ETO 500 SC) causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is equal to 142.3 mg/kg dry weight of the artificial soil (i.e. 65.8 mg of ethofumesate /kg dry weight of the artificial).
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 56 mg/kg dry weight of the artificial soil (i.e. 25.9 mg of ethofumesate /kg dry weight of the artificial).

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 10.0% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 1041 (criterion: ≥100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 19.2% (criterion: ≤ 30%)

A 2.4.2.1.2 Study 2

Comments of zRMS:	The study was conducted to OECD guideline 226 and according to the principles of GLP. Following deviations to the guideline were noted: however they did not affect the results, since all validity criteria were met. 1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.5.6). 2. Due to the use of the temperature extraction method there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution The study is reliable and suitable for the risk assessment with following endpoints: NOEC ≥ 1000 mg formulation/kg dw artificial soil LC_{10} > 1000 mg formulation/kg dw artificial soil

Reference:	KCP 10.4.2/02
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, P. Pieczka; Łukasiewicz Research Network – Institute of Industrial Organic Chemis-try, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-28-20, 2020
Guideline(s):	according to the OECD Guideline No. 226 (2016)
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch number: PFORM03
Test Species:	the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.
Test Design:	exposure period: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Endpoints:	EC10, EC20, EC50, NOEC

	LC10, LC20, LC50, NOEC
Test Concentration:	a control, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry weight of the artificial soil.
Test Conditions:	temperature: 18.0 – 20.0°C pH at the beginning of the test: 5.71 – 5.88 pH at the end of the test: 5.74 – 5.85 soil moisture content at the beginning of the test: 15.5 – 16.6% (44.1 – 47.2% of the maximum water holding capacity) soil moisture content in the middle of the test: 15.7 – 16.4% (44.6 – 46.6% of the maximum water holding capacity) soil moisture content at the end of the test: 15.3 – 16.0% (43.5 – 45.5% of the maximum water holding capacity) light-dark cycle: 16 h light and 8 h dark light intensity at the beginning of the test: 587 – 603 lux light intensity at end of the test: 579 – 612 lux

Results and discussion:

Mortality of adult females

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of the artificial soil was between 2.5% and 10.0%. In the control group mortality of predatory mites was equal to 3.8%. The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is higher than 1000 mg/kg dry weight of the artificial soil (i.e. 462.8 mg/kg dry weight of the artificial soil).

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of ethofumesate/ kg dry weight of the artificial soil]
LC_{10}	>1000	>462.8
LC_{20}	>1000	>462.8
LC_{50}	>1000	>462.8
NOEC	**≥1000**	**≥462.8**

Impact on reproduction

After the application of the test item at the concentrations ranging from 5.6 to 1000 mg/kg dry weight of

the artificial soil, the mean number of juveniles was between 102.8 – 119.3 per replicate. The mean number of juveniles in the control group was equal to 110.5 per replicate.

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of captan/ kg dry weight of the artificial soil]
EC_{10}	>1000	>462.8
EC_{20}	>1000	>462.8
EC_{50}	>1000	>462.8
NOEC	**≥1000**	**≥462.8**

The obtained results led to the following conclusions:
• The concentration of the test item causing a 10% reduction in the number of mites produced within the exposure period (EC10) is higher than 1000 mg/kg dry weight of the artificial soil (i.e. 462.8 mg/kg dry weight of the artificial soil).
• The concentration of the test item causing a 20% reduction in the number of mites produced within the exposure period (EC20) is higher than 1000 mg/kg dry weight of the artificial soil (i.e. 462.8 mg/kg dry weight of the artificial soil).
• The concentration of the test item causing a 50% reduction in the number of mites produced within the exposure period (EC50) is higher than 1000 mg/kg dry weight of the artificial soil (i.e. 462.8 mg/kg dry weight of the artificial soil).
The highest concentration at which the test item is observed to have no statistically significant effects on mite reproduction (NOEC) is higher than or equal to 1000 mg/kg dry weight of the artificial soil (i.e. 462.8 mg/kg dry weight of the artificial soil).

Results of the reference test

Table 10. Reference substance – boric acid. Number of juvenile mites (*Hypoaspis aculeifer*) after 14 days of the exposure period.

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV [%]
0 (control)	1 2 3 4 5 6	254 236 275 211 215 223	235.7 ± 24.8	-	10.5
15	1 2	266 233	249.5 ± 23.3	105.9	9.4
22	1 2	231 262	246.5 ± 21.9	104.6	8.9
32	1 2	250 201	225.5 ± 34.7	95.7	15.4
46	1 2	206 235	220.5 ± 20.5	93.6	9.3
68	1 2	250 208	229.0 ± 29.7	97.2	13.0
100	1 2	221 206	213.5 ± 10.6	90.6	5.0
150	1 2	212 207	209.5 ± 3.5	88.9	1.7
220	1 2	191 155	173.0* ± 25.5	73.4	14.7
320	1 2	0 0	0.0* ± 0.0	0.0	-
460	1 2	0 0	0.0* ± 0.0	0.0	-
680	1 2	0 0	0.0* ± 0.0	0.0	-
1000	1 2	0 0	0.0* ± 0.0	0.0	-
EC_{50}	mg/kg dry weight of the artificial soil	**246.439** (230.912 – 271.712)			

* - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, one-sided smaller.

The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 246.44 mg/kg dry weight of the artificial soil. According to the OECD Guideline No. 226 (2016), the EC50 should be between 100 and 500 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

VALIDITY CRITERIA
The results are considered valid because the following criteria were satisfied in the control:
• mean adult mortality: 3.8% (criterion: ≤ 20%).
• the mean number of juveniles per vessel at the end of the test: 110.5 (criterion: ≥ 50 juveniles at the end of the test.
• the coefficient of variation for the number of juveniles: 21.5 % (criterion: ≤ 30%).

A 2.4.2.2 KCP 10.4.2.1 Species level testing

No additional studies were performed.

A 2.4.2.3 KCP 10.4.2.2 Higher tier testing

No additional studies were performed.

A 2.5 KCP 10.5 Effects on soil transformation

A 2.5.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. Following deviations occurred in the study: - According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower, and the extraction lasted longer - The predicted environmental concentration (PEC) was calculated assuming 2.5 cm of the soil depth according to the German conditions for the substances with the mobility in soil KFoc > 500 mL/g and correspondence with the Sponsor. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000), and EU Method C.21, where the PEC is calculated by using 5 cm of the soil depth. Since all validity criteria were fulfilled these deviations did not affect the results of the study. In the definitive test all the validity criteria were met according to OECD Guideline No. 216: The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) exceeded 25% on 28 day of analysis. Therefore, the experiment was prolonged up to 56th day. After 42 days of incubation there were no statistically significant differences in nitrate concentration between the control and the group treated with the highest concentration of the test item, i.e. 5xPEC. It was concluded that ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils. The study is accepted and can be used in risk assessment.

Reference:	KCP 10.5/01
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Soil Microorganisms: Nitrogen Transformation Test, A. Gierbuszewska; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-29-20, 2020
Guideline(s):	According to OECD Guideline No 216 test method and corresponding EU method C.21
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch no.: PF0RM03
Test Species:	Agricultural soil taken from place adjoining to Institute of Organic Industry, Branch Pszczyna
Test Design:	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 56 days.
Endpoints:	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28, 42 and 56 days of incubation The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42, 0 – 56 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42, 0 – 56 days.
Test Concentration:	control, PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil), 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil)
Test Conditions:	temperature: 20.8 – 22.0°C, soil moisture: 45.7 – 53.4% of the maximum water holding capacity, incubation in darkness

Results and discussion:

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After 0, 7, 14, 28 and 56 days of incubation the statistically significant differences in nitrate concentration between the control and the both groups treated with the test item, i.e. PEC and 5xPEC, were observed. After 42 days of incubation the statistically significant differences in nitrate concentration between the control and the group treated with the test item only at the lower concentration, i.e. PEC were noticed. After 42 days of incubation there were no statistically

significant differences in nitrate concentration between the control and the group treated with the highest concentration of the test item, i.e. 5xPEC)

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	75.888	78.348	80.898	101.258	104.058	99.538	81.168	83.318	87.968
Nitrate ion concentration [mg/kg of dry soil]	379.44	391.74	404.49	506.29	520.29	497.69	405.84	416.59	439.84
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	**391.89 ± 12.53**			**508.09⁺ ± 11.41**			**420.76 ± 17.38**		
CV	**3.2**			**2.2**			**4.1**		

* - values adjusted for the value of the blank sample

⁺ - statistically significant difference between the control and the treatment group (Welch-t test for Inhomogeneous Variances, significance level = 0.05, two-sided)

At the time intervals: 0 – 7, 0 – 14, 0 – 28 and 0 – 56 there were statistically significant differences in nitrate formation rate between the control and the both groups treated with test item, i.e. PEC and 5xPEC. At the time interval 0 – 42 there was statistically significant difference in nitrate formation rate between the control and the group treated with the lower concentration of the test item, i.e. PEC. At the time interval 0 – 42 there was no statistically significant difference in nitrate formation rate between the control and the group treated with the highest concentration of the test item, i.e. 5xPEC.

Time interval [d]	Control				PEC				5 x PEC			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 – 7	23,628	20,278	20,743	21,550 ± 1,82	11,929	11,157	11,457	11,515⁺ ± 0,39	12,205	12,512	11,891	12,203⁺ ± 0,31
0 – 14	19,280	19,837	17,662	18,926 ± 1,13	9,805	9,619	9,419	9,614⁺ ± 0,19	9,596	10,357	10,439	10,131⁺ ± 0,46
0 – 28	13,657	13,638	13,841	13,712 ± 0,11	8,113	8,624	7,818	8,185⁺ ± 0,41	9,678	9,605	10,639	9,974⁺ ± 0,58
0 – 42	7,896	8,189	8,493	8,193 ± 0,30	10,668	11,001	10,463	10,711⁺ ± 0,27	8,013	8,268	8,822	8,368 ± 0,41
0 – 56	9,873	10,167	10,078	10,039 ± 0,15	7,856	7,840	8,579	8,092⁺ ± 0,42	8,194	7,729	8,399	8,107⁺ ± 0,34

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7th, 14th, 28th, 42th and 56th day

⁺ - statistically significant difference between the control and the treatment group (Welch-t test for Inhomogeneous Variances, significance level = 0.05, two-sided)

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) exceeded 25% on 28 day of analysis. Therefore, the experiment was prolonged up to 56 day.

Table 12. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	46.6	43.4
0 – 14	49.2	46.5
0 – 28	40.3	27.3
0 – 42	-30.7	-2.1
0 – 56	19.4	19.2

"-" – nitrate formation rate in the treatment group was lower than in the control group

Values obtained using ToxRat 2.10. computer software.

When the difference in the nitrates formation rate between the lower treatment (PEC) and a control is equal to or less than 25% at any sampling day after day 28, the product can be evaluated as having no long-term influence on nitrogen transformation in soil. On the basis of the results, it was concluded that ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) at the concentrations corresponding to the PEC: 4.82 mg of the test item / kg dry weight of soil (2.23 mg of ethofumesate/kg dry weight of soil) and 5xPEC: 24.10 mg of the test item / kg dry weight of soil (11.15 mg of ethofumesate/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

VALIDITY CRITERON

The coefficients of variation (CV) in the control group were 8.1, 6.4, 5.1, 0.7, 3.2 and 1.4%, after 0, 7, 14, 28, 42 and 56 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

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

No additional studies were performed.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 208 and according to the principles of GLP. Following deviation from the guideline occurred as follows: According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in green-houses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 74.72 and 140.1 µE/m2/s. Good control plant vigour was ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. This deviation did not affect results of the experiment. In the definitive test all the validity criteria were met according to OECD Guide-line No. 208. The study is considered acceptable and can be used in the risk assessment.

Reference:	KCP 10.6/01
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, P. Pieczka; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświadczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-31-20
Guideline(s):	according to the OECD Guideline No. 208 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch number: PFORM03 active substances: ethofumesate: 523 g/L
Test Design:	number of rates: 8 + control; number of replicates/rate: 4 (carrot, red clover, flax, onion) or 7 (pea) or 10 (corn). The total number of seeds per application rate – 20 (carrot, red clover, flax, onion, corn) or 21 (pea). test termination: 14 days after the emergence of 50% of the control seedlings
Endpoints:	ER25, ER50, NOER
Test Concentration:	- a control, - 0.9 mL of the test item /ha (0.5 g ethofumesate/ha), - 2.7 mL of the test item /ha (1.4 g ethofumesate/ha), - 8.2 mL of the test item /ha (4.3 g ethofumesate/ha), - 24.7 mL of the test item /ha (12.9 g ethofumesate/ha), - 74.1 mL of the test item /ha (38.7 ethofumesate/ha), - 222.2 mL of the test item /ha (116.2 g ethofumesate/ha),

- 666.7 mL of the test item /ha (348.7 g ethofumesate/ha),
- 2000 mL of the test item /ha (1046 g ethofumesate/ha),
volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha

Test Conditions: temperature: 18.7 – 24.7°C, humidity: 47.1 – 80.3%, lighting: 16 h light : 8 h dark; light intensity: 74.72 – 140.1 µE/m2/s; carbon dioxide concentration: 320 – 343 ppm

Results and discussion:

Table 36. ER_{25}, ER_{50} and NOER values (mL test item/ha)

	Pea *Pisum sativum*	**Carrot** *Daucus carota*	**Flax** *Linum usitatissimum*	**Red clover** *Trifolium pratense*	**Onion** *Allium cepa*	**Corn** *Zea mays*
Plant number at the end of the experiment						
ER_{25}	>2000	>2000	>2000	431.6 (146.3-1700)	>2000	>2000
ER_{50}	>2000	>2000	>2000	>2000	>2000	>2000
NOER	>2000	≥2000	≥2000	666.7	≥2000	≥2000
Shoot length (plants without roots)						
ER_{25}	656.1 (114.1-1232.6)	1236.2 (438.2->2000)	53.5 (23.0-89.9)	253.2 (98.6-431.2)	>2000	590.4 (255.6-829.1)
ER_{50}	>2000	>2000	208.1 (130.1-339.3)	1186.3 (705.2->2000)	>2000	1001.4 (679.9-1511.5)
NOER	222.2	666.7	24.7	74.1	≥2000	222.2
Plant dry weight (plants without roots)						
ER_{25}	243.0 (96.9-414.0)	171.0 (72.2-279.7)	30.3 (15.0-47.1)	385.6 (147.4-619.3)	1377.2 (656.1->2000)	362.0 (59.9-557.7)
ER_{50}	1207.5 (714.6->2000)	628.6 (400.7-1087.1)	94.3 (63.1-140.9)	1229.3 (779.8->2000)	>2000	657.2 (358.8-1217.4)
NOER	222.2	74.1	24.7	666.7	666.7	222.2

The ER_{25}, ER_{50} and NOER values were calculated using the ToxRat Professional 2.10 computer software.

Table 37. ER_{25}, ER_{50} and NOER values (g of ethofumesate/ha)

	Pea *Pisum sativum*	Carrot *Daucus carota*	Flax *Linum usitatissimum*	Red clover *Trifolium pratense*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{25}	>1046	>1046	>1046	225.7 (76.5-889.1)	>1046	>1046
ER_{50}	>1046	>1046	>1046	>1046	>1046	>1046
NOER	>1046	≥1046	≥1046	348.7	≥1046	≥1046
Shoot length (plants without roots)						
ER_{25}	343.1 (59.7-644.6)	646.5 (229.2->1046)	28.0 (12.0-47.0)	132.4 (51.6-225.5)	>1046	308.8 (133.7-433.6)
ER_{50}	>1046	>1046	108.8 (68.0-177.5)	620.4 (368.8->1046)	>1046	523.7 (355.6-790.5)
NOER	116.2	348.7	12.9	38.8	≥1046	116.2
Plant dry weight (plants without roots)						
ER_{25}	127.1 (50.7-216.5)	89.4 (37.8-146.3)	15.8 (7.8-24.6)	201.7 (77.1-323.9)	720.3 (343.1->1046)	189.3 (31.3-291.7)
ER_{50}	631.5 (373.7->1046)	328.8 (209.6-568.6)	49.3 (33.0-73.7)	642.9 (407.8->1046)	>1046	343.7 (187.7-636.7)
NOER	116.2	38.8	12.9	348.7	348.7	116.2

Pea (Pisum sativum)
After the application of the test item at the rates equal to 8.2, 666.7 and 2000 mL/ha, seedling emergence of pea was delayed by one day when compared with the control. The death of pea plants was not observed. At the control group 100% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha, total number of plants at the end of the experiment was equal to 100% in comparison to the control group . After the application of the test item at the ranging from 0.9 to 2000 mL/ha, the pea shoot length was between 55.0 and 105.0% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the pea shoot weight was between 41.6 and 93.0% of the control shoot weight. After the application of the test item at the rates between 666.7 and 2000.0 mL/ha, the plant damages as deformations and stunted growth were observed.

Carrot (Daucus carota)
After the application of the test item at the rates equal to 0.9, 24.7, 74.1 and 2000 mL/ha, seedling emergence of carrot was delayed by one day when compared with the control. The death of one carrot plant was observed at the concentration equal to 666.7 mL/ha. At the control group 80% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha, total number of plants at the end of the experiment was between 87.5 and 118.8% in comparison to the control group. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the carrot shoot length was between 66.6 and 103.6% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the carrot shoot weight was between 29.4 and 99.3% of the control shoot weight. After the application of the test item at the rates between 222.2 and 2000 mL/ha, the plant damage as stunted growth was observed.

Flax (Linum usitatissimum)
After the application of the test item at the rates from 222.2 to 2000 mL/ha, seedling emergence of flax was delayed by one day when compared with the control. The death of flax plants was not observed. At the control group 85% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha, total number of plants at the end of the experiment was between 70.6 and 111.8% in comparison to the control group.

After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the flax shoot length was between 19.5 and 94.1% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the flax shoot weight was between 12.4 and 105.5% of the control shoot weight. After the application of the test item at the rates between 24.7 and 2000 mL/ha, the plant damages as stunted growth and deformations were observed.

Red clover (Trifolium pratense)
After the application of the test item at the rates from 0.9 to 2000.0 mL/ha, seedling emergence of red clover was not delayed when compared with the control. The death of red clover plants was not observed. At the control group 85.0% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha, total number of plants at the end of the experiment was between 41.2 and 94.1% in comparison to the control group. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the red clover shoot length was between 47.5 and 108.0% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the red clover shoot weight was between 34.0 and 102.0% of the control shoot weight. After the application of the test item at the rates between 222.2 to 2000 mL/ha, the plant damage as stunted growth was observed.

Onion (Allium cepa)
After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, seedling emergence of onion was not delayed when compared with the control. The death of onion plants was not observed. At the control group 100% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha, total number of plants at the end of the experiment was between 95.0 to 100% as in the control group. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the onion shoot length was between 89.9 and 102.8% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the onion shoot weight was between 67.7 and 98.1% of the control shoot weight. After the application of the test item at the rate equal to 2000.0 mL/ha, the plant damage as stunted growth was observed.

Corn (Zea mays)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, seedling emergence of corn was not delayed when compared with the control. The death of one corn plant was observed at the rate equal to 2000 mL/ha. At the control group 100% of plants emerged. At the rates ranging from 0.9 to 2000 mL/ha total number of plants at the end of the experiment ranged from 95.0 to 100% in comparison to the control group. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the corn shoot length was between 18.4 and 96.9% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000 mL/ha, the corn shoot weight was between 17.2 and 97.0% of the control shoot weight. After the application of the test item at the rates between 666.7 to 2000 mL/ha, the plant damages as stunted growth and deformations were observed.

CONCLUSIONS
The test item i.e. ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) had an impact on the process of growth of pea, carrot, flax, red clover and corn. In cultivation of onion the slight impact on the process of growth was observed. The one day delayed seedling emergence was noticed in cultivation of pea (8.2, 666.7 and 2000 mL of the test item/ha), carrot (0.9, 24.7, 74.1 and 2000 mL of the test item/ha), flax (222.2, 666.7 and 2000 mL of the test item/ha). The death of single plants was observed in cultivation of carrot and corn. On the basis of ER25, ER50 and NOER values determined from final number of plants it was proved that the test item inhibited the seedling emergence of red clover. On the basis of ER25, ER50 and NOER values determined from the shoot length it was proved that the test item had an impact on the process of growth of pea, carrot, flax, red clover and corn. On the basis of ER25, ER50 and NOER values determined from the shoot dry weight it was proved that the test item had an impact on the process of growth of all tested plant species. During the experiment the plant damages were noticed in cultivation of all tested plant species. These were stunted growth (pea, carrot, flax, red clover, onion, corn) and deformations (pea, flax, corn).
The plant sensitivity was as follows:
red clover, flax > corn > pea, carrot > onion

VALIDITY CRITERIA
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) on seedling emergence and seedling growth of terrestrial plants were met:
- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
100.0% – pea,
80.0% – carrot,
85.0% – flax,
85.0% – red clover,
100.0% – onion,
100.0% – corn,
- the mean survival of the emerged control seedlings was 100% for pea, carrot, flax, red clover, onion and corn (validity criterion: 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

A 2.6.2.1.2 Study 2

Comments of zRMS:	The study was conducted to OECD guideline 227 and according to the principles of GLP. Following deviation from the guideline occurred as follows: According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m^2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 79.3 – 241.7 µE/m^2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. In the definitive test all the validity criteria were met according to OECD Guideline No. 227. The study is considered acceptable and can be used in the risk assessment.

Reference:	KCP 10.6/02
Report	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) Terrestial Plant Test: Vegetative Vigour Test, A. Wróbel; Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Doświ-adczalna 27, 43-200 Pszczyna, Poland; STUDY CODE: G-30-20, 2020
Guideline(s):	according to the OECD Guideline No. 227 (2006)
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) batch number: PF0RM03 active substances: ethofumesate: 523 g/L
Test Design:	number of rates: 8 + control; number of replicates/rate: 4 (carrot, red clover, flax, onion) or 7 (pea) or 10 (corn). The total number of plants per application rate – 20 (carrot, red clover, flax, onion, corn) or 21 (pea) test termination: 21 days after the spraying
Endpoints:	ER25, ER50, NOER
Test Concentration:	a control, - 0.9 mL of the test item/ha (0.5 g of ethofumesate/ha), - 2.7 mL of the test item/ha (1.4 g of ethofumesate/ha), - 8.2 mL of the test item/ha (4.3 g of ethofumesate/ha), - 24.7 mL of the test item/ha (12.9 g of ethofumesate/ha), - 74.1 mL of the test item/ha (38.7 g of ethofumesate/ha), - 222.2 mL of the test item/ha (116.2 g of ethofumesate/ha), - 666.7 mL of the test item/ha (348.7 g of ethofumesate/ha), - 2000.0 mL of the test item/ha (1046.0 g of ethofumesate/ha), volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha
Test Conditions:	temperature: 18.3 – 24.1°C, humidity: 46.2 – 90.0%, lighting: 16 h light : 8 h dark; light intensity: 87.24 – 241.80 µE/m2/s; carbon dioxide concentration: 359 – 372 ppm.

Results and discussion:

Table 30. ER_{25}, ER_{50} and NOER values (mL test item/ha).

	Pea *Pisum sativum*	Carrot *Daucus carota*	Red clover *Trifolium pratense*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{25}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0*	≥2000.0*	≥2000.0*	≥2000.0*	≥2000.0*	≥2000.0*
Shoot length (plants without roots)						
ER_{25}	>2000.0	>2000.0	1153.3 (425.7 - >2000)	390.1 (363.2 – 416.6)	>2000.0	>2000.0
ER_{50}	>2000.0	>2000.0	>2000.0	1011.6 (961.0 – 1066.5)	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	74.1	74.1	≥2000.0	≥2000.0
Plant dry weight (plants without roots)						
ER_{25}	>2000.0	>2000.0	463.5 (202.4 – 837.0)	356.4 (278.7 – 431.4)	>2000.0	>2000.0
ER_{50}	>2000.0	>2000.0	>2000.0	942.8 (805.6 – 1118.2)	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	74.1	222.2	≥2000.0	≥2000.0

The ER_{25}, ER_{50} and NOER values were calculated using the ToxRat Professional 2.10 computer software.

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 2000.0 mL/ha

Table 31. ER_{25}, ER_{50} and NOER values (g of ethofumesate/ha).

	Pea *Pisum sativum*	Carrot *Daucus carota*	Red clover *Trifolium pratense*	Flax *Linum usitatissimum*	Onion *Allium cepa*	Corn *Zea mays*
Plant number at the end of the experiment						
ER_{25}	>1046.0	>1046.0	>1046.0	>1046.0	>1046.0	>1046.0
ER_{50}	>1046.0	>1046.0	>1046.0	>1046.0	>1046.0	>1046.0
NOER	≥1046.0*	≥1046.0*	≥1046.0*	≥1046.0*	≥1046.0*	≥1046.0*
Shoot length (plants without roots)						
ER_{25}	>1046.0	>1046.0	603.2 (222.6 - >1046)	204.0 (190.0 – 217.9)	>1046.0	>1046.0
ER_{50}	>1046.0	>1046.0	>1046.0	529.1 (502.6 – 557.8)	>1046.0	>1046.0
NOER	≥1046.0	≥1046.0	38.8	38.8	≥1046.0	≥1046.0
Plant dry weight (plants without roots)						
ER_{25}	>1046.0	>1046.0	242.4 (105.9 – 437.8)	186.4 (145.7 – 225.6)	>1046.0	>1046.0
ER_{50}	>1046.0	>1046.0	>1046.0	493.1 (421.4 – 584.8)	>1046.0	>1046.0
NOER	≥1046.0	≥1046.0	38.8	116.2	≥1046.0	≥1046.0

*the value could not be dertermined, it can be probably higher than the highest rate of the test item used in the experiment, i.e. 1046.0 g of ethofumesate/ha

Pea (Pisum sativum)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of pea plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the pea shoot length was between 91.7 and 103.0% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the pea shoot weight was between 103.5 and 130.1% of the control shoot weight. After the application of the test item at the rates ranging from 222.2 to 2000.0 mL/ha, the plant damages were between 5 and 15%. Chlorosis and deformations were observed.

Carrot (Daucus carota)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of carrot plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the carrot shoot length was between 96.0 and 106.7% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the carrot shoot weight was between 105.5 and 125.7% of the control shoot weight. After the application of the test item at the rates between 0.9 to 2000.0 mL/ha, the plant damages were not observed.

Red clover (Trifolium pratense)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of red clover plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the red clover shoot length was between 72.9 and 95.0% of the control shoot length. After

the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the red clover shoot weight was between 60.2 and 113.9% of the control shoot weight. After the application of the test item at the rates ranging from 222.2 to 2000.0 mL/ha, the plant damages were between 5.0 and 15.0 %. Among phytotoxic symptoms stunted growth and necrosis were observed.

Flax (Linum usitatissimum)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of flax plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the flax shoot length was between 30.8 and 108.5% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the flax shoot weight was between 32.8 and 124.4% of the control shoot weight. After the application of the test item at the rates ranging from 222.2 to 2000.0 mL/ha, the plant damages were between 10.0 and 50.0 %. Among phytotoxic symptoms deformations, necrosis, stunted growth and wiltings were observed.

Onion (Allium cepa)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of onion plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the onion shoot length was between 93.7 and 98.5% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the onion shoot weight was between 95.1 and 114.3% of the control shoot weight. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the plant damages were not observed.

Corn (Zea mays)
After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha the mortality of corn plants was not observed. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the corn shoot length was between 90.0 and 104.2% of the control shoot length. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the corn shoot weight was between 98.0 and 134.3% of the control shoot weight. After the application of the test item at the rates ranging from 0.9 to 2000.0 mL/ha, the plant damages were not observed.

CONCLUSIONS
On the basis of the obtained results it was proved that the test item i.e. ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) had no influence on the plant number of the all test species.
On the basis of ER25, ER50 and NOER values determined from the shoot length and plant dry weight, it was proved that the test item inhibited the process of growth of red clover and flax. Growth inhibition of pea, carrot, onion and corn was not observed.
Among phytotoxic symptoms, stunted growth (red clover, flax), deformations (pea, flax), wiliting (flax), chlorosis (pea), necrosis (red clover, flax) were observed.
The following order of the plant sensitivity was noticed:
flax > red clover > pea > carrot, onion, corn.

VALIDITY CRITERIA
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of ETOFUMESATE 500 SC (CHR/H/ETO 500 SC) on vegetative vigour of terrestrial plants were met:
- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
85.7 – 92.9% – pea,
82.5 – 97.5% – carrot,
85.0 – 95.0% – red clover,
85.0 – 97.5% – flax,
90.0 – 95.0% – onion,
87.5 – 90.0% – corn.
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No additional studies were performed.

A 2.8 KCP 10.8 Monitoring data

No additional studies were performed.