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ETHOFUMESATE

Volume 3 – B.9 (PPP) – ETHOFOL 500 SC

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B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

Ethofumesate is an herbicidal active substance and was included into Annex I of Directive 91/414/EEC in 2002 (Directive 2002/37/EC, 3rd May 2002). Directive 91/414/EEC has been repealed by Regulation (EC) No 1107/2009 of 21 October 2009 concerning the placing of plant protection products on the market. Accordingly ethofumesate is deemed to have been approved under Regulation (EC) No 1107/2009, as set out in Part A of the Annex of Commission Implementing Regulation (EC) No 540/2011 as regards the list of approved substances (entry No. 29).

The EU representative formulation is Ethofol 500 SC, a suspension concentrate containing 500 g/L of the active substance ethofumesate.

This renewal assessment report (RAR) contains summaries of studies on ethofumesate, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. In addition, all studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC, were re-evaluated according to the current valid test guidelines and were summarised in the RAR.

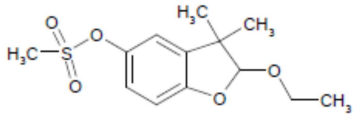
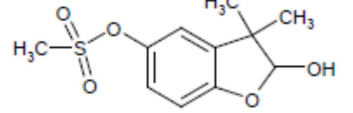
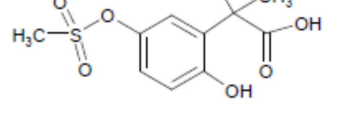
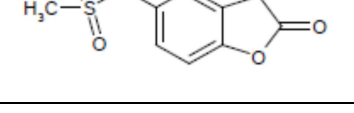
Studies which were submitted for the first EU peer-review of the active substance ethofumesate but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are briefly summarised (text in *italic*).

In case where reliable and adequate literature was found during the literature search, summaries are integrated in the respective sections of the RAR.

Ethofumesate is a racemic mixture of two enantiomers. In degradation studies (non-guideline lysimeter study and in a water sediment study) no significant changes in the ratio of the racemate (1:1) were observed, indicating that the degradation and distribution of both enantiomers is the same in the environment. Therefore it was considered adequate that all studies on the active substance were performed using the racemic mixture.

The different synonyms and codes for the active substance ethofumesate and its metabolites used in the RAR are summarised in the table B.9-1.

Table B.9-1: Substances and metabolites (structure, synonyms and codes)

Codes and synonyms	Description (IUPAC)	Compound found in	Structure
Ethofumesate Synonym: ai NC 8438, AE B049913	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate	All matrices	
Ethofumesate-2-hydroxy Synonym: NC 8493	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate	Soil, groundwater, surface water, sediment	
Ethofumesate-carboxylic acid Synonym: NC 20645	2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methyl propionic acid	Water, sediment	
Ethofumesate-lactone Synonym: NC 9607	2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulfonate	Soil	

The formulation is intended for use as an herbicide against grass weeds and dicotyledonous species in sugar, fodder and red beet. The critical use pattern for this formulation is summarised in Table B.9-2.

Table B.9-2: Intended application pattern

Crop	Timing of application	No. of applications	Application interval [days]	Maximum application rate (formulation) [L/ha]	Maximum application rate (active substance) [kg ai/ha]
Sugar beet, fodder beet	Pre-emergence	1	-	2.0	1.0 ^a
	Post-emergence (until BBCH 18)	6	5	2.0	3 x 0.333 ^b 1.0 ^a

^a The maximum amount of active substance must not exceed 1.0 kg/ha every 3 year.

^b Splitting application with a total maximum rate of 1 kg ai/ha per season. The maximum application rate per treatment is 0.333 kg ai/ha. The critical GAP therefore is 3 applications of 0.333 kg ai/ha (interval 5 days). More applications (max. 6) at lower application rates are possible..

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Several acute, dietary and reproductive toxicity studies with ethofumesate have been performed with mallard duck and bobwhite quail. Studies were already submitted for the first EU peer-review of the active substance ethofumesate. No new studies with the active substance were submitted for the renewal of the EU peer-review. The study summaries are provided under point B.9.1.1 of Volume 3 – B.9 (AS).

In addition, an acute toxicity study with the EU representative formulation Ethofumesate 500 SC was conducted addressing the risk to birds from exposure to the formulated product. A summary is provided under Point B 9.1.1.

A summary of the toxicity of ethofumesate to birds is given in table B.9.1.1-1.

Table B 9.1.1-1: Toxicity of ethofumesate to birds

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	Acute, oral	LD ₅₀ > 2000 mg ai/kg bw LD ₅₀ extrapol = 3776 mg ai/kg bw ^a	██████ et al., 1990b
		LD ₅₀ > 8743 mg ai/kg bw LD ₅₀ extrapol = 16507 mg ai/kg bw ^a	██████ et al., 1977b
Mallard duck		LD ₅₀ > 2000 mg ai/kg bw LD₅₀ extrapol. = 3776 mg ai/kg bw ^a	██████ et al., 1990a
		LD ₅₀ > 3552 mg ai/kg bw LD ₅₀ extrapol = 6706 mg ai/kg bw ^a	██████ et al., 1977a
Bobwhite quail	Short-term, dietary	LC ₅₀ > 5200 ppm LDD ₅₀ > 1003 mg ai/kg bw/d	██████████, 1991b
		LC ₅₀ > 5200 ppm LDD ₅₀ > 1050 mg ai/kg bw/d	██████████, 1990b
		LC ₅₀ > 1000 ppm	██████, 1994a
Mallard duck		LC ₅₀ > 5200 ppm LDD ₅₀ > 1453 mg ai/kg bw/d	██████████, 1991a
		LC ₅₀ > 5200 ppm LDD ₅₀ > 1345 mg ai/kg bw/d	██████████, 1990a
		LC ₅₀ > 1000 ppm	██████, 1994a
Bobwhite quail	20 weeks feeding chronic, reproduction	NOAEC = 3000 ppm NOAEL = 265 mg ai/kg bw	██████ 2001
Mallard duck		NOAEC = 3000 ppm NOAEL = 406 mg ai/kg bw	██████ et al., 2000

Bold values were used for the risk assessment

^a LD₅₀ extrapolated according to the EFSA Guidance Document on Birds and Mammals (2009). 10 birds per group were tested without any mortality during the study. An extrapolation factor of 1.888 was used for the calculation of the extrapolated LD₅₀.

Toxicity of the formulated product

An acute toxicity study with the formulation Ethofumesate 500 SC (██████████ 1988) has been submitted. The formulation Ethofumesate 500 SC is considered comparable to the EU representative formulation Ethofol 500 SC (see Volume 4).

Reference:	Acute Oral Toxicity study with Ethofumesate 500 SC (Ethofumesate: 500 g/L) in Japanese Quails
Author(s), year:	██████████ 1998
Report/Doc. number:	Report no. 2296/97-AOQ, Reference no. IDD00133
Guideline(s):	OECD 401 (1987), EC Directive 96/12/EU, Appendix – 1, Method 8.1.1
GLP:	Yes
Deviations:	None
Acceptability:	Not acceptable

Material and methods:

Test substance:	Ethofumesate 500 SC, SC formulation containing 500 g/L, Batch no.: 1997/4, Purity: 45.53% of ai (analysed)
Test species:	Japanese quail (<i>Coturnix coturnix japonica</i>)
Number of organisms:	10 animals per group (5 males and 5 females)
Age:	12-14 weeks (at start of treatment)
Weight:	143 – 147 g (males) /144 – 174 g (females)
Acclimatisation period:	7 days under laboratory conditions
Type of test:	Acute oral toxicity
Applied concentrations:	2000 mg ai/kg bw, dosage volume: 1.8 mL/kg bw
Type of application:	The undiluted test substance was administered once orally as gavage
Time of exposure:	One single application, monitoring during 15 days
Test conditions:	Test temperature: 23 – 28°C, relative humidity: 50 – 75%, lighting: 12h light and 12h darkness. Feed (broiler starter mash) was provided ad libitum during acclimation and during the test, except of a starvation period of 16 to 18 hours. Drinking water was provided ad libitum.
Observations:	For pre-terminal deaths, birds were observed four times on the test days one and once daily during days 2 – 15. For toxic signs, observations were four times on the test day on and once daily during days 2 – 15. Body weight effects were observed pre administration, on day 7 and 14 or after scarification. Furthermore, necropsy of all the surviving quails was performed at the end of the test.

Findings:

Mortalities:	There were no pre-terminal deaths. No abnormalities were detected.
Body weight:	The body weight of all the quails increased through the study period excepting the interim body weights of 2 quails which were lower. However, the terminal body weights of these 2 quails were higher than their initial weights.
Clinical signs:	Diarrhea was observed in 1 male and in 3 female quails on day 1 and it's persisted in 1 quail on day 2. No toxic effects were observed from day 3 onwards.

Table B 9.1.1-2 : Body weight changes

Test organism		Initial 1st day	Day 07	Day 14
Males	1	147	152	161
	2	143	156	165
	3	146	143	158
	4	147	158	169
	5	145	161	171
Females	1	174	177	213
	2	160	152	175
	3	172	176	220
	4	157	175	194
	5	144	170	225

Conclusion:

The LD₅₀ in Japanese quails for Ethofumesate 500 SC, administered orally as gavage, was estimated to be greater than 2000 mg/kg bw.

Comment RMS:

The study was conducted according to the OECD test guideline 401 (1987). The study is demonstrated to be acceptable, even under consideration of the current test guidelines, e.g. US EPA guideline (OPPTS 850.2100, April 1996) and OECD guideline (OECD 223, July 2010).

No effects on mortality and body weight were observed.

However, some animals showed toxic effects (diarrhoea) after the application of the test substance. The limit test was conducted without a negative control group. Hence, no information on the health and husbandry of the test birds are available and no assessment of the reliability of the results can be done.

In addition, no information on the statistical analysis of the results was provided.

Hence, the study is not considered valid.

Endocrine disruption

The population relevant effects of ethofumesate on birds were studied in reproductive toxicity studies on mallard ducks. For the test species no effects on adult birds, offspring or reproductive parameters up to and including the highest test level of 3000 ppm a.s was observed. As reproduction was not affected in mallard ducks, it is concluded that there are no population relevant adverse effects of ethofumesate. No additional studies are deemed necessary.

Metabolites of ethofumesate

No studies were performed for the metabolites of ethofumesate as the potential risk is covered by the risk assessment for the active substance. This is confirmed by the fact that the metabolites NC 20645, NC 9607 and NC 8493 have been identified in mammalian metabolisms studies. Thus, it can be concluded that neither of these metabolites exhibits toxicity exceeding the adverse effects noted with ethofumesate in the battery of studies on acute and chronic toxicity.

B.9.1.2. Effects on terrestrial vertebrates other than birds

A summary of the toxicity of ethofumesate to mammals is given in the table B.9.1.2-1.

No additional studies were submitted for the renewal of the active substance ethofumesate. Hence, the risk assessment is based on the EU peer review acute and long-term endpoints identified for the first EU approval of the active substance.

For the renewal of the active substance ethofumesate a new reproductive endpoint based on the results from a 2-generation rat study was determined. The NOAEL of 60.9 mg ai/kg bw/d is based on effects on the parent generation (decrease of body weight gain of male adults), the offspring (number of male pups, life birth index P_0 , 21 day survival index in P_0) and the reproduction (decrease of mean litter size in the P_0 -generation, increase of pre-implantation loss in P_0 -generation). The 2-generation study in rats was already submitted for the first EU peer-review of the active substance. For the renewal of the EU peer-review the study was re-evaluated and the calculation of the dietary daily dose was determined (see Section B.6.)

Based on the significant effects on reproduction the long-term risk assessment for mammals should be based on the NOAEL derived from the 2-generation study in rats (■■■■■ 1993).

Table B 9.1.2-1: Toxicity of ethofumesate to mammals

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	Acute, oral	LD ₅₀ > 2000 mg ai/kg bw	██████ 1992
		LD ₅₀ > 7500 mg ai/kg bw	██████ 1991
		LD₅₀ > 5000 mg ai/kg bw	██████ 1988
		LD ₅₀ > 8000 mg ai/kg bw	██████ 1988
Mouse		LD ₅₀ > 5000 mg ai/kg bw	██████ 1992
		LD ₅₀ > 7500 mg ai/kg bw	██████ 1991
		LD ₅₀ > 8000 mg ai/kg bw	██████ 1988
Rat	2-generation reproduction	NOAEC = 1000 ppm NOAEL = 60.9 mg ai/kg bw/d ^a	██████ 1993 ████████████████████, 2013
	2-generation reproduction	NOAEC = 3000 ppm NOAEL _{female} = 256 mg ai/kg bw/d	████████████████████, 1990
	3-generation reproduction	NOAEC = 1000 ppm NOAEL _{male} = 78 mg ai/kg bw/d	██████ at al., 1980
Rabbit	Teratogenicity study	NOAEL = 300 mg ai/kg bw	██████ et al., 1986

Bold values were used for the risk assessment

^a The reproductive endpoint of 1000 ppm is based on adverse effects on the parents (↓ body weight gain), the offspring (number of male pups, life birth index P_0 , 21 day survival index in P_0) and the reproduction (↓ mean litter size in P_0 , ↑ pre-implantation loss in P_0 generation). The actual daily dose of 60.9 mg ai/kg bw/d is based on a statistically significant decrease in body weight gain in male rats (> 10% compared to the control in the P_0 generation males and in the P_1 generation males, mainly at the beginning of the study).

Toxicity of the formulated product:

The acute oral toxicity of the product AD 496 (synonym for Ethofol 500 SC) was determined in a study on rats. Based on the results of the study, the toxicity of the formulation and the active substance ethofumesate is considered to be comparable.

Table B 9.1.2-2: Toxicity of the formulated product to mammals

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	Acute, oral	LD ₅₀ > 2000 mg prod./kg bw	██████, 1996

Endocrine disruption:

A detailed analysis of all the apical toxicological studies (subchronic, chronic / oncogenicity, reproduction and developmental toxicity) on ethofumesate revealed no evidence of any reproducible endocrine effect. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of ethofumesate in mammals.

Relevance and toxicity of the metabolites:

No studies were performed for the metabolites of ethofumesate as the potential risk is covered by the risk assessment for the active substance. This is confirmed by the fact that the metabolites NC 20645, NC 9607 and NC 8493 have been identified in mammalian metabolisms studies. Thus, it can be concluded that neither of these metabolites exhibits toxicity exceeding the adverse effects noted with ethofumesate in the battery of studies on acute and chronic toxicity.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Birds and other terrestrial vertebrates may be exposed to ethofumesate by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey. Another possible route is via drinking water.

The risk assessment for birds and mammals was conducted according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009;7(12):1438).

Ethofol 500 SC is intended to be applied as an herbicide in sugar and fodder beet pre- and post-emergence at a maximum single application rate of 1.0 kg ai/ha. The maximum amount of ethofumesate per season and per hectare must not exceed 1.0 kg every 3 years.

The most critical scenarios are bare soil (BBCH 00-009) with a maximum application rate of 1 x 2.0 L prod./ha (1.0 kg ai/ha) and sugar beet (\leq BBCH 18) with a maximum application rate of 3 x 0.66 L prod./ha (3 x 0.33 kg ai/ha) are used to assess the risk to feeding birds and mammals in sugar and fodder beet.

B.9.2.1. Risk assessment for birds

With regard to the relevant scenarios small granivorous and small omnivorous birds (see Table B.9.2.1-1) are considered as the relevant indicator species for the risk assessment in the categories sugar beet (post-emergence) and bare soils (pre-emergence).

In the screening step it is assumed that animals satisfy all their dietary needs in the treated area feeding on only one food type and do not avoid contaminated food. Therefore, avoidance, fraction of diet obtained in the treated area and fraction of food type in the diet are all set to 1.

Table B. 9.2.1-1: Relevant generic avian focal species for the screening step

Crop	Scenario	Indicator species	SV ₉₀	SV _m
Sugar and fodder beet (pre-emergence)	Bare soils (BBCH < 10)	Small granivorous bird	24.7	11.4
Sugar and fodder beet (post-emergence)	Root and stem vegetables, sugar beet	Small omnivorous bird	158.8	64.8

Acute risk assessment for birds:

The acute risk assessment is based on the endpoints derived from acute toxicity studies with bobwhite quail (■■■■ et al., 1990b) and mallard duck (■■■■ et al., 1990a). The LD₅₀ for all species was determined to be > 2000 mg ai/kg bw. In all acute toxicity tests no mortality was observed. Hence, the LD₅₀ values were

extrapolated taking into account an extrapolation factor (see table B.9.1.1-1). Hence, the acute risk assessment is based on a LD₅₀ extrapolated of 3776 mg ai/kg bw.

Table B. 9.2.1-2: Screening step - acute risk assessment for birds

Crop	Indicator species	SV ₉₀	Application rate [kg ai/ha]	MAF ₉₀	DDD _A	LD ₅₀ [mg ai/kg bw]	TER _A
Bare soils (BBCH < 10)	Small granivorous bird	24.7	1.0	1.0	24.7	3776	153
Root and stem vegetables, sugar beet	Small omnivorous bird	158.8	0.33	1.8	94.3		40

All TER_A values are above the trigger of 10 for acute exposure, indicating an acceptable risk to birds from the use of the product.

Long-term risk assessment for birds:

The long-term endpoint is based on the lowest observed endpoint derived from two long-term reproduction studies with mallard duck (■■■■ et al., 2000).

The acute oral LD₅₀ value used in the acute avian assessment (LD₅₀ = 3776 mg ai/kg bw) divided by 10 to obtain LD₅₀/10 will be compared with the lowest NOAEL from the reproduction study (studies) ignoring purely parental effects (e.g. changes in parental body weight and food consumption).

However, as a conservative approach, the lower endpoint from the reproduction study (NOAEL = 265 mg ai/kg bw/d) will be used in avian reproductive risk assessment.

Table B. 9.2.1-3: Long-term risk assessment for birds – screening step

Crop	Generic focal species	SV _m	Application rate [kg ai/ha]	MAF _m	f _{twa}	DDD _{LT}	NOAEL [mg ai/kg bw/d]	TER _{LT}
Bare soils (BBCH < 10)	Small granivorous bird	11.4	1.0	1.0	0.53	6.0	265.0	44.2
Root and stem vegetables, sugar beet	Small omnivorous bird	64.8	0.33	2.2	0.53	24.9		10.6

All TER_{LT} values are above the trigger of 5 for long-term exposure, indicating an acceptable risk to birds from the use of the product.

Drinking water risk assessment:

The risk to birds from exposure via drinking water was conducted according to the EFSA Guidance Document on Birds and Mammals (2009). The risk assessment for birds is limited to the scenario of puddles formed on the ground after application. The leaf scenario is not relevant considering the intended crops and the time of application (BBCH 10 – 18).

Based on the EFSA Guidance Document on Birds and Mammals (2009) no specific TER calculations are necessary when the ratio of effective application rate (1000 g ai/ha) to relevant acute and long-term endpoint does not exceed 50 for less sorptive substances ($K_{OC} < 500$ L/kg).

Table B. 9.2.1-4: Drinking water risk assessment

K_{OC} [L/kg]	Application rate [kg ai/ha]	MAF	Endpoint [mg ai/kg bw/d]		Ratio effective application rate/endpoint		Conclusion
			Acute	Long-term	Acute	Long-term	
157	1000	1.0	$LD_{50} > 3776$	NOAEL = 265	< 0.3	< 3.8	No concerns

This evaluation confirms that the risk for birds from drinking water that may contain residues from the use of the product is acceptable. Hence, no specific calculation of exposure and TER is deemed necessary.

Secondary poisoning:

Substances with a high potential to bioaccumulate in the food chain could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a $\log P_{OW} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

The $\log P_{OW}$ values of the active substance ethofumesate and its metabolites are below the trigger (see table B.9.2.1-4); hence the risk of secondary poisoning for birds and mammals is not considered necessary.

However, for the first EU approval of the active substance bioaccumulation studies in fish were conducted. Based on the results of the studies ($BCF = 62 - 144$) a risk assessment for fish- and earthworm eating birds and mammals is triggered.

Table B. 9.2.1-5: Log P_{OW} of ethofumesate and its metabolites

Substance	Log P_{OW}	Reference
Ethofumesate	2.7 (pH 6.4, 10°C/25°C)	SANCO/6503/VI/99 final (2002)
NC 8493	1.2	KOWWIN
NC 9607	0.7	KOWWIN
NC 20645	1.18	KOWWIN

Food chain from earthworm to earthworm-eating birds

The risk to earthworm-eating birds from bioaccumulation of ethofumesate is calculated with the following equations in accordance with the EFSA Guidance (2009).

Calculation of the PEC_{worm} for earthworm-eating birds:

$$BCF = (0.84 + 0.012 * K_{OW}) / (f_{OC} * K_{OC})$$

$$PEC_{worm} = PEC_{soil} * BCF$$

Where:

PEC_{worm}	Predicted concentration in earthworms [mg/kg]
PEC_{soil}	Initial PEC_{soil} in soil [mg/kg soil dw]
BCF	Bioconcentration factor in earthworms
K_{OW}	Octanol/water partition coefficient
f_{OC}	Organic carbon content of soil, default = 0.02
K_{OC}	Organic carbon adsorption coefficient

The factor of 1.05 is used to convert the residues in worms to a daily dose based on a bird of 100 g eating 104.6 g worms per day, according to EFSA.

The risk assessment was performed for the single application of 1.0 kg ai/ha.

Table B. 9.2.1-6: Parameters and calculations for the assessment of the long-term risk to earthworm-eating birds

Parameter	Sugar and fodder beet
NOEL _{long-term} [mg ai/kg bw/d]	265
K_{OC} (Organic carbon adsorption coefficient)	157
K_{OW} (Octanol water partition coefficient)	501
f_{OC} (Organic carbon content of soil)	default value: 0.02
PEC_{soil} (plateau) [mg ai/kg]	1.336
BCF_{worm}	2.18
PEC_{worm} [mg ai/kg]	2.91
Daily dose [mg ai/kg bw/d]	3.06
TER	87
Trigger	5

The TER-value following use according to the GAP are above the trigger of 5 for long-term risk, indicating that the use of ethofumesate poses a low risk to earthworm-eating birds.

Food chain from fish to fish-eating birds

The risk to fish-eating birds from bioaccumulation of ethofumesate is calculated with the following equations in accordance with EFSA Guidance (2009):

Calculation of the Daily Dietary Dose (DDD) for fish-eating birds:

$$PEC_{\text{fish}} = PEC_{\text{sw}} * BCF$$

Where:

PEC_{fish}	Predicted concentration in fish [mg/kg]
PEC_{sw}	PEC in surface water [mg/L]
BCF	Bioconcentration factor in fish

The factor of 0.159 is used to convert the residues in fish to a daily dose based on a bird of 1000 g eating 159 g per day, according to EFSA.

The risk assessment is based on the use according to GAP for which the highest initial PEC_{sw} at FOCUS Step 1 was calculated as 0.2972 mg ai/L (Pre-emergence, 1 x 1000 g ai/ha). The initial PEC was used instead of the 21 days twa as it represents a worst-case.

Table B. 9.2.1-7: Parameters and calculations for the assessment of the long-term risk to fish-eating birds

Parameter	Sugar and fodder beet
NOEL _{long-term} [mg ai/kg bw/d]	265
PEC_{water} (initial, FOCUS step 1) [mg ai/L]	0.2972
BCF_{fish}	144
PEC_{fish} [mg ai/kg]	42.8
Daily dose [mg ai/kg bw/d]	6.81
TER	39
Trigger	5

The TER-value following use according to the GAP is above the long-term trigger of 5, indicating that the use of ethofumesate poses a low risk to fish-eating birds.

Biomagnification in terrestrial food chains

Ethofumesate is extensively metabolised to the major metabolite ethofumesate-carboxylic acid and some minor metabolites ethofumesate-lactone and ethofumesate-2-hydroxy. ADME studies conducted with rats and a bioaccumulation study in fish demonstrate that ethofumesate has a low potential to bioaccumulate and biomagnify in vertebrates (see also section toxicology).

B.9.2.2. Risk assessment for mammals

With regard to the relevant scenarios small insectivorous, small omnivorous and large herbivorous mammals (see Table B.9.2.2-1) are considered as the relevant generic focal species for risk assessment in the categories sugar beet and root and stem vegetables.

In the Tier 1 assessment it is assumed that animals satisfy all their dietary needs in the treated area feeding on only one food type and do not avoid contaminated food. Therefore, avoidance, fraction of diet obtained in the treated area and fraction of food type in the diet are all set to 1.

Table B 9.2.2-1: Relevant indicator species for the screening step

Crop	Scenario	Indicator species	SV ₉₀	SV _m
Sugar and fodder beet (pre-emergence)	Bare soils (BBCH < 10)	Small granivorous mammal	14.4	6.6
Sugar and fodder beet (post-emergence)	Root and stem vegetables, sugar beet	Small herbivorous mammal	118.4	48.3

Acute risk assessment for mammals:

The acute risk assessment is based on the lowest acute toxicity endpoint (LD₅₀ > 5000 mg ai/kg bw) derived from standard acute oral laboratory studies with rats (■■■■■ 1988) and mice (■■■■■ 1992).

Table B 9.2.2-2: Acute risk assessment for mammals – Screening step

Crop	Indicator species	SV ₉₀	Application rate [kg ai/ha]	MAF ₉₀	DDD _A	LD ₅₀ [mg ai/kg bw]	TER _A
Bare soils (BBCH < 10)	Small granivorous mammal	14.4	1.0	1.0	14.4	> 5000	> 347
Root and stem vegetables, sugar beet	Small herbivorous mammal	118.4	0.33	1.8	70.3		> 71.1

All TER_A values are above the trigger of 10 for acute exposure, indicating an acceptable risk to mammals from the use of the product.

Long-term risk assessment for mammals:

The long-term risk assessment is based on the reproductive endpoint of NOAEL = 60.9 mg ai/kg bw/d derived from a 2-generation study in rats (Suresh, 1993).

Table B 9.2.2-3: Long-term risk assessment for mammals – Screening step

Crop	Generic focal species	SV _m	Application rate [kg ai/ha]	MAF _m	f _{twa}	DDD _{LT}	NOAEL [mg ai/kg bw/d]	TER _{LT}
Bare soils (BBCH < 10)	Small granivorous mammal	6.6	1.0	1.0	0.53	3.5	60.9	17.4
Root and stem vegetables, sugar beet	Small herbivorous mammal	48.3	0.33	2.2	0.53	18.6		3.3

The long-term risk to small granivorous mammals was identified to be acceptable. The TER_{LT} value is above the trigger of 5, indicating an acceptable risk. However, the TER_{LT} value for small herbivorous mammals (post-emergence application) was identified to be below the trigger of 5. Hence, a Tier 1 risk assessment according to the EFSA Guidance Document (2009) has to be conducted.

Table B 9.2.2-4: Relevant generic mammalian focal species for the Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	SV _m
Sugar beet	BBCH 10-19	Small insectivorous mammal “shrew”	Common shrew	4.2
	BBCH 10-39	Large herbivorous mammal “lagomorph”	Rabbit	14.3
	BBCH 10-39	Small omnivorous mammal “mouse”	Wood mouse	7.8

Table B 9.2.2-5: Tier 1 long-term risk assessment for mammals

Crop	Generic focal species	SV _m	Application rate [kg ai/ha]	MAF _m	f _{twa}	DDD _{LT}	NOAEL [mg ai/kg bw/d]	TER _{LT}
Sugar beet (BBCH ≤ 18)	Small insectivorous mammal “shrew”	4.2	0.33	2.2	0.53	1.6	60.9	38.1
	Large herbivorous mammal “lagomorph”	14.3				5.5		11.1
	Small omnivorous mammal “mouse”	7.8				3.0		20.3

Based on the Tier 1 risk assessment an acceptable risk to feeding mammals in sugar and fodder beet was identified. All TER_{LT} are above the trigger of 5, indicating an acceptable risk.

Drinking water risk assessment:

The risk to mammals from exposure via drinking water was conducted according to the EFSA Guidance Document on Birds and Mammals (2009). The risk assessment for mammals is limited to the scenario of puddles formed on the ground after application.

Based on the EFSA Guidance Document on Birds and Mammals (2009) no specific TER calculations are necessary when the ratio of effective application rate (1000 g ai/ha) to relevant acute and long-term endpoint does not exceed 50 for less sorptive substances ($K_{OC} < 500$ L/kg).

Table B 9.2.2-6: Drinking water risk assessment

K_{OC} [L/kg]	Application rate [kg ai/ha]	MAF	Endpoint [mg ai/kg bw/d]		Ratio effective application rate/endpoint		Conclusion
			Acute	Long-term	Acute	Long-term	
157	1000	1.0	$LD_{50} > 5000$	NOAEL = 60.9	< 0.2	< 16.4	No concerns

Secondary poisoning:

Substances with a high potential to bioaccumulate in the food chain could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a $\log P_{OW} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

The $\log P_{OW}$ values of the active substance ethofumesate and its metabolites are below the trigger (see table B.9.2.1-4); hence the risk of secondary poisoning for birds and mammals is not considered necessary.

However, for the first EU approval of the active substance bioaccumulation studies in fish were conducted. Based on the results of the studies ($BCF = 62 - 144$) a risk assessment for fish- and earthworm eating birds and mammals is triggered.

Food chain from earthworm to earthworm-eating mammals

The risk to earthworm-eating mammals from bioaccumulation of ethofumesate is calculated with the following equations in accordance with the EFSA Guidance (2009).

Calculation of the PEC_{worm} for earthworm-eating mammals:

$$BCF = (0.84 + 0.012 * K_{OW}) / (f_{OC} * K_{OC})$$

$$PEC_{worm} = PEC_{soil} * BCF$$

Where:

PEC _{worm}	Predicted concentration in earthworms [mg/kg]
PEC _{soil}	Initial PEC _{soil} in soil [mg/kg soil dw]
BCF	Bioconcentration factor in earthworms
K _{OW}	Octanol/water partition coefficient
F _{OC}	Organic carbon content of soil, default = 0.02
K _{OC}	Organic carbon adsorption coefficient

The factor of 1.28 is used to convert the residues in worms to a daily dose based on a mammal of 10 g eating 12.8 g worms per day, according to EFSA.

The risk assessment was performed for the single application of 1.0 kg ai/ha (pre-emergence).

Table B. 9.2.2-1: Parameters and calculations for the assessment of the long-term risk to earthworm-eating mammals

Parameter	Sugar and fodder beet
NOEL _{long-term} [mg ai/kg bw/d]	60.9
K _{OC} (Organic carbon adsorption coefficient)	157
K _{OW} (Octanol water partition coefficient)	501
f _{OC} (Organic carbon content of soil)	default value: 0.02
PEC _{soil} (initial) [mg ai/kg]	1.336
BCF _{worm}	2.18
PEC _{worm} [mg ai/kg]	2.91
Daily dose [mg ai/kg bw/d]	3.72
TER	16
Trigger	5

The TER-value following use according to the GAP are above the trigger of 5 for long-term risk, indicating that the use of ethofumesate poses a low risk to earthworm-eating mammals.

Food chain from fish to fish-eating mammals

The risk to fish-eating mammals from bioaccumulation of ethofumesate is calculated with the following equations in accordance with EFSA Guidance (2009):

Calculation of the Daily Dietary Dose (DDD) for fish-eating mammals:

$$PEC_{fish} = PEC_{sw} * BCF$$

Where:

PEC _{fish}	Predicted concentration in fish [mg/kg]
PEC _{sw}	PEC in surface water [mg/L]
BCF	Bioconcentration factor in fish

The factor of 0.142 is used to convert the residues in fish to a daily dose based on a bird of 3000 g eating 425 g per day, according to EFSA.

The risk assessment is based on the use according to GAP for which the highest initial PEC_{sw} at FOCUS Step 1 was calculated as 0.2972 mg ai/L (Pre-emergence, 1 x 1000 g ai/ha). The initial PEC was used instead of the 21 days twa as it represents a worst-case.

Table B. 9.2.2-2: Parameters and calculations for the assessment of the long-term risk to fish-eating mammals

Parameter	Sugar and fodder beet
$NOEL_{long-term}$ [mg ai/kg bw/d]	60.9
PEC_{water} (initial, FOCUS step 1) [mg ai/L]	0.2972
BCF_{fish}	144
PEC_{fish} [mg ai/kg]	42.8
Daily dose [mg ai/kg bw/d]	6.1
TER	10
Trigger	5

The TER-value following use according to the GAP is above the long-term trigger of 5, indicating that the use of ethofumesate poses a low risk to fish-eating mammals.

Biomagnification in terrestrial food chains

Ethofumesate is extensively metabolised to the major metabolite ethofumesate-carboxylic acid and some minor metabolites ethofumesate-lactone and ethofumesate-2-hydroxy. ADME studies conducted with rats and a bioaccumulation study in fish demonstrate that ethofumesate has a low potential to bioaccumulate and biomagnify in vertebrates (see also section toxicology).

B.9.3. EFFECTS ON AQUATIC ORGANISMS

Several studies on the aquatic toxicity of the active substance ethofumesate were already submitted and evaluated for the first EU approval of the active substance. These studies were re-evaluated according to the former and current valid test guidelines and were summarised in Part B.9. (A.S.).

However, additional Annex II studies not peer-reviewed on EU level were considered necessary to account for the new data requirements according to Commission Regulation (EU) No 283/2013.

In addition, studies on the acute toxicity of the metabolites NC 20645 and NC 8493 and studies on the representative formulation Ethofol 500 SC not peer-reviewed on EU level have also been performed and the study summarised are provided in Part B.9. of the RAR.

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

In addition to the acute toxicity studies with the active substance ethofumesate and its metabolites studies with the EU representative formulation (Ethofumesate 500 g/L SC) were conducted with fish, aquatic invertebrates, algae and aquatic plants.

The study summaries are given below.

Reference:	Acute toxicity of ethofumesate 500 SC (500 g/L) against <i>Oncorhynchus mykiss</i>
Author(s), year:	██████████., 1997
Report/Doc. number:	Study no. 5548, Reference no. IDD00134
Guideline(s):	OECD test guideline 203 (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate 500 SC, batch no.: 19970305, purity: 500 g/L
Test species:	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Holding of fish :	Test medium: Reconstituted water, conductivity: 0.2 µS/cm, hardness: 276 mg/L as CaCO ₃ All fish were acclimatised to laboratory conditions for 12 days prior to initiation of the study. Feeding of fish: Daily, except weekends
Number of organisms:	10 fish per controls and test concentrations
Age, length, weight:	Average weight and length: 0.93 g and 4.7 cm, no age of the fish is given
Loading	0.47 g/L fish loading per test vessel
Type of test:	Static, 96 h

Applied concentrations:

Nominal: 0 (control), 1.0, 2.2, 4.8, 10.6, 21.0 and 47.0 mg ai/L

Measured (mean): Not given

Solvent: None

Test conditions:

Water quality: Reconstituted water

Temperature: 15 ± 0.5 °C

pH: 7.5 (0 h), 7.0 – 7.1 (96 h)

O₂ content: 60 – 100% of air saturation

The dissolved oxygen was $\geq 60\%$ of air saturation throughout the test.

Light regime: Light/dark cycle of 12/12

Feeding: The fish were not fed during the 96 hours study period.

Methods: 30 L vessels (all glass) with 20 L test medium were used. The test substance was added directly to the vessels. 10 fish were added per treatment and control group.

Test parameters: All test vessels were monitored for mortality and sub-lethal effects after 3, 24, 48, 72 and 96 hours.

Measurements of temperature, pH and dissolved oxygen were made daily in all treatment solutions. In addition, temperature was continuously measured in the control vessel.

Analytical measurements: The control test substance concentration, analytical controls for three concentrations (control, 21.0 and 47.0 mg ai/L) of the applied test were performed at the start and end of the test using GC/MS analyses.

Statistics: The LC₅₀ was determined using the geometric mean of the concentration causing no mortality and the lowest concentration producing 100% mortality.

Findings:

Analytical data: The chemical analysis showed that the actual concentration of ethofumesate was in the range from 42% to 71% of the nominal concentrations. Hence, the endpoints are based on mean measured concentrations.

Table B. 9.3.1-1: Mortality and sub-lethal effects

Test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)				
	3 h	24 h	48 h	72 h	96 h
Control	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
1.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
2.2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.8	0 (0/10)	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^b
10.6	0 (0/10) ^{bc}	0 (0/10) ^{bc}	0 (0/10) ^{bc}	0 (0/10) ^{bc}	0 (0/10) ^{bc}
21.0	0 (0/10) ^{acd}	0 (0/10) ^{bcef}	0 (0/10) ^{bcf}	0 (0/10) ^{bcf}	10 (1/10) ^{bcf}
47.0	0 (0/10) ^{cef}	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 28.8 mg ai/L					
96 h NOEC = 2.2 mg ai/L (based on behavioural effects)					

^a Slightly reduced swimming activity, ^b Reduced swimming activity, ^c Fish settled at the bottom of the vessel, ^d Gasping for air, ^e Reduced respiration, ^f Loss of equilibrium,

Conclusion:

No mortalities or sublethal effects were recorded at the test concentrations 1.0 and 2.2 mg ai/L over the 96 hour exposure period. Hence, the NOEC was 2.2 mg ai/L. The LC₅₀ was determined to be 28.8 mg ai/L based on nominal concentrations.

Comment RMS:

The study was conducted according to the current valid OECD test guideline 203 (1992).

Taking into account the validity criteria given in the test guideline according to OECD (1992) the acute fish study is considered acceptable.

The mortality in the control was below 10 % (being: 0%) and the environmental conditions (dissolved oxygen, temperature, pH,...) were maintained throughout the test.

However, it should be considered that the information given in the study report is low. Some information is missing, i.e. age of the fish at test start, weight and length of all fish (only average is given), mean measured concentrations.

In addition, no statistical analyses were conducted.

Even though there were identified some uncertainties regarding the reliability of the results of the study, the study is considered acceptable taking into account that the outcome of the study is in line with the results for the active substance. In addition, all validity criteria according to the OECD test guideline 201 are met.

The endpoint given in the study report (LC₅₀ = 28.8 mg ai/L) is based on nominal concentrations. However, under consideration of the analytical measurements conducted for the two highest test concentrations the endpoints should be based on mean measured concentrations.

Considering the mean measured concentrations the LC₅₀ should be > 13.9 mg ai/L and < 22.1 mg ai/L.

Reference:	The effect of ethofumesate 500 SC on the <i>Daphnia magna</i> acute test
Author(s), year:	Pors, J., 1997a
Report/Doc. number:	Study no. 5591, Reference no. IDD00135
Guideline(s):	OECD guideline 202 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate 500 SC, batch no.: 19970305, purity: 500 g/L
Test species:	Water flea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates per treatment and 6 replicate per control, each replicate containing 5 daphnids
Age:	First instar, neonates, < 24 h old
Type of test, duration:	Static test, 48 hours

Applied concentrations:

Nominal:	0 (control), 6, 12, 24, 50, 100 and 200 µL prod./L 0 (control), 13, 6, 12, 25, 50 and 100 mg ai/L
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Mean measured: Not given

Solvent: None

Test conditions:

Water quality:	Natural lake water, pH 7.8
Temperature:	20 ± 2 °C
pH:	8.0 – 8.1 (0 h), 7.6 – 8.1 (48 h)
O ₂ content:	> 90 % of air saturation
Light regime:	16 hours light / 8 hours darkness, light intensity < 50 µmol/m ² /s
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start (0 h) and end of the test (48 h).
Analytical measurement:	The control test substance concentration, analytical controls for two concentrations (3 and 100 mg ai/L) of the applied test were performed at the start and end of the test using GC/MS analyses.
Statistics:	A log/probit regression was performed on the dose response curve giving effect concentrations with 95% confidence limits. Calculations were performed with the software SPSS.

Findings:

Analytical measurements:	Ethofumesate did not degrade significantly during the test period of 72 hours, the values being from 4 to 27%. The mean measured concentrations were between
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80% and 120% of the nominal concentrations. Hence, the results are based on nominal concentrations.

Table B. 9.3.1-2: Effects on daphnids (*D. magna*) exposed to formulated ethofumesate, 500 g/L SC formulation

Ethofumesate [mg ai/L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
3	5	5
6	0	0
12	0	0
25	10	60
50	95	100
100	100	100
24 h EC ₅₀ = 32 mg ai/L 48 h EC ₅₀ = 24 mg ai/L (95 % C.I. = 17 - 28 mg ai/L) 48 h NOEC = 12 mg ai/L (based on immobilisation)		

Conclusion:

The acute toxicity of formulated ethofumesate to *Daphnia magna* has been investigated. No immobilisation was observed at a test concentration of 12 mg a/L, hence the NOEC was determined to be 12 mg ai/L.

The 48-hour EC₅₀ was calculated as 24 mg/L based on nominal concentrations.

Comment RMS:

The study was conducted according to the OECD (OECD 202, 1984). The validity criteria given in the former and current test guidelines according OECD (202, 2004) are met.

The immobilisation in the control group was below 10% (being: 0%) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.

The RMS is of the opinion that the reliability of the results is given. Hence, the results of the study should be used in the risk assessment.

Reference:	The effect of ethofumesate 500 SC on the growth rate of alga <i>Chlorella vulgaris</i>
Author(s), year:	Pors, J., 1997b
Report/Doc. number:	Study no. 5600, Reference no. IDD00136
Guideline(s):	OECD 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate 500 SC, batch no.: 19970305, purity: 500 g/L
Test species:	Green algae, <i>Chlorella</i>
Number of organisms:	1 x 10 ⁴ cells/mL; 3 replicates per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 72 hours

Applied concentrations:

Nominal:	0 (control), 4, 10, 24, 56, 130, 320, 760 and 1800 µL prod./L
Mean measured:	- (control), 2, 5, 12, 28, 65, 160, 380, and 900 mg ai/L
Solvent:	None

Test conditions:

Water quality:	Fresh water medium (according OECD guideline), pH = 8.0
Temperature:	20 ± 2 °C
pH:	8.4 – 9.0
Incubation:	Continuous illumination 76 µmol/cm ² /s
Test parameters:	At the start of the test and after, 24, 48 and 72 h of exposure, the cell mass was determined by fluorescence analyses. Temperature was measured continuously. The pH was measured at the test start and after 72 h.
Analytical measurements:	Samples were taken from the test system and were analysed using GC/MS analyses.
Statistics:	A log/probit regression was performed on the dose response curve giving effect concentrations with 95% confidence limits. Calculations were performed with the software SPSS.

Findings:

Analytical data:	The results of the chemical analyses varied too much to be considered a reliable verification of the nominal test concentrations. For the algal test several steps of taking out a sample were necessary, and this has inevitably caused a great deal of uncertainty. Below the solubility limit (50 mg/L) the analyses showed an approximately
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verification of the nominal test concentration and a degradation of ethofumesate during the test period of 72 hours.

Above the solubility limit, greater values were found after 72 hours of exposition than at the test start. This must be due to the different steps of sampling.

Table B. 9.3.1-3: Effects of Ethofumesate 500 SC on the blue green alga *Chorella vulgaris*

Ethofumesate [mg ai/L] (nominal)	Average specific growth rates	
	Growth rate [h]	% inhibition relative to the control
Control	0.08	-
2	0.07	8
5	0.07	14
12	0.06	19
28	0.06	24
65	0.05	35
160	0.03	57
380	0.01	80
900	0.01	85

Conclusion:

72 h E_rC_{10} = 4 mg ai/L (95% C.I. = 2 – 8 mg ai/L)

72 h E_rC_{50} = 86 mg ai/L (95% C.I. = 57 – 130 mg ai/L)

based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 1984). The used test species, the green algae *Chlorella vulgaris* is stated in the test guidelines (OECD 201, 1984) as proposed test species.

In general the study is in line with the stated test guideline. According to the OECD test guideline (2006) the criterion (16-fold increase of cell density in the control) may not be met when species that grow slower than those mentioned in the test guideline are used.

In the control cultures the increase of the cell density was determined to be greater than the factor of 16 required according to the OECD test guideline.

No statistical analyses considering the effects on biomass were conducted. In addition, no information on the NOEC is given in the study report.

A re-evaluation of the study results by the RMS using the software ToxRat was not possible as important information is not given in the study report (information on calibration).

Based on the low solubility of the active substance the mean measured concentrations were below 80% of the nominal test concentrations. Nevertheless,

the results in the study report are based on nominal test concentrations. The use of the nominal endpoint might underestimate the risk to algae from exposure to the formulated active substance.

The RMS is of the opinion that the reliability of the results is not given. Hence, the results of the study should not be used in the risk assessment.

Reference: **Alga growth inhibition test with Ethofumesate 500 g/L SC**

Author(s), year: Ruymen, V., 2003

Report/Doc. number: Study no. WE-06-391

Guideline(s): OECD 201 (1984), EC test guideline C.3 (1992)

GLP: Yes

Deviations: None relevant

Validity: Acceptable

Material and methods:

Test substance: Ethofumesate 500 SC, batch no.: 012053, purity: 481.9 g/L

Test species: Green algae, *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*)

Number of organisms: 9.5×10^3 cells/mL; 3 replicates per treatment group and control (algal medium with and without algae)

Type of test, duration: Static test, 72 hours

Applied concentrations:

Nominal: 0 (control), 1.9, 4.3, 9.4, 21, 45 and 100 mg prod./L corresponding to 0 (control), 0.92, 2.1, 4.5, 10, 22 and 48 mg ai/L

Mean measured: - (control), 2.0, 4.5, 9.7, 22, 46 and 103 mg prod./L

Solvent: None

Test conditions:

Water quality: Fresh water medium (according OECD guideline)

Temperature: 21.9 – 23.3 °C

pH: 7.91 – 7.95 (test start), 7.77 – 8.30 (test end)

Incubation: Continuous illumination, 6000 lux

Test parameters: At the start of the test and after, 24, 48 and 72 h of exposure, the cell mass was determined by fluorescence analyses.

The light intensity was measured at the start of the test. Temperature was recorded daily and the pH was measured at the test start and after 72 h.

Analytical measurements: At the start and at the end of the test samples were taken from the test system and were analysed using Liquid Chromatography with Diode Array Detection

(LC/DAD).

Statistics: The concentration-effect curve fittings and EC_{50} were based on the model of Bruce and Versteeg (1992).

The NOEC is calculated using a one-way analysis of variance followed by a Dunnett's-t-test or by a Welch-test (SAS Procedure GLM). Because the variances for the endpoint growth rate were not homogeneously distributed and the logarithmic transformation did not adequately homogenize the variances, the Welch-t-test was performed.

Findings:

Analytical data: The concentration of the test substance remained with 80 and 120% of the nominal concentrations throughout the test. Hence, the results are based on nominal test concentrations.

Table B. 9.3.1-4: Effects of Ethofumesate 500 SC on the green alga *Raphidocelis subcapitata*

Ethofumesate [mg prod./L] (nominal)	Average growth		Average growth rate	
	Growth (biomass)	% inhibition relative to the control	Growth rate [h]	% inhibition relative to the control
Control	2.50	-	0.049	-
1.9	2.39	4.3	0.048	1.1
4.3	2.08	16.8	0.046	5.1
9.4	1.42	43.3	0.041	16.5
21	0.52	79.2	0.026	45.9
45	0.26	89.6	0.018	63.6
100	0.18	92.8	0.011	76.9

Conclusion: 72 h E_bC_{10} = 10.74 mg prod./L (95% C.I. = 9.81 – 11.75 mg prod./L)
 72 h E_rC_{50} = 28.23 mg prod./L (95% C.I. = 25.18 – 31.65 mg prod./L)
 72 NOEC = 1.9 (growth rate) and < 1.9 (biomass) mg prod./L
 based on nominal concentrations

Comment RMS: The study was conducted according to the OECD guideline 201 (1984). The used test species, the green algae *Raphidocelis subcapitata* is stated in the test guidelines (OECD 201, 1984) as proposed test species.

In general the study is in line with the stated test guideline. According to the current valid OECD test guideline (2006) a 16-fold increase of cell density in the control has to be shown.

In the control cultures the increase of the cell density was determined to be greater than the factor of 16 required according to the OECD test guideline (actual: 33).

Under consideration of an active substance content of 481.9 g ai/L the relevant endpoints based on the active substance are:

72 h E_rC_{50} = 13.6 mg ai/L

72 h E_bC_{50} = 5.2 mg ai/L

NOEC = 0.92 mg ai/L

The RMS is of the opinion that the reliability of the results is given. Hence, the results of the study should be used in the risk assessment.

Reference:	Toxicity of Ethofol 500 SC (HBX01) to the aquatic plant <i>Lemna gibba</i> in a static growth inhibition test
Author(s), year:	Hoffmann, K. and Deierling, T., 2010
Report/Doc. number:	Study no.: 56172240, Reference no.: IDD00137
Guideline(s):	OECD test guideline 221 (2006), EC guideline No 761/2009, Part C, C.26 (2009)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: Ethofol 500 SC (HBX01), batch no.: 241Y, purity: 485.6 g ai/L (analysed), CAS no.: 26225-79-6

Test species: *Lemna gibba*, duckweed (floating aquatic plant)

Number of organisms: 3 replicates per controls and treatments, colonies consisting of 4 fronds, total of 12 fronds per vessel

Type of test, duration: Static test, 7 days

Applied concentrations:

Nominal: 0 (control), 1.0, 3.2, 10, 32, 100 mg prod./L
0 (control), 0.49, 1.55, 4.86, 15.54 and 48.56 mg ai/L

Measured (mean): 0 (control), 0.442, 1.414, 4.545, 13.91 and 40.87 mg ai/L

Solvent: None

Test conditions:

Water quality: 20X AAP growth according to the OECD guideline, pH = 7.4, conductivity < 5 μ S/cm

Temperature: 23 - 24 °C

pH: 7.4 – 7.6 (test start), 8.6 – 8.9 (test end)

O₂ content: Not given

Light regime: Continuous light, light intensity 7550 – 8310 lux (mean: 8038)

Test parameters:	Frond numbers were counted at the start, at days 3, 5 and 7. Dry weight of plants was determined at test start and at the end of the test. pH was measured at the beginning and at the end of the test in all vessels per concentration. The temperature was measured every day in a vessel without plants.
Analytical measurements:	Samples for analytical measurements were taken at the start of the test and at the end of the test. Duplicate samples were taken from all test concentrations and controls. The analytical measurements were conducted using the HPLC method.
Statistics:	The EC ₅₀ values (yield and growth rate) and their 95% confidence limits were calculated by Probit analyses. For the determination of the NOEC values significant difference at the test concentrations compared to the control values were tested by the Williams Multiple Sequential t-test. The software used to perform the statistical analyses was ToxRat Professional, Version 2.10.

Findings:

Analytical data:	At the start and at day 3, turbidity of the test item was observed in the highest test concentration of 100 mg/L. The mean measured concentrations were in range of 94 and 104% of the nominal test concentration. Hence the results of the test are based on nominal test concentrations.
Morphological findings:	The shape of fronds and colonies after the test period of 7 days showed no differences compared to the control up to and including the nominal test concentration of 10 mg/L. At the highest test concentrations the fronds showed deviations from the control replicates, i.e. smaller fronds, gibbeous growth and shortened roots (32 and 100 mg/L) and additionally necrosis (100 mg/L).

Table B. 9.3.1-5: Mean growth rate and yield (based on fronds numbers)

Ethofol 500 SC [mg/L] (nominal)	Frond number at day 7 (mean) ^a	Mean growth rate		Mean yield	
		0-7 d [1/d]	% inhibition relative to the control	0-7 d	% inhibition relative to the control
Control	185.7	0.390	-	173.7	-
1.0	189.3	0.394	-0.9	177.3	-2.1
3.2	186.3	0.391	-0.3	174.3	-0.4
10	175.7	0.383	1.8	163.7	5.8
32	128.3	0.338	13.4 *	116.3	33.0 *
100	35.7	0.155	60.2 *	23.7	86.4 *

* Statistically significant difference from control, Williams t-test, $\alpha = 0.05$, one-sided

Negative values indicate an increase in growth relative to that of the control

^a Starting number of fronds (day 0): 12 fronds/test vessel

Table B. 9.3.1-6: Mean growth rate and yield (based on dry weight)

Ethofol 500 SC [mg/L] (nominal)	Dry weight after 7 d (mean) ^a [mg]	Mean growth rate		Mean yield	
		0-7 d [1/d]	% inhibition relative to the control	After 7 days [mg]	% inhibition relative to the control
Control	24.4	0.528	-	23.8	-
1.0	24.3	0.528	0.0	23.7	0.4
3.2	23.5	0.523	1.0	22.9	3.6
10	20.6	0.505	4.3	20.0	15.7 *
32	14.9	0.458	13.2 *	14.3	39.7 *
100	6.8	0.346	34.7 *	6.2	74.1 *

* Statistically significant difference from control, Williams t-test, $\alpha = 0.05$, one-sided

Negative values indicate an increase in growth relative to that of the control

^a Initial dry weight at test start: 0.6 mg dry weight/vessel

Conclusion:

Endpoints based on frond number:

7 d E_rC_{50} = 80.6 mg prod./L (95% C.I. = 77.4 – 84.0 mg/L)

7 d E_yC_{50} = 44.0 mg prod./L (95% C.I. = 39.5 – 49.1 mg/L)

7 d E_rC_{10} = 27.5 mg prod./L (95% C.I. = 24.8 – 29.9 mg/L)

7 d E_yC_{10} = 16.3 mg prod./L (95% C.I. = 12.6 – 19.6 mg/L)

NOEC = 10 mg prod./L (base on growth rate and yield)

Endpoints based on dry weight:

7 d E_rC_{50} > 100 mg prod./L

7 d E_yC_{50} = 43.1 mg prod./L (95% C.I. = 38.4 – 48.5 mg/L)

7 d E_rC_{10} = 23.2 mg prod./L (95% C.I. = 20.0 – 26.3 mg/L)

7 d E_yC_{10} = 7.35 mg prod./L (95% C.I. = 5.55 – 9.17 mg/L)

NOEC = 10 mg prod./L

NOEC = 3.2 mg prod./L

Comment RMS:

The study was conducted according to the OECD test guideline 221 (2006) and the EC test guideline C.26 (2009).

The study is considered acceptable as the validity criteria given in the test guideline are met.

The doubling time of the frond number in the control was less than 2.5 days, corresponding to approx. a seven-fold increase in seven days and an average specific growth rate of 0.275 per day.

The doubling time of the frond number in the control was 1.77 days, corresponding to a mean growth rate of 0.390.

The RMS is of the opinion that the reliability of the results is given. Hence, the results of the study should be used in the risk assessment.

Reference:	Ethofol 500 SC: Phytotoxicity test with the rooted submerse aquatic macrophytes <i>Myriophyllum aquaticum</i>, static 7-10 d
Author(s), year:	Scheerbaum, D., 2013
Report/Doc. number:	Study no.: SMA15459, Reference no.: IDD00138
Guideline(s):	Draft OECD test guideline (May 2013)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: Ethofol 500 SC, code: HBX01, batch no.: 710B, purity: 516.2 g ai/L, CAS no.: 26225-79-6

Test species: *Myriophyllum spicatum* (Eurasian water milfoil), rooted macrophytes

Number of organisms: 3 replicates per treatment groups and 6 replicates per control groups, 3 plants per replicate

Type of test, duration: Static, 10 days (preliminary submerse rooting phase: 3 d, exposure phase: 7 d)

Applied concentrations:

Nominal: 0 (control), 0.02, 0.2, 2.0, 20 and 200 mg prod./L
0 (control), 0.010, 0.103, 1.032, 10.32 and 103.2 mg ai/L

Measured (mean): 0 (control), 0.00933, 0.0899, 0.908, 8.72 and 69 mg ai/L

Solvent: None

Test conditions:

Water quality: Smart & Barko medium according to the guideline, pH = 7.5 – 8.0

Sediment: Artificial soil according to OECD guideline 219
5% pear, 20% kaolin and 75% quartz sand, pH = 7.0 ± 0.5

Temperature: 20 ± 2°C

pH: 7.84 – 7.96 (Day 0), 7.22 – 9.77 (Day 14)

O₂ content: 97 – 100% of air saturation (Day 0), 35 – 100% of air saturation (Day 7)

Light regime: 16 hours light, 8 hours dark, light intensity 160 ± 20 µE/m²/s

Methods: Glass beakers with a volume of 2000 mL with small plant pots were used. The ratio of sediment surface area to water surface area was approx. 1:2.
Three days before application of the test item, healthy shoot tips from the pre-culture were clipped off at a length of 6 ± 1 cm. All shoots were weighed individually. Three uniform shoot tips were planted into each pot containing the sediment.

	<p>After an acclimation period of 3 days, the plants were exposed to the test solution for 7 days. All test vessels were contained in an environmentally controlled study area.</p>
Test parameters:	<p>The shoot length of plants, number and length of side shoots were determined at test start and test end and at least once during the exposure phase. Visual observations (growth abnormalities, chlorosis, necrosis,...) were included on every determination day.</p> <p>At test end the fresh weight and dry weight of shoots was determined.</p> <p>pH-values, temperature and oxygen concentration were measured at test start and end and on each observation interval. Light intensity was determined prior to start of the rooting phase.</p>
Analytical measurements:	<p>Analytical evaluation of the test concentrations of ethofumesate and the control was carried out via LC-MS/MS on day of application as well as on day 7 from the sediment layer and the water layer.</p>
Statistics:	<p>The statistical analyses were conducted using software like Excel, SigmaPlot and GraphPad Prism.</p>
<u>Findings:</u>	
Analytical data:	<p>The mean measured concentrations in the water layer were between 91 and 102% of nominal concentration. For the 200 mg prod./L sample, the recovery rate at the start and at the end of the exposure was 84% and 65%, respectively. Probably, the recovery rate for this concentrations level is the result of the low solubility of the test substance in water.</p> <p>In the sediment only minor concentrations were measured at test start, at test end the measured concentrations in the sediment were in the range of < LOQ to 5%.</p> <p>The results of the study are based on mean measured concentrations.</p>
Morphological observations:	<p>In two treatment groups (0.00933 and 0.0899 mg ai/L) an algal contamination was observed after 4 and 7 days. The data from these replicates are reported but were not included in the evaluation.</p> <p>Visual symptoms on the plant shoots were observed at test concentrations equal and up to 0.0809 mg ai/L. The symptoms observed were alteration of form and appearance, curling/twisting or other deformation of whorls, reduced development of head whorls and intermodal stretching. A NOEC of 0.00933 mg ai/L based on visual symptoms was determined.</p>

Table B. 9.3.1-7: Mean yield and growth rate for plant shoots length

Mean measured concentration [mg ai/L]	Length (Day 7)		Average specific growth rate of shoot length		Yield of shoot length	
	[mm]	% inhibition	0 – 7 d [1/d]	% inhibition	[mm]	% inhibition
Control	146 ± 655	-	0.119	-	82.8	-
0.00933	136 ± 12.3	6.85	0.108	9.67	72.2	12.9
0.0899	212 ± 3.11	-45.2	0.169 *	-41.6	146 *	-76.8
0.908	199 ± 11.7	-36.3	0.165 *	-29.2	131 *	-58.2
8.72	128 ± 7.38	12.3	0.094 *	21.3	61.2 *	26.1
69.0	75.2 ± 5.78	48.5	0.021 *	82.1	10.3 *	87.5
7 d E _r C ₅₀ = 20.7 mg ai/L (95% C.I. = 17.1 – 25.5 mg ai/L) 7 d E _r C ₁₀ = 6.11 mg ai/L (95% C.I. = 5.24 – 7.03 mg ai/L) 7 d E _y C ₅₀ = 24.7 mg ai/L (95% CI = 17.1 – 34.9 mg ai/L) 7 d E _y C ₁₀ = 7.52 mg ai/L (95% C.I. = 4.57 – 11.7 mg ai/L) 7 d NOEC = 0.00933 mg ai/L (growth rate and yield)						

* Statistically significant difference from control, Dunnett's test, $p \leq 0.05$

Table B. 9.3.1-8: Mean yield and growth rate for plant fresh weight

Mean measured concentration [mg ai/L]	Fresh weight (Day 7)		Average specific growth rate of fresh weight		Yield of fresh weight	
	[g]	% inhibition	0 – 7 d [1/d]	% inhibition	[mg]	% inhibition
Control	1.095 ± 0.18	-	0.158	-	737	-
0.00933	0.886 ± 0.26	19.1	0.127	19.9	528	28.4
0.0899	1.141 ± 0.03	-4.2	0.166	-4.75	782	-6.18
0.908	0.812 ± 0.13	25.8	0.116	26.8	454	38.4
8.72	0.677 ± 0.06	38.2	0.091 *	42.6	319 *	56.7
69.0	0.558 ± 0.07	49.0	0.062 *	60.6	200 *	72.9
7 d E _r C ₅₀ = 16.1 mg ai/L 7 d E _r C ₁₀ = 0.256 mg ai/L 7 d E _y C ₅₀ = 2.87 mg ai/L 7 d E _y C ₁₀ = 0.183 mg ai/L 7 d NOEC = 0.908 mg ai/L (growth rate and yield)						

Initial biomass fresh weight was 358 mg/plant

* Statistically significant difference from control, Dunnett's test, $p \leq 0.05$

Table B. 9.3.1-9: Mean yield and growth rate for plant dry weight

Mean measured concentration [mg ai/L]	Dry weight (Day 7)		Average specific growth rate of dry weight		Yield of dry weight	
	[mg]	% inhibition	0 – 7 d [1/d]	% inhibition	[mg]	% inhibition
Control	68.0 ± 16.6	-	0.131	-	41.5	-
0.00933	49.9 ± 9.05	26.6	0.09	32.1	23.3	43.7
0.0899	66.2 ± 3.75	2.64	0.13	0.38	39.7	4.27
0.908	55.2 ± 10.2	18.8	0.103	21.1	28.7	30.8
8.72	53.4 ± 5.89	21.5	0.099	24.2	26.9	35.2
69.0	49.3 ± 5.61	27.5	0.088	33.1	22.8	45.1
<p>7 d $E_rC_{50} > 69$ mg ai/L 7 d $E_rC_{10} = 0.233$ mg ai/L</p> <p>7 d $E_yC_{50} > 69$ mg ai/L 7 d $E_yC_{10} = 0.131$ mg ai/L</p> <p>7 d NOEC = 69 mg ai/L (growth rate and yield)</p>						

Initial biomass dry weight was 26.5 mg/plant

* Statistically significant difference from control, Dunnett's test, $p \leq 0.05$

The acute toxicity of the reference item 3,5-dichlorophenol to the non-target aquatic species *Myriophyllum aquaticum* was determined over a period of 7 d. Based on growth rate EC_{50} -values of 4.26 (shoot length), 2.59 (fresh weight) and 2.7 (dry weight) mg/L were determined, respectively.

Conclusion:

The lowest E_yC_{50} and E_rC_{50} in the 7 d exposure of formulated ethofumesate to the rooted macrophytes *Myriophyllum spicatum* was fresh weight. The statistical EC_{50} for this endpoint was 2.87 mg ai/L (based on yield) and 16.1 mg ai/L (based on growth rate).

Comment RMS:

The study was conducted according to the draft OECD test guideline "Water-sediment *Myriophyllum spicatum* toxicity test", published in May 2013. Even though the test guideline is available as a draft version only, the given validity criteria were used for the evolution of the study.

According to the draft OECD guideline the study is considered valid if the following points are met:

- The mean total shoot length and mean shoot fresh weight in control plants must at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures must not

exceed 35% between replicates.

The validity criteria given in the draft OECD test guideline are met in the study.

The doubling time of the total shoot length and mean shoot fresh weight in control plants was 5.85 days and 4.49 days after 7 days in the control (required: ≤ 7 days), corresponding to an average specific growth rate of 0.119 and 0.158, respectively.

In addition, no visual symptoms were observed in the control group.

The mean coefficient of variation for average specific growth rates based on measurement of the shoot length and weight in the control groups was 8.4% (shoot length), 12.7% /fresh weight), and 22.9% (dry weight), respectively (required: < 35%).

The study was well conducted and also covers the methods and requirements given in the draft OECD test guideline. However, it has to be considered that effects on the roots and root development of the test species were not assessed at the end of the test.

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms are not required since it is possible to extrapolate from data of the active substance.

B.9.3.3. Further testing on aquatic organisms

In view of the risk assessment indicating acceptable risk to aquatic organisms, further testing on aquatic organisms is not required.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The aquatic risk assessment includes ethofumesate (as active substance and formulated in the EU representative product) and the environmentally relevant metabolites NC 8493 and NC 20645 (see Table B.9-1).

A summary of the toxicity studies conducted with the active substance, the representative formulation and its metabolites are provided in the following tables.

Table B.9.4-1: Endpoints: Acute toxicity of ethofumesate to aquatic organisms

Test organism	Test condition	Time	Endpoint	Test conc.	NOEC [mg/L]	EC ₅₀ /LC ₅₀ [mg/L]	Reference
Fish							
<i>Oncorhynchus mykiss</i> Rainbow trout	Semi-static	96 h	Mortality	n	9.7	26.5	██████ 1991a ^a
<i>Oncorhynchus mykiss</i> Rainbow trout	Semi-static	96 h	Mortality	mm	4.125	11.91	██████ et al., 1989
<i>Lepomis macrochirus</i> Bluegill sunfish	Semi-static	96 h	Mortality	n	15.0	21.2	██████ 1991b
<i>Lepomis macrochirus</i> Bluegill sunfish	Semi-static	96 h	Mortality	mm	3.55	12.37	██████ et al., 1990
<i>Cyprinodon variegatus</i> Sheepshead minnow	Static	96 h	Mortality	n	12.0	25.0	██████ ██████ 1992
<i>Cyprinus carpio</i> Mirror carp	Semi-static	96 h	Mortality	mm	6.51	10.92	██████ et al., 1989
<i>Leuciscus idus</i> Golden orfe	Static	96 h	Mortality	n	9.3	22.0	██████ 1993 ^a
<i>Danio rerio</i> Zebrafish	Flow-through	FFLC	Reproduction Growth	mm	1.25 0.156	-	██████ 2013
<i>Pimephales promelas</i> Fathead minnow	Flow-through	ELS (28 d)	Mortality Growth	mm	13.3 4.17	-	██████ 1991
Aquatic invertebrates							
<i>Daphnia magna</i> Waterflea	Static	48 h	Immobility	n	13.0	28.1	Thun, S., 1993
<i>Americamysis bahia</i> Mysid shrimp	Static	96 h	Immobility	mm	< 2.5	5.4	Schupner, J.K. & Stachura, B.J., 1992
<i>Crassostrea virginica</i> Eastern oyster	Flow-through	96 h	Mortality Shell growth	mm	5.6 < 0.81	> 9.0 1.7	Yurk, J.J. & Ache, B.W., 1992
<i>Daphnia magna</i> Waterflea	Semi-static	21 d	Reproduction	n	0.32	0.77	Douglas, M.T., James, C.M. & Macdonald, I.A., 1990
<i>Daphnia magna</i>	Semi-static	21 d	Reproduction	n	1.0	2.7	Bellmann, W.,

Test organism	Test condition	Time	Endpoint	Test conc.	NOEC [mg/L]	EC ₅₀ /LC ₅₀ [mg/L]	Reference
Waterflea							1992 ^a
<i>Daphnia magna</i> Waterflea	Semi-static	21 d	Reproduction	n	0.32	1.2	Adema, D.M.M. & de Rulter, A., 1989 ^a
Sediment dwelling organisms							
<i>Chironomus riparius</i> Midge	Static	28 d	Emergence	mm	3.2	> 3.2	Mattock, S.D., 1998
<i>Chironomus riparius</i> Midge	Static	28 d	Emergence Development	mm	2.42	> 2.42	Desmares-Koopmans, M.J.E., 2002
<i>Chironomus riparius</i> Midge	Static	28 d	Emergence Development	mm	12.9	> 33.0	Stäbler, D., 2003
Algae							
<i>Pseudokirchneriella subcapitata</i> Green algae	Static	72 h	Growth rate Yield	mm	5.91	16.3 9.68	Bruns, E., 2008
<i>Anabaena flos-aquae</i> Blue green algae	Static	96 h	Growth rate Biomass	n	20.0	> 20.0	Banman, C.S., Daly, R.A. & Lam, C.V., 2009a
<i>Skeletonema costatum</i> Saltwater diatom	Static	72 h	Growth rate Biomass	n	5.0 2.5	> 20.0 14.5	Banman, C.S., Daly, R.A. & Lam, C.V., 2009b
		96 h	Growth rate Biomass	n	10.0 5.0	> 20.0 17.1	
Aquatic macrophytes							
<i>Lemna minor</i> Duckweed	Semi-static	14 d	Growth rate Biomass	mm	4.3	> 52.8 50.4	Scheerbaum, D., 1998
<i>Lemna minor</i> Duckweed	Semi-static	7 d	Growth rate Biomass	mm	26.0 17.0	> 42.0 35.0	Bogers, M., 2001
<i>Myriophyllum spicatum</i> Water milfoil	Static	14 d	Growth rate Yield	mm	0.036	0.479 0.25	Banman, C.S., 2013

n...nominal, mm...mean measured

^a Due to deficiencies observed in the study the results of study should be used as additional information only.

Effects to aquatic organisms from exposure to the metabolites NC 8493 and NC 20645 were tested for the aquatic invertebrates and algae. No studies were conducted with fish and aquatic macrophytes.

Under consideration of the high sensitivity of the parent compound ethofumesate to algae and aquatic macrophytes no studies on fish are considered necessary. However, to address the risk to aquatic macrophytes a 10 time higher toxicity of the metabolites compared to the parent compound is considered in the risk assessment.

An additional metabolite was found in a water sediment study submitted by the Task Force Ethofumesate. The metabolite was measured at concentrations > 10% and hence an aquatic risk assessment has to be conducted.

The notifier Task Force Ethofumesate submitted studies with aquatic invertebrates and algae. For the aquatic macrophytes a ten times higher toxicity compared to the parent compound was assumed.

United Phosphorous Ltd. submitted no additional studies as the metabolite Ethofumesate acetic acid was not identified in the water sediment study conducted by UPL.

Table B.9.4-2: Endpoints: Acute toxicity of metabolites to aquatic organisms

Test substance	Test organism	Test condition	Time	Endpoint	Test conc.	NOEC [mg/L]	EC ₅₀ /LC ₅₀ [mg/L]	Reference
Aquatic invertebrates								
Metabolite NC 8493	<i>Daphnia magna</i> Waterflea	Semi-static ^a	48 h	Immobility	n	10	> 10	Riebschläger, T., 2012a
Metabolite NC 8493	<i>Daphnia magna</i> Waterflea	Static ^a	48 h	Immobility	n	100	> 100	Juckeland, D., 2013a
Metabolite NC 20645	<i>Daphnia magna</i> Waterflea	Semi-static ^a	48 h	Immobility	n	10	> 10	Riebschläger, T., 2012b
Metabolite NC 20645	<i>Daphnia magna</i> Waterflea	Static ^a	48 h	Immobility	n	100	> 100	Juckeland, D., 2013b
Metabolite Ethofumesate acetic acid	<i>Daphnia magna</i> Waterflea	Static ^a	48 h	Immobility	n	10	> 10	König, N., 2013
Algae								
Metabolite NC 8493	<i>P. subcapitata</i> Green algae	Static	72 h	Growth rate Yield	n	0.367	20.7 0.865	Bruns, E., 2012a
Metabolite NC 8493	<i>D. subspicatus</i> Green algae	Static	72 h	Growth rate Yield	mm	1.33	4.83 1.87	Juckeland, D., 2013c
Metabolite NC 20645	<i>P. subcapitata</i> Green algae	Static ^a	72 h	Growth rate Yield	n	10.0	> 10.0	Bruns, E., 2012b
Metabolite NC 20645	<i>D. subspicatus</i> Green algae	Static	72 h	Growth rate Yield	mm	1.25	52.4 8.83	Juckeland, D., 2013d
Metabolite Ethofumesate acetic acid	<i>P. subcapitata</i> Green algae	Static	72 h	Growth rate Yield	N	25	> 100 98.98	Sobxzyk, H., 2013

n...nominal, mm...mean measured

^a Limit test

Table B.9.4-3: Endpoints: Acute toxicity of Ethofol 500 SC to aquatic organisms

Test organism	Test condition	Time	Endpoint	Test conc.	NOEC [mg ai/L]	EC ₅₀ /LC ₅₀ [mg ai/L]	Reference
Fish							
<i>Oncorhynchus mykiss</i> Rainbow trout	Static	96 h	Mortality	mm	2.2	> 13.9 < 22.1	██████████ 1997
<i>Cyprinus carpio</i> Mirror carp	Semi-static	96 h	Mortality	mm	12.8	14.4	██████████ et al., 1989 ^a
<i>Dario rerio</i> Zebra fish	Semi-static	96 h	Mortality	n	9.0	34.0	██████████ et al., 1988 ^a
Aquatic invertebrates							
<i>Daphnia magna</i> Waterflea	Static	48 h	Immobility	n	12.0	24.0	Pors, J., 1997a
<i>Daphnia magna</i> Waterflea	Static	48 h	Immobility	n	19.57	26.8	Cameron, B.D., et al., 1989 ^a
<i>Daphnia magna</i> Waterflea	Semi-static	21 d	Reproduction	n	0.32	1.2	Barber, I., 1991 ^a
Algae							
<i>Desmodesmus subspicatus</i> Green algae	Static	72 h	Growth rate Biomass	n	2.2	9.7 6.7	Knacker, T., 1989 ^a
<i>Raphidocelis capricornutum</i> Green algae	Static	72 h	Growth rate Biomass	n	0.92 < 0.92	13.6 5.2	Ruymen, V., 2003
Aquatic macrophytes							
<i>Lemna minor</i> Duckweed	Static	7 d	Growth rate	n	10.0	80.6	Hoffmann, K. & Deierling, T., 2010
			Yield (frond number)		10.0	44.0	
			Growth rate Yield (dry weight)		10 3.2	> 100 43.1	
<i>Myriophyllum aquaticum</i> Water milfoil	Static	7 d	Growth rate Yield (fresh weight)	mm	0.908 0.908	16.1 2.87	Scheerbaum, D., 2013

n...nominal, mm...mean measured

^a Studies were conducted with the comparable formulation Ethofumesate 500 SC

The risk assessment for aquatic organisms is based on the recommendations of the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4 (final), 17 October 2002) and follows a tiered approach.

The standard risk assessment (first tier) is based on the calculation of the toxicity/exposure ratios (TER) taking into consideration the most sensitive organism of each group. TER values will be estimated as the ratio of EC₅₀/LC₅₀ or NOEC to PEC_{SW} (exposure, see Section B.8) calculated with the FOCUS surface water model. This model based on scenarios considering a tiered sequence of exposure assessment steps (Step 1, 2, 3 and 4). The trigger values for the acute risk are TER_A > 100 for fish and invertebrates, TER_A > 10 for algae and aquatic macrophytes and for the chronic risk TER_{LT} > 10 for fish and aquatic invertebrates.

Table B.9.4-4: Summary of maximum observed PEC_{SW} values of ethofumesate and metabolites, FOCUS Step 1 and 2

Substance	Sugar beet (pre-emergence), Application rate: 1 x 1.0 kg ai/ha			Sugar beet (post-emergence), Application rate: 3 x 0.333 kg ai/ha		
	FOCUS Step 1 [µg/L]	FOCUS Step 2 [µg/L]		FOCUS Step 1 [µg/L]	FOCUS Step 2 [µg/L]	
		N-EU	S-EU		N-EU	S-EU
Ethofumesate	297.2	60.0	111.7	296.9	42.3	78.7
NC 8493	70.81	nc		70.81	nc	
NC 20645	7.43	nc		7.43	nc	
Ethofumesate acetic acid	1.36	nc		1.36	nc	

nc...not calculated

^a FOCUS Step 2 calculations is based on input parameters for Southern Europe (worst-case)**B.9.4.1. Acute risk****TER_A for fish:**

The most sensitive species was observed to be the mirror carp with a 96 h LC₅₀ of 10.92 mg ai/L (■■■■■ et al., 1989).

No studies with the metabolites NC 8493, NC 20645 and ethofumesate acetic acid were submitted. However, under consideration that the most sensitive groups of aquatic organisms were identified to be aquatic invertebrates, algae and aquatic macrophytes acute toxicity studies with fish are not considered necessary.

Table B. 9.4.1-1: Acute toxicity exposure ratios (TER_A) for fish based on worst case PEC_{SW} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.2972	36.7	100
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.2969	36.8	100

T**Table B. 9.4.1-2: Acute toxicity exposure ratios (TER_A) for fish based on worst case PEC_{SW} from FOCUS Step 2 (Southern EU) – mentioned are only these organisms which failed the trigger at Step 1**

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.1117	97.8	100
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.0787	139	100

Table B. 9.4.1-3: Acute toxicity exposure ratios (TER_A) for fish based on worst case PEC_{sw} from FOCUS Step 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L] 1 x 1000 g ai/ha	TER _A values
		<i>Cyprinus carpio</i> LC ₅₀ = 10.92 mg ai/L
D3 ditch	0.0052	2100
D4 pond	0.0004	27300
D4 stream	0.0043	2540
R1 pond	0.0045	2427
R1 stream	0.0476	229
R3 stream	0.0115	950
Trigger		100

Under consideration of FOCUS Step 3 PEC_{sw} values an acceptable acute risk to fish was identified based on the most sensitive species, the mirror carp.

TER_A for aquatic invertebrates:

The new guidance document on aquatic organisms (EFSA Journal 2013;11(7):3290) proposes the usage of a geometric mean approach when results from more than one species are available.

Acute toxicity studies with three aquatic invertebrate species are available. The most sensitive species were observed to be the marine species *Mysidopsis bahia* (EC₅₀ = 5.4 mg ai/L based on mortality) and *Crassostrea virginica* (EC₅₀ = 1.7 mg ai/L based on shell growth).

The endpoint derived for the freshwater species *Daphnia magna* was determined to be EC₅₀ = 28.1 mg ai/L based on immobilisation.

Studies with the representative formulation Ethofol 500 SC and the comparable formulation Ethofumesate 500 SC were also submitted indicating a comparable toxicity to daphnids than observed in the studies with the technical active substance.

In addition studies with the metabolites NC 8493 and NC 20645 were conducted addressing the risk to aquatic invertebrates. No studies were submitted with the metabolite ethofumesate acetic acid which was identified in a water sediment study submitted by the notifier Task Force Ethofumesate. Hence, the risk assessment is based on the acute toxicity study conducted by the notifier Task Force Ethofumesate.

Table B. 9.4.1-4: Acute toxicity exposure ratios (TER_A) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	EC ₅₀ = 28.1	48 h	0.2972	94.5	100
<i>Americamysis bahia</i>	Ethofumesate	EC ₅₀ = 5.4	96 h	0.2972	18.2	100
<i>Crassostrea virginica</i>	Ethofumesate	EC ₅₀ = 1.7	96 h	0.2972	5.7	100
<i>Daphnia magna</i>	NC 8493	EC ₅₀ > 10.0	48 h	0.0708	> 141	100

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
<i>Daphnia magna</i>	NC 20645	EC ₅₀ > 10.0	48 h	0.00743	> 1346	100
<i>Daphnia magna</i>	Ethofumesate acetic acid	EC ₅₀ > 10.0	48 h	0.00136	> 7353	100
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	EC ₅₀ = 28.1	48 h	0.2969	94.6	100
<i>Americamysis bahia</i>	Ethofumesate	EC ₅₀ = 5.4	96 h	0.2969	18.2	100
<i>Crassostrea virginica</i>	Ethofumesate	EC ₅₀ = 1.7	96 h	0.2969	5.7	100
<i>Daphnia magna</i>	NC 8493	EC ₅₀ > 10.0	48 h	0.0708	> 141	100
<i>Daphnia magna</i>	NC 20645	EC ₅₀ > 10.0	48 h	0.00743	> 1346	100
<i>Daphnia magna</i>	Ethofumesate acetic acid	EC ₅₀ > 10.0	48 h	0.00136	> 7353	100

Table B. 9.4.1-5: Acute toxicity exposure ratios (TER_A) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 2 (Southern EU) – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	EC ₅₀ = 28.1	48 h	0.1117	252	100
<i>Americamysis bahia</i>	Ethofumesate	EC ₅₀ = 5.4	96 h	0.1117	48.3	100
<i>Crassostrea virginica</i>	Ethofumesate	EC ₅₀ = 1.7	96 h	0.1117	15.2	100
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	EC ₅₀ = 28.1	48 h	0.0787	357	100
<i>Americamysis bahia</i>	Ethofumesate	EC ₅₀ = 5.4	96 h	0.0787	68.6	100
<i>Crassostrea virginica</i>	Ethofumesate	EC ₅₀ = 1.7	96 h	0.0787	21.6	100

The TER calculations based on FOCUS Step 3 were conducted using the lowest endpoint, i.e. the EC₅₀ of 1.7 mg ai/L based on effects on shell growth of oysters. The risk to aquatic invertebrates (*Mysidopsis bahia*) is covered by the following risk assessment.

Table B. 9.4.1-6: Acute toxicity exposure ratios (TER_A) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L] 1 x 1000 g ai/ha	TER _A values	PEC [mg/L] 3 x 333 g ai/ha	TER _A values
		<i>Crassostrea virginica</i> EC ₅₀ = 1.7 mg ai/L		<i>Crassostrea virginica</i> EC ₅₀ = 1.7 mg ai/L
D3 ditch	0.0052	327	0.0013	1308
D4 pond	0.00043	3953	0.00051	3333
D4 stream	0.0043	395	0.0011	1545
R1 pond	0.0045	378	0.00047	3617
R1 stream	0.0476	35.7	0.0078	218
R3 stream	0.0115	148	0.0192	88.5
Trigger		100		100

Based on the risk assessment a high acute risk to aquatic invertebrates was identified considering the FOCUS scenarios R1 and R3 stream. Hence, risk mitigation measures are required addressing the risk to aquatic invertebrates.

Table B. 9.4.1-7: Acute toxicity exposure ratios (TER_A) based on worst case PEC_{sw} from FOCUS Step 4 – mentioned are only these organisms which failed the trigger at Step 3

GAP uses	FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	
				<i>Crassostrea virginica</i> EC ₅₀ = 1.7 mg ai/L
1 x 1000 g ai/ha	R1 stream	10	0.021692	78.4
		20	0.011377	149
3 x 333 g ai/ha	R3 stream	10	0.008718	195
		20	-	-
Trigger				100

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 20 m (pre-emergence, 1 x 1000 g ai/ha) and 10 m (post-emergence, 3 x 333 g ai/ha) an acceptable risk to aquatic invertebrates was identified.

B.9.4.2. Chronic risk

TER_{LT} for fish:

A fish full life cycle test with the zebra fish (■■■■■ 2013) was submitted addressing the chronic risk to fish. A NOEC of 0.156 mg ai/L based on effects on growth was determined.

Table B. 9.4.2-1: Chronic toxicity exposure ratios (TER_{LT}) for fish based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.2972	0.52	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.2969	0.53	10

Table B. 9.4.2-2: Chronic toxicity exposure ratios (TER_{LT}) for fish based on worst case PEC_{sw} from FOCUS Step 2 – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.1117	1.4	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.0787	2.0	10

Table B. 9.4.2-3: Chronic toxicity exposure ratios (TER_{LT}) for fish based on worst case PEC_{sw} from FOCUS Step 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L] 1 x 1000 g ai/ha	TER _{LT} values	PEC [mg/L] 3 x 333 g ai/ha	TER _{LT} values
		<i>Danio rerio</i> NOEC = 0.156 mg ai/L		<i>Danio rerio</i> NOEC = 0.156 mg ai/L
D3 ditch	0.0052	30	0.0013	120
D4 pond	0.00043	363	0.00051	306
D4 stream	0.0043	36	0.0011	142
R1 pond	0.0045	35	0.00047	332
R1 stream	0.0476	3.3	0.0078	20
R3 stream	0.0115	14	0.0192	8.1
Trigger		10		10

Based on the risk assessment an acceptable risk to fish was identified. For two FOCUS scenario (i.e. R1 and R3 stream) the TER_{LT} value was calculated to be below the trigger of 10.

Hence, a refined risk assessment based on risk mitigation measured was conducted.

Table B. 9.4.2-4: Chronic toxicity exposure ratios (TER_{LT}) for fish based on worst case PEC_{sw} from FOCUS Step 4 – mentioned are only these organisms which failed the trigger at Step 3

GAP uses	FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _{LT} values
				<i>Danio rerio</i> NOEC = 0.156 mg ai/L
1 x 1000 g ai/ha	R1 stream	10	0.021692	7.2
		20	0.011377	14
3 x 333 g ai/ha	R3 stream	10	0.008718	18
		20	-	-
Trigger				10

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 20 m (pre-emergence, 1 x 1000 g ai/ha) and 10 m (post-emergence, 3 x 333 g ai/ha) an acceptable risk to fish was identified.

TER_{LT} to aquatic invertebrates:

Chronic toxicity studies with daphnids were submitted addressing the long-term risk to aquatic invertebrates. The lowest endpoint was determined to be NOEC = 0.32 mg ai/L based on effects on reproduction (Douglas et al., 1990). In addition a reproduction study with the representative formulation was submitted indicating a comparable toxicity to daphnids than observed in the chronic toxicity studies with the active substance.

Table B. 9.4.2-5: Chronic toxicity exposure ratios (TER_{LT}) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.2972	1.1	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.2969	1.1	10

Table B. 9.4.2-6: Chronic toxicity exposure ratios (TER_{LT}) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 2 – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.1117	2.9	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.0787	4.1	10

Table B. 9.4.2-7: Chronic toxicity exposure ratios (TER_{LT}) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L] 1 x 1000 g ai/ha	TER _{LT} values	PEC [mg/L] 3 x 333 g ai/ha	TER _{LT} values
		<i>Daphnia magna</i> NOEC = 0.32 mg ai/L		<i>Daphnia magna</i> NOEC = 0.32 mg ai/L
D3 ditch	0.0052	61.5	0.0013	246
D4 pond	0.00043	744	0.00051	627
D4 stream	0.0043	74	0.0011	291
R1 pond	0.0045	71	0.00047	681
R1 stream	0.0476	6.7	0.0078	41
R3 stream	0.0115	28	0.0192	17
Trigger		10		10

Based on the risk assessment an acceptable risk to aquatic invertebrates was identified. For one FOCUS scenario (i.e. R1 stream) the TER_{LT} value was calculated to be below the trigger of 10.

Hence, a refined risk assessment based on risk mitigation measured was conducted.

Table B. 9.4.2-8: Chronic toxicity exposure ratios (TER_{LT}) for aquatic invertebrates based on worst case PEC_{sw} from FOCUS Step 4 – mentioned are only these organisms which failed the trigger at Step 3

GAP uses	FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _{LT} values
				<i>Daphnia magna</i> NOEC = 0.32 mg ai/L
1 x 1000 g ai/ha	R1 stream	10	0.021692	15
		20	0.011377	-
Trigger				10

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 10 m an acceptable risk to aquatic invertebrates was identified.

TER_{LT} for sediment dwelling organisms:

Studies on effects on chironomids (*Chironomus riparius*) were submitted by the notifiers addressing the risk to sediment dwelling organisms. The lowest endpoint was derived from the study by Desmares-Koopmans, 2002. A NOEC of 2.42 mg ai/L based on mean measured concentrations in the overlaying water was determined.

Table B. 9.4.2-9: Chronic toxicity exposure ratios (TER_{LT}) for chironomids based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.2972	8.1	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.2969	8.2	10

Table B. 9.4.2-10: Chronic toxicity exposure ratios (TER_{LT}) for chironomids based on worst case PEC_{sw} from FOCUS Step 2 – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.1117	22	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.0787	31	10

The risk to sediment dwelling organisms is considered acceptable based on FOCUS Step 2 PEC_{sw} values.

TER_{LT} for algae:

Several studies with algae were submitted for the risk assessment. Most of the studies submitted for the first EU approval of ethofumesate were identified to be not valid considering deficiencies observed in the studies. Hence, the notifier UPL has only access to one algae study conducted with the comparable formulation Ethofumesate 500 SC (Knacker, 1989). As the active substance ethofumesate is an herbicide at least two different algae species have to be tested. The notifier UPL submitted an additional algae study conducted with the formulation Ethofumesate 500 SC and the green algae *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*). Both studies were conducted with green algae (*Raphidocelis subcapitata* and *Desmodesmus subspicatus*), hence, an additional study with an additional algae species from a different taxonomic group has to be submitted.

The notifier Task Force Ethofumesate submitted studies with three different algae species (*P. subcapitata*, *Anabaena flos-aquae* and *Skeletonema costatum*), tested with the technical active substance. The most sensitive species was observed to be the green algae *P. subcapitata*.

In addition, a study with the representative formulation was submitted. The endpoints derived from the study with the green algae *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) indicate a similar toxicity than observed in the studies with the active substance.

The risk assessment for algae is based on the lowest endpoint derived from the study with the formulation Ethofumesate 500 SC and the green algae *Raphidocelis subcapitata* ($E_bC_{50} = 5.2$ mg ai/L).

In addition studies with the metabolites NC 8493 and NC 20645 were conducted addressing the risk to algae. No studies were submitted with the metabolite ethofumesate acetic acid which was identified in a water sediment study submitted by the notifier Task Force Ethofumesate. Hence, the risk assessment is based on the acute toxicity study conducted by the notifier Task Force Ethofumesate.

The risk assessment was conducted according to the SANCO guidance document. Hence, endpoints based on biomass and yield were also considered in the risk assessment.

Table B. 9.4.2-11: Chronic toxicity exposure ratios (TER) for algae based on worst case PECsw from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>P. subcapitata</i>	Ethofumesate	$E_yC_{50} = 9.68$	72 h	0.2972	32.6	10
<i>P. subcapitata</i>	Ethofumesate 500 SC	$E_bC_{50} = 5.2$	72 h	0.2972	17.5	10
<i>P. subcapitata</i>	NC 8493	$E_yC_{50} = 0.865$	72 h	0.07081	12.2	10
<i>D. subspicatus</i>	NC 20645	$E_yC_{50} = 8.83$	72 h	0.00743	1188	10
<i>P. subcapitata</i>	Ethofumesate acetic acid	$E_yC_{50} = 98.98$	72 h	0.00136	72779	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>P. subcapitata</i>	Ethofumesate	$E_yC_{50} = 9.68$	72 h	0.2969	32.6	10
<i>P. subcapitata</i>	Ethofumesate 500 SC	$E_bC_{50} = 5.2$	72 h	0.2969	17.5	10
<i>P. subcapitata</i>	NC 8493	$E_yC_{50} = 0.865$	72 h	0.07081	12.2	10
<i>D. subspicatus</i>	NC 20645	$E_yC_{50} = 8.83$	72 h	0.00743	1188	10
<i>P. subcapitata</i>	Ethofumesate acetic acid	$E_yC_{50} = 98.98$	72 h	0.00136	72779	10

Based on the risk assessment a low risk to algae was identified. At FOCUS Step 1 all TER values were above the relevant trigger values.

TER for aquatic macrophytes:

Studies with the aquatic macrophytes *Lemna* spp. and *Myriophyllum* sp. were submitted to address the risk to aquatic macrophytes. Based on the results of the studies with the technical active substance and the representative formulation Ethofol 500 SC the water milfoil *Myriophyllum aquaticum* was observed to be the most sensitive species. Hence, the risk assessment is based on the lowest endpoint derived from the 14 day studies with water milfoil.

Based on the available data with the technical active substance and the representative formulation the active substance was observed to be of higher toxicity to aquatic macrophytes than the technical active substance. However, it should be considered that the study with the active substance was conducted with *Myriophyllum spicatum* whereas the study with the formulation was conducted with *Myriophyllum aquaticum*.

No studies with the metabolites NC 8493, NC 20645 and ethofumesate acetic acid was submitted by the notifiers. Hence, the risk assessment for the metabolites is based on a ten times higher toxicity of the metabolites compared to the parent compound.

Table B. 9.4.2-12: Chronic toxicity exposure ratios (TER_{LT}) for aquatic macrophytes based on worst case PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Myriophyllum spicatum</i>	Ethofumesate	$E_yC_{50} = 0.25$	14 d	0.2972	0.84	10
<i>Myriophyllum aquaticum</i>	Ethofol 500 SC	$E_yC_{50} = 2.87$	7 d	0.2972	9.7	10
<i>Myriophyllum spicatum</i>	NC 8493	$E_yC_{50} = 0.025^a$	14 d	0.07081	0.35	10
<i>Myriophyllum spicatum</i>	NC 20645	$E_yC_{50} = 0.025^a$	14 d	0.00743	3.4	10
<i>Myriophyllum spicatum</i>	Ethofumesate acetic acid	$E_yC_{50} = 0.025^a$	14 d	0.00136	18	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Myriophyllum spicatum</i>	Ethofumesate	$E_yC_{50} = 0.25$	14 d	0.2969	0.84	10
<i>Myriophyllum aquaticum</i>	Ethofol 500 SC	$E_yC_{50} = 2.87$	7 d	0.2969	9.7	10
<i>Myriophyllum spicatum</i>	NC 8493	$E_yC_{50} = 0.025^a$	14 d	0.07081	0.35	10
<i>Myriophyllum spicatum</i>	NC 20645	$E_yC_{50} = 0.025^a$	14 d	0.00743	3.4	10
<i>Myriophyllum spicatum</i>	Ethofumesate acetic acid	$E_yC_{50} = 0.025^a$	14 d	0.00136	18	10

^a A ten times higher toxicity to aquatic macrophytes compared to the parent compound is assumed.

Table B. 9.4.2-13: Chronic toxicity exposure ratios (TER_{LT}) for aquatic macrophytes based on worst case PEC_{sw} from FOCUS Step 2 (Southern EU) – mentioned are only these organisms which failed the trigger at Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
Sugar and fodder beet, pre emergence, 1 x 1000 g ai/ha						
<i>Myriophyllum spicatum</i>	Ethofumesate	E _y C ₅₀ = 0.25	14 d	0.1117	2.2	10
<i>Myriophyllum aquaticum</i>	Ethofol 500 SC	E _y C ₅₀ = 2.87	7 d	0.1117	26	10
<i>Myriophyllum spicatum</i>	NC 8493	E _y C ₅₀ = 0.025 ^a	14 d	< 0.001	> 25	10
<i>Myriophyllum spicatum</i>	NC 20645	E _y C ₅₀ = 0.025 ^a	14 d	0.0017	15	10
Sugar and fodder beet, post-emergence, 3 x 333 g ai/ha						
<i>Myriophyllum spicatum</i>	Ethofumesate	E _y C ₅₀ = 0.25	14 d	0.0787	3.2	10
<i>Myriophyllum aquaticum</i>	Ethofol 500 SC	E _y C ₅₀ = 2.87	7 d	0.0787	36	10
<i>Myriophyllum spicatum</i>	NC 8493	E _y C ₅₀ = 0.025 ^a	14 d	< 0.001	> 25	10
<i>Myriophyllum spicatum</i>	NC 20645	E _y C ₅₀ = 0.025 ^a	14 d	0.0012	21	10

^a A ten times higher toxicity to aquatic macrophytes compared to the parent compound is assumed.

Table B. 9.4.2-14: Chronic toxicity exposure ratios (TER_{LT}) for aquatic macrophytes based on worst case PEC_{sw} from FOCUS Step 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L] 1 x 1000 g ai/ha	TER _{LT} values	PEC [mg/L] 3 x 333 g ai/ha	TER _{LT} values
		<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L		<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L
D3 ditch	0.0052	48	0.0013	192
D4 pond	0.00043	581	0.00051	490
D4 stream	0.0043	58	0.0011	227
R1 pond	0.0045	56	0.00047	532
R1 stream	0.0476	5.3	0.0078	32
R3 stream	0.0115	22	0.0192	13
Trigger		10		10

Based on the risk assessment a high risk to aquatic macrophytes was identified considering the FOCUS scenario R1 stream. Hence, risk mitigation measures are required addressing the risk to aquatic macrophytes.

Table B. 9.4.2-15: Chronic toxicity exposure ratios (TER_{LT}) for aquatic macrophytes based on worst case PEC_{sw} from FOCUS Step 4 – mentioned are only these organisms which failed the trigger at Step 3

GAP uses	FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _{LT} values
				<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L
1 x 1000 g ai/ha	R1 stream	10	0.021692	12
		20	0.011377	-
Trigger				10

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 10 m an acceptable risk to aquatic macrophytes was identified.

B.9.4.3. Bioaccumulation

Although the $\log P_{ow}$ of the active substance ethofumesate and its metabolites is below the trigger (< 3), the accumulation and elimination of ethofumesate has been determined in two fish bioaccumulation studies.

The bioconcentration factor (BCF) of ethofumesate was between 67 and 144 in whole fish, edible and non-edible tissues. Depuration was very rapid with a calculated elimination half-life of < 1 day.

According to the Guidance Document on Aquatic Organisms (SANCO/3268/2001) a higher tier risk assessment is required when the maximum BCF is greater than 100 for substances which are not readily biodegradable. To address the risk of bioaccumulation in the food chain the following exposure routes were considered:

- Direct long-term effects in fish due to bioconcentration (see fish full life cycle study, [REDACTED] 2013)
- Secondary poisoning for birds and mammals (see section B.9.2)
- Biomagnification in aquatic food chains (see section B.9.2)

B.9.5. EFFECTS ON ARTHROPODS**B.9.5.1. Effects on bees**

Regarding the toxicity data on the technical ethofumesate please refer to Volume 3, B.9. (AS).

In addition studies with the representative formulation Ethofol 500 SC and the comparable formulation Ethofumesate 500 SC were submitted by the notifier United Phosphorus Ltd.

B.9.5.1.1. Acute toxicity to bees

Reference:	Honeybee – Acute oral toxicity test with Ethofumesate 500 SC (Ethofumesate: 500 g/L)
Author(s), year:	Mallikarjunappa, S., 1998
Report/Doc. number:	Report No. 2295/97, Reference No. IDD00139
Guideline(s):	EPPO Guideline 170 (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate 500 SC, Batch no. 1997/4, Purity: 500 g/L (declared)
Reference:	Dimethoate technical, 0.3 µg ai/L
Solvent:	None
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Acute oral test
Number of organisms:	Three replicates with 10 bees for control (solvent control with 50% sucrose solution), the reference item treatments and the test item treatment groups
Food:	50% sucrose solution

Oral toxicity test:

Applied concentrations:	Control: 50% (w/w) aqueous sucrose solution Test item: 40, 80, 120, 160 and 200 µg ai/bee Reference item: 0.3 µg ai/bee
Exposure route:	The test item was dissolved in sucrose solution was added and offered to the bees. The test bees were starved for 2 hours before they were fed with the solutions. 0.2 mL of the test solution was fed to groups of 10 bees via feeding tubes assuming that each bee will consume a dose of 20 µL.
Test conditions:	Temperature: 25 - 26 °C
Test parameter:	The mortality of honey-bees was recorded at 24 and 48 h.
Analytical measurements:	After preparing the test concentrations, the highest concentration was sampled for analysis of the active substance using the HPLC method.

Findings:**Table B. 9.5.1-1: Effects of Ethofumesate 500 SC on *Apis mellifera* following 48-h oral exposure in an acute toxicity test**

Nominal dose [$\mu\text{g ai/bee}$] (analysed)	Mortality [%]	
	24 h	48 h
Control (sugar solution)	0.0	0.0
Treatment		
40	0.0	3.33
80	0.0	0.0
120	3.33	3.33
160	0.0	0.0
200 (184.3)	3.33	3.33
Reference		
0.3	100	100
Test substance: 24h / 48h $\text{LD}_{50} > 184.3 \mu\text{g ai/bee}$ Reference: 24 h $\text{LD}_{50} < 0.3 \mu\text{g dimethoate/bee}$ 48 h $\text{LD}_{50} < 0.3 \mu\text{g dimethoate/bee}$		

Conclusions:

No substance related dose-response effects was observed in the study. Low numbers of mortalities were
 48 h $\text{LD}_{50} > 184.3 \mu\text{g ai/bee}$ (oral toxicity)

Comment RMS:

The study was conducted according to the EPPO Guideline 170 (1992) and is in general agreement with the current valid test guideline according to OECD 213 (1998). No specific validity criteria are given in the EPPO guideline. However, taking into account the validity criteria stated in the OECD guideline 2013 (1998) the study is considered valid.

The mean mortality of the control (solvent) in the oral toxicity test was 0 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.

The 24 h LD_{50} values of the reference item (dimethoate) in the oral (24 h $\text{LD}_{50} < 0.3 \mu\text{g ai/bee}$) toxicity test were within the recommended range of 0.10 – 0.35 $\mu\text{g ai/bee}$.

Some deviations to the test guidelines (EPPO and OECD guidelines) were identified (no information on the relative humidity). However, these deviations are not considered of relevance for the results of the acute oral toxicity test.

Reference:	Effects of Ethofol SC (HBX01) (Acute Contact Test) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory
Author(s), year:	Schmitzer, S., 2010
Report/Doc. number:	IBACON project no.: 56173035, Reference No. IDD00140
Guideline(s):	OECD 214 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol SC (HBX01), Batch no. 241Y, Content of ai: 485.6 g/L (analysed)
Reference:	Perfekthion (BAS 152 11 I), Batch no.: 90924-06, Dimethoate 414.8 g/L (analysed)
Wetting agent	Adhäsit, 100 g/L Marlopon (nominal), Batch no.: 0150207
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Acute oral and contact limit test
Number of organisms:	Five replicates with 10 bees for controls, the reference item treatments and the test item treatment groups
Food:	Ready-to-use syrup (Apiinvert) containing 30% sucrose, 31% glucose and 39% fructose
Applied concentrations:	Control: tap water with 0.5% Adhäsit (wetting agent to improve spreading of the test droplet on the water-repellent hairs on the thorax of bees) Test item: 200 µg Ethofol SC (HBX01)/bee Reference item: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee (nominal)
Test parameter:	A 5.0 µL droplet of Ethofol SC (HBX01) in an appropriate carrier (tap water + 0.5% Adhäsit) was administered to the thoracic surface of CO ₂ -anaesthetised bees with a hand-held applicator. The control bees were similarly dosed with tap water containing the wetting agent Adhäsit (0.5%). The reference item was also applied in a 5 µL droplet (dimethoate made up in tap water containing 0.5% Adhäsit). After application the bees were returned to the test cages and feed with ready-to-use syrup <i>ad libitum</i> .
Test conditions:	Temperature: 25 °C, Relative humidity: 30 - 62 %, Darkness (except during observation)
Test parameter:	Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive cleaning, vomiting) were made after exposure for 4, 24 and 48 h.

Findings:

No test item induced behavioural effects were observed at any time in the contact toxicity test.

Table B. 9.5.1-2: Effects of Ethofol SC (HBX01) on *Apis mellifera* following 48-h contact exposure in an acute toxicity test (averages from 5 replicates per dosage/control)

Nominal dose	Mortality [%]		
	4 h	24 h	48 h
Control (tap water + wetting agent)	0.0	0.0	0.0
Treatment [$\mu\text{g prod./bee}$]			
200	0.0	0.0	0.0
Reference item [$\mu\text{g ai/bee}$]			
0.10	0.0	4.0	6.0 ^a
0.15	0.0	16.0	28.0 ^a
0.20	0.0	32.0	52.0
0.30	2.0 ^a	66.0	72.0
Test substance: 24 h / 48 h LD ₅₀ > 200 $\mu\text{g prod./bee}$ Reference: 24 h LD ₅₀ = 0.26 $\mu\text{g dimethoate/bee}$ (95 % C.I. 0.20 – 0.33 $\mu\text{g ai/bee}$) 48 h LD ₅₀ = 0.21 $\mu\text{g dimethoate/bee}$ (95 % C.I. 0.17 – 0.25 $\mu\text{g ai/bee}$)			

^a moving coordination problemsConclusions:48 h LD₅₀ > 200 $\mu\text{g prod./bee}$ (contact toxicity), corresponding to 48 h LD₅₀ > 87.4 $\mu\text{g ai/bee}$ Comment RMS:

The study is considered acceptable. All validity criteria according to the OECD guidelines 214 are met. The mean mortality of the control (water + wetting agent) in the contact toxicity test was 0 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.

The 24 h LD₅₀ value of the reference item (dimethoate) in the contact (24 h LD₅₀ = 0.26 $\mu\text{g ai/bee}$) toxicity tests was within the recommended range of 0.10 – 0.30 $\mu\text{g ai/bee}$.

Some deviations to the OECD guidelines were identified, e.g. the relative humidity is between 30 - 62% which is below the recommendations given in the OECD guidelines (relative humidity between 50 and 70%). However, these deviations are not considered of relevance for the results of the acute contact toxicity test.

B.9.5.1.2. Chronic toxicity to bees

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) the chronic risk to adult honeybees has to be evaluated. However, no valid test guidelines are available to address this point. In the draft EFSA guidance document on risk assessment on honeybees (EFSA Journal 2013;11(7):3295) a study protocol (Appendix O) is given as support on how to perform a chronic oral toxicity test. The protocol is based on information from Decourtye et al. (2005), Suchail et al. (2001), Thompson H. (Food and Environment Research Agency, 2012) and CEB (2012).

In addition, to the adult chronic toxicity study conducted with the representative formulation Ethofol 500 SC a 10 day chronic oral toxicity study was also submitted for the technical active substance ethofumesate; the corresponding summary is given under point B.8.3.1.2., Volume 3 – B.9 (AS).

Reference:	Chronic toxicity of Ethofol 500 SC to the honeybee <i>Apis mellifera</i> L. under laboratory conditions
Author(s), year:	Kleebaum, K., 2014
Report/Doc. number:	Study No.: 13 10 48 017 B, Reference No. IDD00141
Guideline(s):	None
GLP:	Yes (certified laboratory)
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol 500 SC, Batch no. 710B, Content of ai: 516.2 g/L (analysed)
Reference:	Dimethoate 400 EC, Content of ai: 411.7 g/L (analysed)
Solvent:	None
Test species:	<i>Apis mellifera carnica</i> P., adult worker honeybees (2 - 3 days old)
Type of test:	Chronic 10 days continuous feeding test
Number of organisms:	Three replicates with 20 bees for control, reference item and the test item treatment groups
Applied concentrations:	Control: 50% (w/v) aqueous sucrose solution Test item: 54.9, 109.7, 219.5, 438.9 and 877.8 µg prod./bee/day 25.0, 50.0, 100, 200 and 399.9 µg ai/bee/day (corresponding to 0.642, 1.284, 2.568, 5.135 and 10.27 g ai/kg food) Toxic reference: 4.3, 7.2, 12.0 and 20.1 ng dimethoate/bee/day (corresponding to 0.1, 0.2, 0.3 and 0.5 mg ai/kg food)
Exposure route:	Over a period of 10 days, honeybees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing concentrations of the test item by continuous and ad libitum feeding.

The application (feeding) solutions were offered ad libitum to each cage of 20 bees in plastic syringes. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new syringes, filled with freshly prepared application solution over a period of 10 days. The weight of the syringes was determined before and after feeding to determine the mean food consumption of the bees per replicate.

Test conditions: Temperature: 34 – 35 °C °C, Relative humidity: 57 - 63 %, Darkness (except during observation)

Test parameter: Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive cleaning, vomiting) were made every 24 hour during the 10 days test period. After the end of the 10 day feeding period a possible sub-lethal effect of the test item on the hypopharyngeal glands (HPG) of bees was tested by measuring the diameters of the glands acini. During assessment the glands were extracted from the bee heads and the diameters of ten acini per bee were measured (VisiCam Image Analyser). The resulting data were analysed using a statistical program (ToxRat Professional 2.10.06, 2010).

Statistics: Fisher's Exact Binomial Test (with Bonferroni correction) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group and to determine the NOEC based on mortality.

Statistical calculations were made using a statistical program (ToxRat Professional 2.10.06, 2010).

Findings:

During the study only one bee treated with test item (test concentration 1284 mg ai/kg food) showed unnormal behaviour (at D7). It was classified as moribund and found dead the following day.

After 10 days of exposure none of the surviving bees treated with the test item showed adverse effects compared to the control.

Based on the statistical analyses of the results of the measurements of the hypopharyngeal glands no statistically significant difference of the test item concentrations if compared to the control was observed, suggesting a NOEC at the highest test concentration, 10270 mg ai/kg food.

Table B. 9.5.1-3: Mean cumulative mortality following 10 days oral exposure in a chronic toxicity test

Nominal dose [mg ai/kg]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Cumulative mortality [%]										
Control	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3	5.0	6.7
Treatment										
642	0.0	0.0	1.7	3.3	3.3	3.3	5.0	6.7	6.7	6.7
1284	0.0	0.0	0.0	0.0	0.0	1.7	5.0	6.7	6.7	8.3
2568	0.0	1.7	1.7	1.7	1.7	1.7	3.3	3.3	3.3	3.3
5135	0.0	1.7	3.3	3.3	5.0	5.0	5.0	5.0	5.0	6.7
10270	0.0	1.7	1.7	1.7	1.7	1.7	1.7	1.7	3.3	3.3
Reference										
0.111	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.186	0.0	0.0	0.0	0.0	0.0	1.7	3.3	3.3	5.0	6.7
0.309	0.0	1.7	1.7	1.7	6.7	8.3	11.7	21.7	28.3	33.3
0.516	0.0	3.3	3.3	13.3	21.7	41.7	55.0	65.0	78.3	86.7

In the chronic toxicity test, the control group showed a mean mortality of 6.7% after 10 days of testing. In the test item groups, none of the doses consumed by the bees revealed a mean mortality, which is statistically significantly increased compared to the control. The highest mortality (8.3%) was observed at a test concentration of 1284 mg ai/kg food.

Based on the observed effects a 10 d LD₅₀ of greater than 10270 mg ai/kg food (> 311.6 µg ai/bee/d) was determined.

A LD₅₀ value after 10 days for the reference item was calculated to be 9.6 ng dimethoate/bee/day.

Table B. 9.5.1-4: Daily and overall food consumption following 10 days oral exposure in a chronic toxicity test

Nominal dose [mg ai/kg]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	% ^a
Daily mean food uptake [mg/bee]											
Control	26.6	27.3	40.1	24.3	42.9	30.9	39.2	36.5	38.5	31.0	86.6
Treatment											
642	24.6	41.2	26.2	34.3	38.0	36.0	37.2	36.8	39.7	32.4	89.0
1284	30.1	30.3	41.4	44.3	30.6	45.5	39.6	32.3	46.6	34.8	96.4
2568	28.2	22.1	38.8	33.2	36.0	39.8	31.6	41.2	38.8	33.9	88.2
5135	22.0	35.2	35.3	32.5	38.4	34.6	35.7	36.1	35.1	30.7	86.2
10270	12.9	31.0	30.4	33.0	35.9	36.3	34.0	27.5	34.7	27.6	77.9
Reference											
0.111	27.9	28.2	31.9	23.5	31.2	27.8	33.3	27.9	33.1	27.7	75.2
0.186	33.0	20.6	26.4	29.2	28.0	28.1	32.9	22.6	29.9	28.5	71.6
0.309	30.9	24.5	37.6	22.3	24.2	28.8	23.9	24.7	33.6	25.0	70.6
0.516	25.7	23.2	29.3	19.5	32.0	16.1	26.8	20.3	25.6	36.8	65.5

^a Overall mean relative food uptake compared to expected amount.

In the test item group the food consumption ranged between 30.3 and 37.6 mg solution per bee and day which is 77.8 to 96.6% of the expected amount (control: on average 33.7 mg ai/bee/day = 86.5% of the expected amount). It was also observed that in the course of the study with growing age of the bees more test item solution was consumed.

Table B. 9.5.1-5: Mean cumulative uptake of test and reference item amount following 10 days oral exposure in a chronic toxicity test

Nominal dose [mg ai/kg]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Daily dose
Mean cumulative uptake [$\mu\text{g ai/bee}$]											
Control	-	-	-	-	-	-	-	-	-	-	-
Treatment											
642	15.8	42.2	59.1	81.1	105.5	128.6	152.5	176.1	201.6	222.4	22.2
1284	38.7	77.6	130.7	187.6	226.9	285.3	336.2	377.6	437.5	482.2	48.2
2568	72.3	129.1	228.7	313.9	406.5	508.7	589.9	695.8	795.4	882.5	88.2
5135	112.9	293.8	475.0	641.7	839.1	1017	1200	1385	1566	1723	172.3
10270	132.4	451.3	763.8	1103	1471	1844	2194	2476	2833	3166	311.6
Reference											
0.111	3.1	6.2	9.8	12.4	15.9	18.9	22.6	25.7	29.4	32.5	3.2
0.186	6.1	10.0	14.9	20.3	25.5	30.7	36.9	41.1	46.6	51.9	5.2
0.309	9.5	17.1	28.7	35.6	43.1	52.0	59.4	67.0	77.4	85.1	8.5
0.516	13.3	25.2	40.4	50.5	67.0	75.3	89.1	99.6	112.8	131.8	13.2

Conclusion:

Based on the results of the study a 10 day LD₅₀ of greater than 10270 mg ai/kg food (corresponding to 311.6 $\mu\text{g ai/bee/d}$) and a NOEC of equal to 10270 mg ai/kg food was determined. No statistically significantly effects on the survival and the food consumption of the bees and on the length of the hypopharyngeal glands were observed.

Comment RMS:

No test guideline is available to address the chronic risk to adult honeybees. Hence, no validity criteria can be met. However, the study is considered valid because the mean mortality in the control was $\leq 15\%$ at the end of the test. The criterion of 15% is taken from the acute toxicity study outlined in the EPPO test guideline 170.

The study protocol given in the draft EFSA Guidance Document on honeybees (EFSA Journal 2013;11(7):3295) was used to evaluate the chronic oral toxicity test. The study is considered valid as it is well in line with the study protocol given in the EFSA Guidance Document.

B.9.5.1.3. Effects on honeybee development and other honeybee life stages

A honeybee brood study with the solo formulation Ethofol 500 SC according to the test guideline by Oomen *et al.* was conducted to address the risk on the honeybee brood.

Reference:	Study on the Effects of Ethofol 500 SC (HBX01) on Honey Bee Brood (<i>Apis mellifera</i> L.) – Brood Feeding Test
Author(s), year:	Schmitzer, S., 2012
Report/Doc. number:	IBACON project no.: 63831031, IDD00142
Guideline(s):	Oomen <i>et al.</i> (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol 500 SC (HBX01), Batch no.: 025A, Content of ai: 484.4 g/L (analysed)
Reference:	Insegar (insect growth regulator), ai: fenoxycarb, Batch no.: L125262 0096/PM SYN GERM/9J PPE 216499, Content 25% (nominal)
Test species:	Honey bees (<i>Apis mellifera</i> L.), all ages and all stages Honey bee colonies were maintained according to normal beekeeping practice, containing two magazines with 11 combs, each. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply of nectar and pollen. The mean strength of the colonies per treatment group, two days before application, was similar and ranged between 9000 and 13000 adult bees. Colonies were free flying, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area.
Location:	Rossdorf, Darmstadt-Dieburg, Germany Test site: Uncultivated fields, surrounding area underlies agricultural use mainly with arable crops and meadow.
Test conditions:	Natural field conditions. Temperature, relative humidity and rain were recorded during the experimental time. Temperature (daily mean values): 8.8 – 32.9 °C (mean: 14.6 – 26.0 °C) Rain: 0.0 – 30.6 mm (total precipitation per day) Rel. humidity (daily mean values): 69.9 – 98.9%
Feeding:	Natural food and water sources
Test design:	One single application per colony during the afternoon in order to prevent robbery. 1 L contaminated (test item and reference item) or untreated (control) commercial ready-to-use sugar syrup per colony was used.

Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine. Pure sugar syrup (Apiinvert) was used for the control group.

Each feeding trough was weighed before introduction to the bee colonies and after uptake of the contaminated food. About 27 hours after application, the uptake of the colonies was complete.

Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 21 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) from of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again, the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 21 days after application (i.e. 22 days following BFD0). Mortality of adult bees and pupae was also assessed.

Test concentrations: Control: 1 L untreated ready-to-use syrup (Apiinvert containing 30% sucrose, 31% glucose and 39% fructose) per hive.

Test item: 1.16 kg prod./ha (= 500 g ai/ha), corresponding to 2.9 g product in 1 L sugar solution (ready-to-use syrup, Apiinvert)

Reference item: 3.0 g reference item (Insegar; 25 % fenoxycarb) in 1 L commercial ready-to-use sugar syrup per hive, corresponding to a nominal active substance concentration of 0.75 g ai/L.

Test parameter: Mortality of adult bees as well as pupae or larvae: between 3 days before to 21 days after application (= end of the trial);

Bee brood development (eggs, young- and old larvae): one day before (= BFD0) and 4 (= BFD5), 8 (= BFD9), 15 (= BFD16), 21 (= BFD) 22 days after the application.

Behavioural abnormalities (e.g. intensive cleaning, restlessness or moving coordination problems) were observed during the assessment of mortality.

Statistics: Statistical evaluation was done for mortality and the brood termination rates using Shapiro-Wilk's test (check for normal distribution), Levene's test (check for homogeneity of variance), Student's t-test (pairwise).

Software: ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH.

Findings:

During the study the mean temperature was between 14.8 and 21.1 °C (minimum: 9.9 °C, maximum: 29.0 °C) and the mean relative humidity was between 67.5 and 100%. The total precipitation per day was between 0 and 21 mm on day 7 after treatment.

No behavioural impairments were noted at any time in any of the test or reference item treatment groups until test end. No abnormal behaviour could be observed in the control groups.

Mortality of adult honey-bees:

Following the treatment with Ethofol 500 SC no direct toxicity occurred after ingestion of the test item treated sugar solution. Mortality levels in the treated colonies were comparable to the control colonies for the first 3 days. Over the whole post-application period from day 0 to day 21 a mean of 13.8 dead bees per colony and day was found in the traps of the test item treated colonies. In comparison, a mean of 14.2 dead bees per colony and day was found in the control groups.

Treatment with the reference item led to a slightly increased number of dead bees (22.1 dead bees per colony and day) following the treatment from day 0 to test end on day 21 following application.

Mortality of pupae and larvae:

During the entire trial almost no larva was found in any of the colonies. Therefore the mortality values reflect the pupae mortality only.

Over the whole post-application period from day 0 to day 21 a mean of 4.2 dead pupae per colony and day was found in the traps of the test item treated colonies. In comparison, a mean of 1.5 dead pupae per colony and day was found in the control groups.

Treatment with the reference item led to no increased number of dead pupae (3.0 dead pupae per colony and day) following the treatment from day 0 to test end on day 21 following application.

Development of the bee brood:

Following the assessment of single cells from the egg stage to the hatched worker bee, the mean termination rate in the test item group was 27.1% compared with 15.6% in the control group. The difference between treatment and control groups was not statistically significant.

Comparing the development success of the young larvae after the treatment with the test item, a clearly lower mean termination rate could be observed, when compared to the control group. 6.4% of the marked young larvae in the test item colonies did not reach the adult stage, whereas terminated rate in the control group was 32.5%. Accordingly, the difference was not statistically significant.

No effect of the test item on old larvae could be found when 1.6% of the marked old larvae did not undergo a complete development compared to 7.9% in the control group (not statistically significant).

Treatment with the reference item led to a statistically significant loss of brood development of the marked eggs, young and old larvae, resulting in a terminate rate of 99.8% (eggs), 99.8% (young larvae) and 74.8% (old larvae), respectively.

Table B. 9.5.1-6: Effects of Ethofol 500 SC on honey bee brood

Treatment	Untreated control	Ethofol 500 SC	Reference item
Rate per L sugar solution [ai]	-	2.9 g ai/L	0.75 g ai/L
Termination rate of eggs [%] ^a	15.6%	27.1%	99.8%
Termination rate of the young larvae [%] ^a	32.4%	6.4%	99.8% *
Termination rate of the old larvae [%] ^a	7.9%	1.6%	74.8% *
Mean mortality of worker bees/colony/d during pre-application phase ^b during the entire post-application phase ^b	2.6 ± 4.4 14.2 ± 14.3	3.0 ± 3.0 13.8 ± 9.0	3.0 ± 2.6 22.1 ± 22.5
Mean mortality of pupae/colony/d during pre-application phase ^c during the entire post-application phase ^c	0.0 1.5 ± 2.7	0.0 4.2 ± 10.7	0.0 3.0 ± 6.4
Mean number of bees before application (range)	13425 (10665 - 14940)	11670 (10530 - 12870)	8835 (6660 - 12420)

^a Mean terminate rate of 3 colonies per treatment group, 22 days after BFD0

^b Mean number of dead honey bees per day and colony found in dead bee traps

^c Mean number of dead pupae/larvae per day and colony found in dead bee traps

* Statistically significantly different compared to the control, Student t-test, $\alpha = 0.05$

Conclusions:

Overall, it can be concluded according to the results of this study that Ethofol 500 SC does neither adversely affect honey bee colonies nor bee brood development at test concentrations of 500 g ai/ha.

Comment RMS:

The study was conducted according to the test guideline by Oomen *et al.* (1992). No validity criteria are given in the test guideline. The study was well conducted and is therefore considered valid and acceptable for use in the risk assessment.

According to Oomen *et al.* the colonies should have a size between 10 000 and 15 000 bees per colony. However, two colonies used in the test (reference treatment group) have a size of below 10000 bees (6660 and 7425 bees). However, considering the results of the study, this deviation might not have an impact on the outcome of the study.

B.9.5.1.4. Cage and tunnel tests

Based on the results reported in the available laboratory studies (acute oral and contact toxicity to honey-bees, chronic toxicity to adult honey-bees and effects on larvae of honey-bees), no further studies are required addressing the risk to honey-bees.

B.9.5.1.5. Field tests

As Ethofol 500 SC does not pose an unacceptable risk to honey-bees, further tests are not necessary.

In order to support the risk assessment, an expert statement concerning "Ethofumesate - exposure of honeybees to residues in nectar, pollen and guttation fluid in sugar and fodder beets" is summarised below.

Report:	KCA 6.10.1/01, Lückmann, J. (2013)
Title:	Ethofumesate - exposure of honeybees to residues in nectar, pollen and guttation fluid in sugar and fodder beets
Document No:	P13096
Guidelines:	-
GLP:	-

The risk for honeybees to get in contact with contaminated nectar and pollen is negligible as sugar and fodder beets do not build flowers within the first year. Sugar and fodder beets are harvested by the end of the first year. In the rare case that shoots with flowers are produced in the first year or beets are flowering in the second year (if beets are grown for seed production) no risk for honeybees is expected as flowers are wind pollinated. Sugar and fodder beet flowers are not mentioned in any standard or handbook on honey bee foraging plants (e.g. Maurizio & Schaper 1994, Pritsch 2007).

The beet structure does not allow formation of water reservoirs in leaf axils. The risk for honeybees to get in contact with Ethofumesate residues in guttation fluid being present at the leaf edges as guttation droplets at sugar and fodder beets after Ethofumesate treatment is very low as well, since beets display guttation in a very low frequency and intensity (Joachimsmeier et al. 2012, Pistorius et al. 2012). Hence, beets are a very unattractive water sources for honeybees.

In the case that weeds are present in or next to beet fields treated with ethofumesate 500 g/L SC or other herbicides, it is not very likely that weeds will have reached flowering stage at the time of application, and thus will be attractive for honeybees.

B.9.5.2. Effects on non-target arthropods other than bees

Several laboratory studies with formulated ethofumesate have been performed with the arthropod species *Aleochara bilineata*, *Poecilus cupreus* and *Chrysoperla carnea*, representing various beneficial arthropod taxa. The studies were already submitted and evaluated for the first EU approval of the active substance ethofumesate. However, no studies investigating the effects on the two representative species *Aphidius rhopalosiphi* and *Typhlodromus pyri* have been evaluated in the DAR. In order to fulfil the data requirements for the active substance and the representative formulation, two laboratory studies with Ethofol 500 SC, not-peer reviewed on EU level have been performed.

Reference:	Effects of Ethofol 500 SC on the parasitoid <i>Aphidius rhopalosiphi</i> in the laboratory – dose response test
Author(s), year:	Moll, M., 2011
Report/Doc. number:	Report No. 63551001, Reference No. IDD00149
Guideline(s):	Mead-Briggs et al., 2000 and 2009 (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable
<u>Material and Methods:</u>	
Test substance:	Ethofol 500 SC, Content of ai: 484.4 g/L (analysed), Batch no. 025A
Toxic reference:	Perfekthion (BAS 152 11 I), Content of dimethoate: 414.8 g/L (analysed)
Test species:	<i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), adults, < 48 h old
Type of test:	Acute contact laboratory test
Number of organisms:	Exposure period: 4 replicates with 10 adult wasps (7 females, 3 males) per replicate Post-exposure period: max. 20 replicates/treatment group; 1 female per unit
Treatments:	Control: deionised water Toxic reference: 0.3 mL Perfekthion/ha (corresponding to ~ 0.13 g ai/ha taking into account a density of 1.074 g/cm ³ and an analysed content of 414.8 g dimethoate/L). Test item: 125, 250, 500, 1000 and 2000 mg prod./ha; deionised water as diluent; Treatments applied with a calibrated sprayer in 200 L water/ha
Exposure route, duration:	Exposure units: 2 treated glass plates, held apart by an untreated aluminium frame and held together with at least 2 clamps. Post-exposure units: Untreated pots with 12-20 barley seedlings infested with approx. 100-200 host aphids (<i>Rhopalosiphum padi</i>) enclosed within a clear polyacrylic cylinder. Exposure time: 48 h, parasitisation period: 24 h, post-parasitisation period: 11 - 12 days.
Feeding:	A solution of fructose (10%) in small test tubes provided as source of food, <i>ad libitum</i> .
Test conditions:	Temperature: 19 - 21 °C; relative humidity: 70 – 79% (acclimatization, exposure period) and 74 - 82 % (post-exposure period), 16 h light, 759 – 1620 lux (acclimatization, exposure, parasitisation period) and 9800 - 10900 lux (post-parasitisation period)
Test parameters:	Mortality and behavioural abnormalities were assessed 2, 24 and 48 h after introduction of the wasps to the test units.

The parasitisation rate was determined at the end of the parasitisation phase by counting the number of mummies for each individual wasp.

Reproduction phase was performed where the corrected mortality was $\leq 50\%$.

No reproduction testing was performed with the reference item group.

Statistics:

Mortality data were analysed for significance by using the Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).

Reproduction data were tested for normal distribution and homogeneity of variance using the Kolmogorov-Smirnov test ($\alpha = 0.05$) and the Bartlett's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous, Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used.

Software: ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH

Findings:

After 48 hours exposure period no mortality was observed in the control as well as in all test item treatment groups. 100% mortality was observed in the reference item group. No abnormal behaviour was observed.

Reproduction was assessed in the control and in all test item treatment groups.

There was no statistically significant effect on reproduction up to and including 1000 mL product/ha compared to the control and the reduction in reproduction was below the trigger value of 50% (5.2-12.2%). At 2000 mL product/ha reproduction was statistically significantly reduced compared to the control; however the effect on reproduction was with 34.3% below the trigger value of 50%. Therefore it can be summarized that there was no effect on reproduction up to and including 2000 mL product/ha.

Table B.9.5.2-1: Summary of the effects of Ethofol 500 SC on *A. rhopalosiphi* following exposure on glass plates for 48 h under laboratory conditions

Nominal application rate (mL prod./ha)	Mortality		Parasitisation efficiency	
	Cumulative [%]	Control corrected [%] ^a	Parasitisation rate [mummies/female]	% Reduction relative to control
Control	0.0	-	40.7 ± 19.0	-
125	0.0	0.0	36.3 ± 17.5	10.9
250	0.0	0.0	37.7 ± 15.6	7.4
500	0.0	0.0	38.6 ± 16.6	5.2
1000	0.0	0.0	35.7 ± 23.6	12.2
2000	0.0	0.0	26.8 ± 12.4 **	34.3
Reference item	100	100 *	-	-

* Statistically significant compared to the control, according to Fisher's Exact Test, $\alpha = 0.05$

** Statistically significant compared to the control, according to Dunnett's t-Test, $\alpha = 0.05$

Conclusion:

48 h LR₅₀ > 2000 mL prod./ha

48 h ER₅₀ > 2000 mL prod./ha

<u>Comment RMS:</u>	<p>The study was conducted according to the IOBC test guidelines (Mead-Briggs et al., 2000, Mead-Briggs et al., 2009).</p> <p>The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline are met.</p> <p>The mortality of the adult wasps in the control was below 13% (being: 0%). For the fecundity assessments, wasps in the control group produced more than 5 mummies per female (being: mean 40.7 mummies per female).</p> <p>The number of wasps in the control producing no mummies was not more than two wasps (being: 0 wasps).</p> <p>The mortality observed in the toxic reference group (100% at ~ 0.13 g ai/ha) was in line with the IOBC test guideline.</p> <p>Hence, the RMS is of the opinion that the study is considered valid and acceptable for the use in the risk assessment.</p>
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Reference:	Effects of Ethofol 500 SC on the predatory mite <i>Typhlodromus pyri</i> in the laboratory – dose response test
Author(s), year:	Schwarz, A., 2011
Report/Doc. number:	Report no. 63552063, Reference no. IDD00144
Guideline(s):	Blümel et al., 2000 (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol 500 SC, Content of ai: 484.4 g/L (analysed), Batch no. 025A
Toxic reference:	Perfekthion (BAS 152 11 I), Content of dimethoate: 414.8 g/L (analysed)
Test species:	<i>Typhlodromus pyri</i> protonymphs, < 24 h old
Type of test:	Acute contact laboratory test, 14 days
Number of organisms:	3 replicates with 20 individuals per replicate
Treatments:	Control: deionised water
	Toxic standard: 8.0 mL prod./ha (corresponding to ~ 3.6 g ai/ha taking into account a density of 1.074 g/cm ³ and an analysed content of 414.8 g dimethoate/L).
	Test item: 125, 250, 500, 1000 and 2000 mL prod./ha.
	The test item was sprayed in 200 L/ha on the glasses.
Exposure route:	Formed by two cover slides fixed by gluing small cover slides to both side-ends. A

glue barrier was placed on the test unit to keep the mites on this test arena.

Plastic trays half-filled with water, with a foam rubber and a glass-plate on top, covered by tissue paper. Tissue paper in contact with water. Test units were placed on the tissue paper.

Feeding: Pollen (mixture of pine and birch, 3:1) *ad libitum*

Test conditions: Temperature: 24 - 27°C; relative humidity: 70 – 80%, 16 h photo period, 1300 lux (acclimatization) and 240 – 550 lux (exposure)

Test parameter: Mortality was recorded on day 3 and 7 after test initiation.

If necessary, the sex-ratio was adjusted to at least 1 male : 5 females on day 7.

Reproduction was assessed by counting eggs, number of live and dead juvenile stages per female from day 7 on with a maximum interval of 3 days up to and including day 14. Reproduction phase was performed where the corrected mortality was $\leq 50\%$.

No reproduction testing was performed with the reference item group.

Statistics: Mortality data were analysed for significance by using the Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).

Reproduction data were tested for normal distribution and homogeneity of variance using the R/S-Test ($\alpha = 0.05$) and the Cochran's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous, Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used.

Software: ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH

Findings:

A statistically significant mortality as well as reduction in reproduction capacity was observed in different test item treatment groups. However, mean mortality and effects on reproduction were below the trigger value of 50% in all test item treatment groups. 100% mortality was observed in the reference item group.

Table B.9.5.2-2: Summary of the effects of Ethofol 500 SC on *Typhlodromus pyri* following exposure on glass plates for 7 days under laboratory conditions

Nominal application rate [mL prod./ha]	Mortality after 7 days ^a		Reproduction (days 7 - 14)	
	Cumulative [%]	Control corrected [%]	Mean no. of eggs per female	% Reduction relative to control
Control	1.7 ± 2.9	-	10.6 ± 0.6	-
125	8.3 ± 10.4	6.8	8.6 ± 1.8	18.9
250	48.3 ± 36.9 *	47.5	7.4 ± 1.3 **	30.2
500	13.3 ± 11.5 *	11.9	7.9 ± 1.3 **	25.5
1000	18.3 ± 5.8 *	16.9	7.3 ± 1.6 **	31.1
2000	11.7 ± 2.9 *	10.2	8.8 ± 0.3	17.0
Reference item	100 ± 0.0 *	100	-	-

* Statistically significant compared to the control, Fisher's exact Test, $\alpha = 0.05$

* Statistically significant compared to the control, Dunnett's t-Test, $\alpha = 0.05$

^a Number of dead mites including escaped mites

Conclusion:

Statistically significant effects on the mortality of adults and the reproduction was observed in the study at test concentrations between 250 and 2000 mL prod./ha. However, these effects were below 50% relative to the control.

7 d LR₅₀ > 2000 mL prod./ha

14 d ER₅₀ > 2000 mL prod./ha

Comment RMS:

The study was conducted according to the IOBC test guidelines (Blümel et al., 2000). The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline.

The arithmetic mean mortality (dead and escaped) in the control treatment was below 20% on day 7 after treatment application (being: 1.7%). The cumulative mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female (being: 10.6 eggs/ female).

The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the reference item was 100% and therefore in the recommended range between 50% and 100%.

Hence, the RMS is of the opinion that the study is considered valid and acceptable for the use in the risk assessment.

The high mortality of mites at the test rate of 250 mL prod./ha is based on the high rate of escaped mites in one replicate. This outlier is not considered to be substance related.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for honeybees

Honeybees may be exposed to formulated ethofumesate by direct spraying of the plant protection product while bees are foraging on flowers and weeds present in or adjacent to the crop treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

The LD₅₀-values (48 h) for oral and contact toxicity of the active substance and the representative formulation Ethofol 500 SC are above 100 µg ai/bee, indicating a low toxicity. Based on the available data no higher toxicity of the formulation is expected.

The EU representative use is a pre- and post-emergence application (single and splitting applications) on sugar and fodder beets. The maximum total rate of the active substance must not exceed 1 kg ai/ha/year. The risk assessment for honeybees is based on the maximum single application rate of 1 kg ai/ha.

Acute risk assessment:

Table B. 9.6.1-1: Summary of effects of ethofumesate on honeybees (acute exposure)

Test substance	Exposure route	Endpoint	Toxicity	Reference
Ethofumesate tech.	Acute oral	48 h LD ₅₀	> 50 µg ai/bee	Barrett, K.L., 1991
	Acute contact		> 50 µg ai/bee	
	Acute oral	48 h LD ₅₀	> 100 µg ai/bee	Cole, J.H., 1992
	Acute contact		> 100 µg ai/bee	
	Acute oral	48 h LD ₅₀	> 106.3 µg ai/bee	Schmitzer, S., 2011a
	Acute contact		> 100 µg ai/bee	
Ethofumesate 500 SC	Acute oral	48 h LD ₅₀	> 184.3 µg ai/bee	Mallikarjunappa, S., 1998
Ethofol 500 SC	Acute contact	48 h LD ₅₀	> 87.4 µg ai/bee	Schmitzer, S., 2010

Under consideration of the deficiencies identified in the acute oral and contact toxicity study by Barrett (1991) and Cole (1990) the risk assessment for the active substance is based on the newly submitted acute oral and contact toxicity study by Schmitzer (2011).

The acute risk from oral and contact exposure was conducted in accordance with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). The acute risk for bees was expressed as a Hazard Quotient (Q_H) calculated by the following formula (single application rate in g/ha, LD₅₀ in µg/bee):

$$\text{Hazard quotient (Q}_H\text{)} = \text{application rate [g ai/ha]} / \text{LD}_{50} [\mu\text{g ai/bee}]$$

Table B. 9.6.1-2: Risk to honeybees from oral and contact exposure to ethofumesate

Test substance	Exposure route	Application rate [g ai/ha]	Endpoint [µg ai/bee]	Q _H
Ethofumesate tech.	Oral	1 x 1000	48 h LD ₅₀ > 106.3	< 9.4
	Contact		48 h LD ₅₀ > 100	< 10
Ethofumeaste 500 SC	Oral	1 x 1000	48 h LD ₅₀ > 184.3	< 5.4
	Contact		48 h LD ₅₀ > 87.4	< 11.4

The resulting Hazard Quotients are clearly below the trigger of 50 indicating a low risk to honeybees after the use of ethofumesate according to representative uses.

Chronic risk assessment:

Table B. 9.6.1-3: Summary of effects of ethofumesate on honeybees (chronic exposure)

Test substance	Exposure route	Endpoint	Toxicity	Reference
Ethofumesate tech.	Chronic oral	10 d LC ₅₀ 10 d NOEC	> 120 mg ai/kg 120 mg ai/kg (= 4.4 µg ai/bee/d)	Kling, A., 2013
Ethofol 500 SC	Chronic oral	10 d LC ₅₀ 10 d NOEC	> 311.6 µg ai/bee/d 311.6 µg ai/bee/d	Kleebaum, K. 2014

Based on the available chronic toxicity studies with the active substance and the representative formulation Ethofol 500 SC a chronic risk assessment considering chronic oral toxicity and effects on the hypopharyngeal glands (HPGs) was conducted.

The chronic study with the representative formulation was designed as dose-response test by exposing adult honey-bees for 10 consecutive days to a maximum concentration of nominally 10270 mg ai/kg in aqueous sugar solution.

No adverse lethal-, sub-lethal, behavioural or delayed effects were observed by exposing adult honeybees for ten consecutive days exclusively to sugar solution, containing 10270 mg ethofumesate/kg, corresponding to 311.6 µg ai/bee/day.

A chronic oral toxicity study with the active substance ethofumesate was submitted by the Task Force Ethofumesate. Based on the results of the study a 10 d LDD₅₀ of > 4.4 µg ai/bee/d was determined.

The following risk assessment is based on the endpoint for the active substance.

No guidance is available how to assess the risk to adult honey-bees from chronic exposure to the active substance. Hence, the chronic risk assessment was conducted based on the draft EFSA guidance document on bees (EFSA Journal 2013;11(7):3295).

Chronic oral toxicity - screening step:

The chronic exposure-toxicity ratio (ETR_{chronic adult oral}) is calculated using the following formula:

$$ETR_{\text{chronic adult oral}} = AR * SV / 10 \text{ d LDD}_{50}$$

With

AR...Application rate [kg ai/ha]

SV...Short-cut value for the respective kind of application

LDD₅₀...Lethal dietary dose [µg ai/bee/day]

Table B. 9.6.1-4: Chronic oral toxicity to bees

Crop	Endpoint	SV	ETR	Trigger
Sugar and fodder beets 1 x 1 kg ai/ha	10d LDD ₅₀ > 4.4 µg ai/bee/d	7.6 (down-ward application)	1.7	ETR > 0.03
	10d LDD ₅₀ > 311.6 µg ai/bee/d	7.6 (down-ward application)	0.024	ETR > 0.03

The $ETR_{\text{chronic adult oral}}$ for the active substance is above the trigger value of 0.03 indicating a potential chronic risk to adult honey-bees. Hence a refined chronic risk assessment taking into account various exposure routes has to be conducted.

Chronic oral toxicity - Tier 1:

The following exposure routes have to be considered in the tier 1 risk assessment according to the draft EFSA guidance document on bees.

- Risk from foraging on the treated crop
- Risk from foraging on an adjacent crop
- Risk from foraging on weeds on the treated field
- Risk from foraging in the field margin
- Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops

As the likelihood of bees being in early post-emergence sugar, red or fodder-beet field is low, the Tier 1 risk assessment focuses on the exposure of foraging bees visiting plants in the field margin or on an adjacent crop.

Therefore the ETR is recalculated using the following formula:

$$ETR_{\text{chronic adult oral}} = AR * Ef * SV * twa / 10 \text{ d LDD}_{50}$$

With

AR...Application rate [kg ai/ha]

Ef...Exposure factor

tw...Time weighted average

SV...Short-cut value for the respective kind of application

LDD₅₀...Lethal dietary dose [µg ai/bee/day]

Table B. 9.6.1-5: Risk from foraging in the field margin and adjacent crops

Exposure	Crop	Endpoint	SV	Ef ^a	tw	ETR	Trigger
Field margin	Beets 1 x 1 kg ai/ha	10d LDD ₅₀ = 4.4 µg ai/bee/d	5.9	0.0092	0.72	0.009	ETR > 0.03
Adjacent crop			9.9	0.0033	0.72	0.005	

^a Plants at the field margin/ adjacent crops of field crops (sugar, red and fodder beets)

Based on the Tier 1 risk assessment an acceptable chronic risk to adult honey-bees foraging in the field margins and adjacent crops was identified. No further information are required addressing the chronic risk to honey-bees.

Effects on the hypopharyngeal gland:

The exposure-toxicity ratio (ETRR_{hpg}) relevant for the development of the hypopharyngeal glands (HPGs) is calculated using the following formula according to the draft EFSA guidance document on bees.

$$ETR_{hpg} = AR * SV / NOEL_{hpg}$$

With

AR...Application rate [kg ai/ha]

SV...Short-cut value for the respective kind of application

NOEL...No observed effect concentration [µg ai/bee/day]

Table B. 9.6.1-6: Effects on the development of the hypopharyngeal glands (HPGs) of bees

Crop	Endpoint	SV	ETR	Trigger
Beets 1 x 1 kg ai/ha	10d NOEL _{hpg} = 311.6 µg ai/bee/d	7.6 (down-ward application)	0.024	ETR > 1

The ETR_{hpg} value for chronic oral exposure is below the trigger value of 1. Thus, an acceptable risk by chronic oral exposure can be concluded for the development of the hypopharyngeal glands (HPGs) of bees.

Risk assessment for honeybee brood:**Table B. 9.6.1-7: Summary of effects of formulated ethofumesate (500 g/L, SC formulation) on honeybees and honeybee brood**

Test substance	Exposure route	Results	Reference
Ethofol 500 SC	Honeybee brood feeding	No adverse effects on mortality, bee brood development (eggs, young larvae, old larvae, pupae) and colony development by feeding honey bee colonies sugar syrup at a concentration of 1.16 kg prod./ha, equivalent to 500 g ai/ha.	Schmitzer, S., 2013

In order to reveal whether ethofumesate poses a risk to immature honey bee life stages, a bee brood feeding study has been conducted by following the provisions/method of Oomen *et. al.* (OEPP/EPPO Bulletin 22:613-616 (1992)), which require, amongst other parameters to “...use formulated products only... products are fed at a concentration recommended for high-volume use...”. The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.

The administration of 1 litre sugar solution per colony, containing 2.9 g ai/L has not resulted in adverse effects. There were neither adverse acute or chronic effects on adult honey bees nor adverse effects on immature honey bee life stages (eggs, young larvae, old larvae, pupae) or on the colony itself. Neither mortality of worker bees and pupae (as assessed via dead bee traps) nor the termination rate of eggs, young larvae and old larvae (as assessed via digital imaging of individual marked cells) was statistically significantly different from the untreated control.

B.9.6.2. Risk assessment for non-target arthropods

The notifier United Phosphorous Ltd. submitted standard laboratory studies with the two standard arthropod species *Aphidius rhopalosiphi* and *Typhlodromus pyri*. In addition, laboratory studies with three additional arthropod species, *Aleochara bilineata*, *Chrysoperla carnea* and *Poecilus cupreus* which were submitted for the first EU approval are available.

Table B. 9.6.2-1: Summary of effects of ethofumesate on non-target-arthropods (laboratory studies)

Test species	Exposure	Test item	Rate [mL/ha]	Type of effect	Effect [%]	Reference
<i>Aphidius rhopalosiphi</i> (adults)	contact with dried residues on treated glass plates	Ethofol 500 SC	125	Corrected mortality / Reproduction	0.0 / 10.9	Moll, M., 2011
			250		0.0 / 7.4	
			500		0.0 / 5.2	
			1000		0.0 /12.2	
			2000		0.0 / 34.3	
			48 h LR ₅₀ > 2000 mL prod./ha 48 h ER ₅₀ > 2000 mL prod./ha			
<i>Typhlodromus pyri</i> (protonymphs)	contact with dried residues on treated glass plates	Ethofol 500 SC	125	Corrected mortality / Reproduction	6.8 / 18.9	Schwarz, A., 2011
			250		47.5 / 30.2	
			500		11.9 / 25.5	
			1000		16.9 / 31.1	
			2000		10.2 / 17.0	
			7 days LR ₅₀ > 2000 mL prod./ha 14 d ER ₅₀ > 2000 mL prod./ha			

Non-target arthropods may be exposed to formulated ethofumesate by direct spraying, contact with fresh or dry residues or by oral uptake of contaminated food.

A risk assessment for non-target arthropods was performed according to the recommendations of ESCORT II. In the first tier hazard quotients were calculated for exposure in in-field and off-field areas according to the following formulas:

$$HQ_{\text{in-field}} = \frac{\text{application rate} \times \text{MAF}}{LR_{50}}$$

$$HQ_{\text{off-field}} = \text{correction factor} \times \frac{\text{application rate} \times \text{MAF} \times \left(\frac{\text{drift factor}}{\text{vegetation distribution factor}} \right)}{LR_{50}}$$

drift factor = % drift/100 (90 %ile drift according to Ganzelmeier et al. 1995)

The correction factor and the vegetation distribution factor were set to 10. Drift figures were chosen according to crop type in 1 m distance.

The worst case intended use for sugar and fodder beet (pre and post-emergence) is a single rate of 2 L prod./ha (1 kg ai/ha), hence no MAF is applied. This use patterns is considered to cover also the multiple applications, i.e. 2 x 500 g ai/ha and 3 x 333 g ai/ha.

Table B. 9.6.2-2: HQ calculations for *Aphidius rhopalosiphi* and *Typhlodromus pyri* in beets (post-emergence)

species	Application rate [mL prod./ha]	MAF	Drift [%]	LR ₅₀ [g ai/ha]	HQ _{in-field}	HQ _{off-field}
<i>Aphidius rhopalosiphi</i>	2000	1	2.77	> 1000	< 1	< 0.03
<i>Typhlodromus pyri</i>		1		> 1000	< 1	< 0.03

All HQ-values for both indicator species and the in-field and off-field area are below the trigger of 2 and indicate a low and acceptable risk.

In addition to the two standard laboratory studies, studies with three additional arthropod species were conducted.

Table B. 9.6.2-3: Summary of effects of ethofumesate on non-target-arthropods (laboratory studies)

Test species	Exposure	Test item	Rate [g ai/ha]	Type of effect	Effect [%]	Reference
<i>Aleochara bilineata</i> (adults)	contact with dried residues on sand	Tramat 500 SC	1252.5 (2.5 L prod./ha)	Mortality / Reproduction	8.0% ↑ no. of eggs ↑ no. of hatched eggs	Mead- Briggs, M., 1991
				48 h ER ₅₀ > 1252.5 g ai/ha		
<i>Poecilus cupreus</i> (adults)	contact with dried residues on sand	Tramat 500 SC	2000 (4 L prod./ha)	Corrected mortality	3.3%	Römbke, J., 1990
				14 d LR ₅₀ > 2000 g ai/ha		
<i>Chrysoperla carnea</i> (larvae)	contact with dried residues on treated glass plates	Tramat 500 SC	2000 (4 L prod./ha)	Corrected mortality / Reproduction	0.2% ↑ no. of eggs ↑ egg-laying performance	Kühner, C., 1990
				LR ₅₀ > 2000 g ai/ha		

Under consideration of a field rate of 1000 g ai/ha and a drift rate of 2.77 g ai/ha the risk to non-target arthropods is considered acceptable. No statistically significant adverse effects were observed at the field rate or at the drift rate.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

A study on the toxicity of the active substance ethofumesate (tested as Ethofumesate 500 SC) to earthworms was already submitted for the first EU approval of the active substance (Addendum to the DAR, 2000). Additional studies on the reproductive toxicity of the representative formulation Ethofol 500 SC were submitted by the notifier for the renewal of the EU approval of ethofumesate.

New studies on other soil macro-organisms (*Hypoaspis aculeifer*, *Folsomia candida*) were submitted with the formulation Ethofol 500 SC and the comparable formulation Ethofumesate 500 SC.

Studies with the soil metabolite NC8493 were conducted addressing the risk soil organisms. Therefore, laboratory studies with earthworms and the other soil macro-organisms *Hypoaspis aculeifer* and *Folsomia candida* were conducted.

In addition, studies with the metabolite NC 20645 were conducted. However, no risk assessment was conducted for the metabolite NC 20645 as the metabolite was found only in a lysimeter leachate and hence is not considered relevant for the soil risk assessment.

The study summaries for studies with the active substance ethofumesate and the soil metabolite NC 8493 are provided in the RAR, Volume 3, B.9 (A.S.). The study summaries for the studies with the formulation Ethofol 500 SC are given below.

B.9.7.1. Earthworms

For the first EU approval of the active substance several earthworm reproduction studies with the formulated active substance has been submitted addressing the risk for soil organisms.

Reference:	Effects of Ethofol 500 SC on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat (spraying application)
Author(s), year:	Witte, B., 2013
Report/Doc. number:	Study no. 81041022, Reference no. ID000147
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofol 500 SC, Batch no.: 710B, 516.2 g/L ethofumesate (analysed)
Test species:	Earthworm <i>Eisenia fetida</i> (Savigny, 1826)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, each with 10

	individuals.
Weight, age:	Mean: 324 - 600 mg/worm, adults with clitellum, approximately 8 months
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)
Applied concentrations:	Nominal: 0 (control, quartz sand), 3.4, 6.8, 9.6, 19.2, 38.4, 76.8 and 153.6 kg prod./ha corresponding to 5.7, 11.3, 16.1, 32.2, 64.3, 128.7 and 257.3 mg ai/kg soil dw, test substance was sprayed on the soil
Solvent:	None
Toxic standard:	Luxan Carbendazim 500 FC, tested at concentrations of 5 and 10 mg prod./kg soil dw
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	500 g dry weight/test container
Temperature:	18.0 – 22.0 °C
Light regime:	16 hours light (400-800 lx) / 8 hours dark
Water content:	Test start: 19.3-21.6% (equivalent to 50.8-56.8% of WHC) Test end: 21.5-24.7% (equivalent of 56.5-65.1% of WHC)
pH:	Test start: 6.1 Test end: 5.7 – 6.0
Feeding:	Finely ground cattle manure was used as food; 5 g per replicate was added each week for the first 4 weeks.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	Mortality data were analysed for significance by using the Fisher's Exact Test (one-sided greater, $\alpha = 0.05$). The EC_{50} values and their 95% confidence limits were calculated by applying Probit-Analysis. The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk's test and the Levene's test, respectively. As the data for body weight changes and reproduction were normally distributed and homogenous in both cases, Williams t-test was used to compare treatment and control values (multiple comparison, one-sided smaller, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Findings:

Biological effects:

Feeding activity: The turnover of biomass of those earthworms exposed to the different rates of the test item was comparable to the control, except at the highest test rate of 153.6 kg prod./ha (corresponding to 257.3 mg ai/kg soil dw), where the food intake was slightly reduced.

No behavioural abnormalities were observed and all worms burrowed into soil within 15 minutes after introduction.

Table B. 9.7.1-1: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	Ethofol 500 SC [kg prod./ha]						
Exposure	-	3.4	6.8	9.6	19.2	38.4	76.8	153.6
Mortality of adult earthworms [%] after 28 d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0
Mean change of body weight of the adults from day 0 to day 28 [mg] (Standard deviation)	64 (24)	97 (10)	54 (28)	71 (11)	54 (18)	9 * (18)	-5 * (25)	-4 * (15)
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	13.8 (5.6)	20.6 (2.7)	11.5 (6.6)	14.9 (2.7)	11.5 (4.1)	1.8 * (3.7)	-1.0 * (5.2)	-0.8 * (3.1)
Mean number of offspring per treatment group after 56 d (Standard deviation)	213 (38)	233 (38)	223 (30)	165 (50)	180 (55)	183 (27)	112 * (59)	91 *(36)
Reproduction compared to control [%]	-	109.5	104.6	77.4	84.7	85.8	52.4 *	42.8 *

* Significantly different compared to the control, Williams t-test, α 0.05, one-sided smaller

In the positive control (Carbendazim) the number of juveniles was significantly reduced at test concentrations between 3.0 – 6.7 mg test item/kg soil dw. The mean number of juveniles was between 5 (highest test concentration) and 279 (lowest test concentration) after 8 weeks of test duration. The mean number of juveniles in the control was 263. Based on the results of the study a NOEC (reproduction) of 0.87 mg ai/kg soil dw was determined. The EC₅₀ was calculated to be 1.7 mg ai/kg soil dw based on effects on the reproduction of earthworms.

Conclusion:

NOEC_{mortality} = 153.6 kg prod./ha (257.3 mg ai/kg soil dw)

NOEC_{body weight} = 19.2 kg prod./ha (32.2 mg ai/kg soil dw)

NOEC_{reproduction} = 38.4 kg prod./ha (64.3 mg ai/kg soil dw)

EC₁₀ (reproduction) = 12.4 kg prod./ha (20.7 mg ai/kg soil dw)

EC₅₀ (reproduction) = 113.0 kg prod./ha (189.3 mg ai/kg soil dw)

Comment RMS:

The earthworm reproduction study was conducted according to the OECD test guideline 222 (2004). Based on the validity criteria stated in the guideline the study was considered acceptable. The mortality of adults in the control was below 10% (being 0%). The number

of juveniles per control replicate was greater than 30 (being 164-270 juveniles per replicate). The coefficient of variation of reproduction in the control was $\leq 30\%$ (being 17.8%).

Based on results of the study a NOEC of 19.2 kg prod./ha (corresponding to 32.2 mg ai/kg soil dw) based on statistically significant effects on the body weight was determined.

Reference:	Effects of Ethofol 500 SC on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat
Author(s), year:	Lühns, U., 2011a
Report/Doc. number:	Study no. 63554022, Reference no. ID000145
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofol 500 SC (code: HBX01), Batch no.: 025A, 484.4 g/L ethofumesate (analysed)
Test species:	Earthworm <i>Eisenia fetida</i> (Savigny, 1826)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, each with 10 individuals.
Weight, age:	Mean: 320 - 599 mg/worm, adults with clitellum, approximately 9 to 10 months
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)
Applied concentrations:	Nominal: 0 (control, quartz sand), 7.75, 15.5, 31.0, 62.0 and 124.0 mg prod./kg soil dw corresponding to 0 (control), 3.34, 6.67, 13.3, 26.7 and 53.4 mg ai/kg soil dw, test substance was incorporated into the soil
Solvent:	None
Toxic standard:	Luxan Carbendazim 500 FC, tested at concentrations of 5 and 10 mg prod./kg soil dw
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	500 g dry weight/test container
Temperature:	18.0 – 22.0 °C
Light regime:	16 hours light (400-800 lx) / 8 hours dark
Water content:	Test start: 19.3-21.7% (equivalent to 50.8-57.1% of WHC) Test end: 22.6-24.6% (equivalent to 59.5-64.8% of WHC)

pH:	Test start: 6.3 – 6.4, test end: 6.3 – 6.4
Feeding:	Finely ground cattle manure was used as food; 5 g per replicate was added each week for the first 4 weeks.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk's test and the Cochran's test, respectively. As the data for body weight changes and reproduction were normally distributed and homogenous in both cases, Williams t-test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Findings:

Biological effects:	Feeding activity: The turnover of biomass of those earthworms exposed to the different rates of the test item was comparable to the control. No behavioural abnormalities were observed and all worms burrowed into soil within 15 minutes after introduction.
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Table B. 9.7.1-2: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	Ethofol 500 SC [mg prod./ha]				
Exposure	-	7.75	15.5	31.0	62.0	124
Mortality of adult earthworms [%] after 28 d	0.0	0.0	0.0	0.0	0.0	0.0
Mean change of body weight of the adults from day 0 to day 28 [mg] (\pm SD)	229 (12)	241 (34)	222 (26)	226 (15)	211 (20)	148 * (13)
Mean change of body weight of the adults from day 0 to day 28 [%] (\pm SD)	52.1 (3.5)	55.4 (10.4)	50.9 (7.0)	51.1 (5.3)	48.3 (6.7)	36.0 * (4.0)
Mean number of offspring per treatment group after 56 d (\pm SD)	215 (27)	201 (30)	204 (23)	158 (28)	227 (57)	146 ** (64)
Reproduction compared to control [%]	-	93.5	94.5	73.2	105.2	67.8 **

* Significantly different compared to the control, Williams t-test, α 0.05, two sided

** Significantly different compared to the control, Williams t-test, α 0.05, one sided smaller

In the positive control (Carbendazim) the number of juveniles was significantly reduced at all test concentrations (2.3 – 7.5 mg test item/kg soil dw). The mean number of juveniles was between 1 (highest test concentration) and 233 (lowest test concentration) after 8 weeks of test duration. The mean number of juveniles in the control was 335. Based on the results of the study a NOEC (reproduction) of < 1.0 mg ai/kg soil dw was determined. The EC₅₀ was calculated to be 1.21 mg ai/kg soil dw based on effects on the reproduction of earthworms.

Conclusion: NOEC (mortality, body weight, reproduction) = 62 mg prod./kg soil dw (= 26.7 mg ai/kg soil dw)

Comment RMS: The earthworm reproduction study was conducted according to the OECD test guideline 222 (2004). Based on the validity criteria stated in the guideline the study was considered acceptable. The mortality of adults in the control was below 10% (being 0%). The number of juveniles per control replicate was greater than 30 (being 179-254 juveniles per replicate). The coefficient of variation of reproduction in the control was \leq 30% (being 12.6%).

Based on results of the study a NOEC of 26.7 mg ai/kg soil dw based on statistically significant effects on the body weight and reproduction was determined.

Reference:	Ethofol 500 SC: Assessment of effects on reproduction and growth on <i>Eisenia fetida</i> in artificial soil
Author(s), year:	Stäbler. D., 2002
Report/Doc. number:	Study no. 20021052/01-NREf, Reference no. IDD00146
Guideline(s):	BBA Guideline Part VI, 2-2 (1994), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofol 500 SC, Batch no.: 354L, 47.68% (analysed)
Test species:	Earthworm <i>Eisenia fetida andrei</i> (Michaelson)
Number of organisms:	4 replicates per treatment, control and reference, each with 10 individuals.
Weight, age:	Mean: 300 - 600 mg/worm, adults with clitellum, approximately > 2 months and < 12 months
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)

Applied concentrations:	Nominal: 0 (control, quartz sand), 0.75, 2.63, 5.25, 7.88 and 10.5 kg ai/ha corresponding to 1.0, 3.5, 7.0, 10.5 and 14.0 mg ai/kg soil dw, test substance was sprayed on the soil
Solvent:	None
Toxic standard:	Derosal flüssig (360 g Carbendazim/L), Batch no.: 03398, tested at an application rate of 0.468 kg ai/ha corresponding to 0.624 mg ai/kg soil dw
Test substrate:	Artificial soil, 10 % sphagnum peat, 20 % kaolin clay, 69 % industrial quartz sand, 1% calcium carbonate
Substrate/test vessel:	600 g dry weight/test container
Temperature:	20 ± 2 °C
Light regime:	16 hours light (~ 600 lx) / 8 hours dark
Water content:	Test start: 28.4 – 30.3% (> 50% of WHC) Test end: 27.9 – 30.1% (> 50% of WHC)
pH:	Test start: 6.4 – 6.5 Test end: 6.4 - 6.6
Feeding:	Finely ground manure was used as food; 5 g per replicate was added each week for the first 4 weeks.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	The analysis of data included the no observed adverse effect concentration (NOAEL) and the lowest observed effect concentration (NOEC) by a multiple t-test procedure according to Dunnett ($p \leq 0.05$, one-sided smaller). Statistical calculations were done using SAS® or EASY ASSAY multiple testing program.
<u>Findings:</u>	
Biological effects:	Feeding activity: The turnover of biomass of those earthworms exposed to the different rates of the test item was comparable to the control.

Table B 9.7.1-1: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Exposure	Reference	Control	Ethofol 500 SC [mg ai/kg soil dw]				
			1.0 *	3.5	7.0	10.5	14.0
Mortality of adult earthworms [%] after 28 d	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean change of body weight of the adults from day 0 to day 28 [mg]	34.0	89.0	117	80.0	74.0	107	67.0
Mean change of body weight of the adults from day 0 to day 28 [%]	108.5**	122.4	130.2	120.8	119.0	127.3	117.4
Mean number of offspring per treatment group after 56 d (± SD)	142 ** (33)	299 (37)	280 (37)	325 (24)	295 (20)	243 (18)	218 ** (70)

* One earthworm escaped during the exposure period.

** Significantly different compared to the control, Dunnett, α 0.05, one sided smaller

In the positive control (Carbendazim) the number of juveniles was significantly reduced at the test concentration. The mean number of juveniles was about 142 after 8 weeks of test duration. The mean number of juveniles in the control was 299. Based on the results of the study a NOEC (reproduction) of < 0.624 mg ai/kg soil dw was determined.

Conclusion:

NOAEC (mortality, body weight) = 14.0 mg ai/kg soil dw

NOAEC (reproduction) = 10.5 mg ai/kg soil dw

Comment RMS:

The earthworm reproduction study was conducted according to the BBA guideline (Teil VI, 2-2). Based on the validity criteria stated in the guideline the study was considered acceptable. The mortality of adults in the control was below 10% (being: 0%). The reduction of body weight of the adults in the control was \leq 20% (being: + 22.4%). The number of juveniles per control replicate was greater than 30 (being 299 juveniles per replicate). The coefficient of variation of the mean of juveniles in the control was \leq 30% (being: 12.4%).

Under the current valid OECD test guideline (OECD 222) the study is also considered to be valid. The validity criteria given in the OECD test guideline (\geq 30 juveniles per replicate, coefficient of variation of reproduction \leq 30% and adult mortality over the initial 4 weeks \leq 10%) are well covered by the BBA test guideline.

Based on the results of the study the NOAEC was determined to be 10.5 mg ai/kg soil dw based on significant effects on the reproduction.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Reference:	Effects of Ethofol 500 SC on reproduction of the Collembola <i>Folsomia candida</i> in artificial soil with 5% peat
Author(s), year:	Lühns, U., 2011b
Report/Doc. number:	Study no. 63553016, Reference no. IDD00148
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofol 500 SC (code: HBX01), Batch no.: 025A, 484.4 g/L ethofumesate (analysed)
Test species:	Collembola <i>Folsomia candida</i> (Willem, 1902)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, 1 additional replicate per treatment to check the pH and water content of the test substrate after 28 days, each with 10 individuals.
Life stage, age:	Juveniles / adults, 10-12 days old
Type of test, duration:	Laboratory sub-lethal test, 28 days
Applied concentrations:	Nominal: 0 (control), 3.88, 7.75, 15.5, 31.0 and 62.0 mg prod./kg soil dw corresponding to 1.67, 3.34, 6.67, 13.35 and 26.7 mg ai/kg soil dw
Solvent:	None
Toxic standard:	Boric acid, Purity: 100.3%
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	200 g dry weight/test container
Temperature:	18.0 – 22.0 °C
Light regime:	16 hours light (400-800 lx) / 8 hours dark
Water content:	Test start: 21.2 – 21.9% (equivalent to 53.0 – 54.9% of WHC) Test end: 20.5 – 21.8% (equivalent of 51.2 – 54.5% of WHC)
pH:	Test start: 5.8 – 5.9 Test end: 5.6
Feeding:	Approximately 2 mg of granulated dry yeast were spread over the soil surface at test start. After 14 days, 2 mg of granulated dry yeast were added.
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked on day 14 after application. Mortality of adults, behavioural effects and number of juvenile Collembola were assessed after 28 days

Statistics: Mortality data were statistically analysed using Fisher's Exact Test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test and the Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Findings:

Biological effects: No abnormal behaviour was observed with the surviving Collembola.

Table B 9.7.2-1: Effects on mortality and reproduction of *Folsomia candida* in a sub-chronic test

	Control	Ethofol 500 SC [mg prod./ha]				
Exposure	-	3.88	7.75	15.5	31.0	62.0
Mortality of adult Collembola [%] after 28 d	11.0	15.0	18.0	10.0	8.0	20.0
Mean number of juveniles per treatment group after 28 d (\pm SD)	718 (193)	608 (158)	865 (193)	739 (117)	746 (147)	774 (115)
Reproduction compared to control [%]	-	85	120	103	104	108

SD...Standard Deviation

The reference item boric acid showed statistically significant effects on reproduction at concentrations of ≥ 59.3 mg ai/kg soil. The EC_{50} for reproduction was calculated to be 70.7 mg ai/kg soil dw. Mortality was statistically significantly higher compared to the control at 88.9 mg ai/kg soil dw and above.

Conclusion: NOEC (mortality, reproduction) = 62 mg prod./kg soil dw (= 26.7 mg ai/kg soil dw)

Comment RMS: The Collembola reproduction study was conducted according to the OECD test guideline 232 (2009). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being: 11%). The mean number of juveniles per control replicate was greater than 100 (being: 506 - 1016 juveniles per replicate). The coefficient of variation of reproduction in the control was $\leq 30\%$ (being: 26.9%).

Based on results of the study a NOEC of 62 mg prod./kg soil dw based on the highest tested concentrations was determined.

Reference:	Effects of Ethofumesate 500 g/L SC on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat
Author(s), year:	Ganßmann, M., 2012
Report/Doc. number:	Report no. 674803089, Reference no. IDD00149
Guideline(s):	OECD 226 (2008)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate 500 g/L SC, Batch no.: 0058263020 03/2012, 491.3 g/L (analysed)
Test species:	Predatory mites, <i>Hypoaspis aculeifer</i> (Canestrini, 1883)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, 1 additional replicate per treatment to check the pH and water content of the test substrate after 14 days, each with 10 female adults.
Life stage, age:	Adult females, approximately 14 days after reaching the adult stage
Type of test, duration:	Laboratory sub-lethal test, 14 days
Applied concentrations:	Nominal: 0 (control), 8.6, 15.4, 27.8, 50.0 and 90.0 mg prod./kg soil dw corresponding to 4.2, 7.6, 13.7, 24.6 and 44.2 mg ai/kg soil dw
Solvent:	None
Toxic standard:	BAS 152 11 I (dimethoate), 411.7 g/L (analysed)
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	200 g dry weight/test container
Temperature:	18.0 – 22.0 °C
Light regime:	16 hours light (400-800 lx) / 8 hours dark
Water content:	Test start: 20.6 – 21.6% (equivalent to 55.5 – 58.3% of WHC) Test end: 18.2 – 20.2% (equivalent of 49.1 – 54.5% of WHC)
pH:	Test start: 5.7 – 6.1 Test end: 5.6 – 5.7
Feeding:	One spatula of cheese mite (<i>Tyrophagus putrescentiae</i>) at start and day 2, 4, 7, 9 and 11.
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked on day 7 after application. Mortality of adults, differences in morphology, behavioural effects and number of juveniles were assessed after 14 days
Statistics:	Mortality data were statistically analysed using Fisher's Exact Test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test and the Levene's test (α

=0.05). Further statistical evaluation was performed using Willimas t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Findings:

Biological effects: No differences in morphology of the mites between the test item groups and the control were observed.

Table B 9.7.2-2: Effects on mortality and reproduction of *Hypoaspis aculeifer* in a sub-chronic test

Exposure	Control	Ethofumesate 500 g/L SC [mg prod./ha]				
		8.6	15.4	27.8	50.0	90.0
Mortality of adult mites [%] after 14 d	0	5	5	3	3	3
Mean number of juveniles per treatment group after 14 d (\pm SD)	166 (34)	155 (6)	173 (26)	174 (19)	164 (24)	152 (16)
Reproduction compared to control [%]	-	93	104	105	99	92

SD...Standard Deviation

The reference item dimethoate showed statistically significant effects on the adult mortality and reproduction at concentrations of ≥ 1.7 mg ai/kg soil. The EC_{50} for reproduction was calculated to be 4.0 mg ai/kg soil dw. The LC_{50} was determined to be 2.1 mg ai/kg soil dw.

Conclusion:

NOEC (mortality, reproduction) = 90 mg prod./kg soil dw (= 44.2 mg ai/kg soil dw)

Comment RMS:

The predatory mite reproduction study was conducted according to the OECD test guideline 226 (2008). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being 0%). The mean number of juveniles per control replicate was greater than 50 (being 115-220 juveniles per replicate). The coefficient of variation of reproduction in the control was $\leq 30\%$ (being 20.5%).

Based on results of the study a NOEC of 90 mg prod./kg soil dw (corresponding to 44.2 mg ai/kg soil dw) based on the highest tested concentrations was determined.

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA**Table B.9.8-1: Summary of effects on soil meso- and macrofauna**

Species	Substance	Endpoint	Reference
<i>Eisenia fetida</i>	Ethofol 500 SC	56 d NOEC = 32.2 mg ai/kg soil dw	Witte, B., 2013
		56 d NOEC = 26.7 mg ai/kg soil dw	Lührs, U., 2011a
		56 d NOAEC = 10.5 mg ai/kg soil dw	Stäbler, D., 2002
	Ethofumesate 500 SC	56 d NOEC = 25 mg ai/kg soil dw	Sowing, P. & Gosch, H.
	Metabolite NC8493	56 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012a
		56 d NOEC = 16 mg/kg soil dw	Lührs, U., 2011
<i>Folsomia candida</i>	Metabolite NC 20645	56 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012b
	Ethofol 500 SC	28 d NOEC = 26.7 mg ai/kg soil dw	Lührs, U., 2011b
	Metabolite NC8493	28 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012c
		28 d NOEC = 556 mg/kg soil dw	Friedrich, S., 2013a
<i>Hypoaspis aculeifer</i>	Metabolite NC 20645	28 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2013b
	Ethofumesate 500 SC	14 d NOEC = 44.2 mg ai/kg soil dw	Ganßmann, M., 2012
	Metabolite NC8493	14 d NOEC = 309 mg/kg soil dw	Schulz, L., 2013

Bold written values were used for the risk assessment.

The risk assessment for soil organisms was conducted according to the Terrestrial Guidance Document (SANCO/10329/2002).

TER values for earthworms were calculated as the ratio between sublethal no observed effect concentrations (NOEC), and the maximum initial PEC_{soil} . The PEC_{soil} used for the 1st tier risk assessment is based on a single application (1 x 1.0 kg ai/ha) on sugar beets (0% interception) and was calculated to be 1.336 mg ai/kg soil (see fate section B.8). The other uses (post-emergence, multiple applications) are covered by the risk assessment for the pre-emergence use.

The endpoints for the active substance / the representative formulation were corrected by a factor of 2 irrespective of the peat content in the study. During an EFSA expert meeting (PRAS 91, April 2012) the use of a correction factor for substances with a $\log P_{ow} > 2$ was discussed and it was agreed that the correction factor should always be used for those substances. Unless it can be demonstrated that toxicity to earthworms is independent of f_{oc} .

Table B. 9.8-2: TER long-term for earthworms and other soil macro-organisms

GAP use	Test substance	NOEC [mg/kg soil dw]	max PEC _{soil} [mg/kg soil dw]	TER _{LT}	Trigger
Earthworms					
Sugar beets, fodder beets (pre-emergence) 1 x 1 kg ai/ha	Ethofol 500 SC	NOAEC = 5.25 ai *	1.336	3.9	5
	Metabolite NC 8493	NOEC = 16.0	0.291	55	5
Sugar beets, fodder beets (post-emergence) 3 x 0.333 kg ai/ha	Ethofol 500 SC	NOAEC = 5.25 ai *	0.357	15	5
<i>Folsomia candida</i>					
Sugar beets, fodder beets (pre-emergence) 1 x 1 kg ai/ha	Ethofol 500 SC	NOEC = 13.35 ai *	1.336	10	5
	Metabolite NC 8493	NOEC = 100	0.291	344	5
<i>Hypoaspis aculeifer</i>					
Sugar beets, fodder beets (pre-emergence) 1 x 1 kg ai/ha	Ethofumesate 500 SC	NOEC = 22.1 ai *	1.336	17	5
	Metabolite NC 8493	NOEC = 309	0.291	1062	5

* corrected by a factor of 2 due to the log P_{OW} of ethofumesate > 2

Based on the risk assessment a high long-term risk to earthworms from exposure to the formulated active substance was identified. The TER_{LT} value for the pre-emergence application (1 x 1 kg ai/ha) was calculated to be 3.9 based on a corrected NOAEC of 5.25 mg ai/kg soil dw. The long-term risk to earthworms considering a post-emergence application (3 x 0.333 kg ai/ha) was identified to be acceptable.

The risk to the other soil macro-organisms, *Folsomia candida* and *Hypoaspis aculeifer* was identified to be acceptable. In addition, the risk to earthworms and other soil organisms from exposure to the soil metabolite NC 8493 was determined to be low. The TER_{LT} values are below the relevant trigger indicating an acceptable risk.

Refined risk assessment:

The notifier provided a refined risk assessment considering the chronic toxicity to earthworms. The notifier proposed to use a mean NOEC value considering the endpoints of all available earthworm reproduction studies conducted with the representative formulation Ethofol 500 SC.

Taking into account all four long-term endpoints (see Table B.9.8-1) a mean NOEC of 19.2 mg ai/kg soil dw was determined. Based on this endpoint the long-term risk to earthworms was identified to be acceptable (TER_{LT} = 14.4).

The RMS does not agree on this approach to refine the chronic risk to earthworms due to the following reasons:

- The use of a mean NOEC is not a validated approach. The approach was already discussed for other groups of organisms, e.g. aquatic organisms and birds and mammals and especially for chronic endpoints no general agreement within the Member States could be reached.
- The calculated mean NOEC of 19.2 mg ai/kg soil dw is based on four studies with the representative formulation Ethofol 500 SC and the comparable formulation Ethofumesate 500 SC. It should be considered that

the lowest endpoints of each earthworm reproduction study are based on different kind of effects (growth, reproduction). In addition, the type of application (overspraying, incorporation into the soil) is not comparable within the studies.

- The use of a mean endpoint should reduce the uncertainties regarding differences in inter- and intra-species sensitivities and the extrapolation from the laboratory to the field conditions. However, the available studies were conducted with the same earthworm species. Hence, even under consideration of a mean NOEC some uncertainties
- The calculation of the mean NOEC should be based on corrected NOEC values. At an EFSA expert meeting it was agreed that the endpoints of substances with a $\log P_{ow} > 2$ should be always corrected by a factor of 2 irrespective of the peat content.

Based on the risk assessment a high long-term risk to earthworms considering the pre-emergence application of 1 x 1 kg ai/ha was identified. Further data are required addressing the risk to earthworms.

The risk to earthworms considering the post-emergence application (multiple applications) was identified to be acceptable.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Several studies on the effects of the active substance ethofumesate on nitrogen transformation and carbon mineralisation were submitted for the first EU approval of the active substance.

According to the EU data requirements for active substances (Regulation 283/2013) and plant protection products (Regulation 284/2013) the impact on soil microbial activity should be evaluated, in terms of nitrogen transformation. Hence, the available studies on micro-flora respiration (carbon transformation) are given as additional information only. Please refer to RAR, Volume 3, B.9 (A.S.).

In addition to the studies with the active substance a new nitrogen and carbon mineralisation study with the representative formulation Ethofol 500 SC was submitted. A summary of the study is provided below.

No nitrogen mineralisation studies with the relevant soil metabolites were provided addressing the risk to soil micro-organisms.

Reference:	Effects of Ethofol 500 SC (HBX01) on the activity of the soil microflora in the laboratory
Author(s), year:	Feil, N., 2010
Report/Doc. number:	Report no.: 56176080, Reference no.: IDD00150
Guideline(s):	OECD 216 (2000) and OECD 217 (2000)
GLP:	Yes
Deviations:	- Measurement of the soil respiration rate was conducted on day 8 instead on day 7 due to technical reasons. This deviation has no impact on the outcome of the study.
Validity:	Acceptable

Material and methods:

Test substance:	Ethofol 500 SC (code: HBX01), batch no: 241Y, content: 485.6 g ai/L, CAS no: 26225-79-6
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation and carbon mineralisation test, 28 days
Applied concentrations:	0 (control, deionised water), 2.96 and 14.81 mg/kg soil dw corresponding to 1.29 and 6.47 mg ai/kg soil dw
Solvent/vehicle:	None Sodium chloride, 16 g/kg soil dw
Toxic standard:	The reference item (sodium chloride) had a retarding or stimulating effect of more than $\pm 25\%$ compared to the control at days 28 and 98 after application.
Test substrate:	Soil from fallow grassland (mid loamy sand), from a field located in Darmstadt, Germany. No application of fertilisers and plant protection products for at least 4 years prior to the study. Sampling depth between 0.05 and 0.2 m

	Soil texture: 9.4% clay, 33.1% silt, 57.5% sand, 0.5% lucerne meal (nitrogen transformation test)
	Total C _{ORG} 1.16 %, pH: 6.9, Microbial biomass: 1.55% of total organic carbon, Content of NH ₄ ⁺ -N, NO ₂ ⁻ -N, NO ₃ ⁻ -N and N _{min} -N: 0.389, 0.156, 16.425 and 16.97 mg/kg soil dw
	Water holding capacity (WHC): 41.6%
Test units:	Carbon transformation: 750 – 1000 g soil per test vessel (0.5 L plastic box) Nitrogen transformation: 250 – 500 g soil per tests vessel (0.5 L plastic box) 3 units per treatment and control group
Incubation:	20 - 22°C, darkness
Water content	45 – 52% of the WHC _{max}
pH:	7.0 – 7.2
Test parameters:	Soil pH, dry weight and water content were determined at test start and at each sampling date. The temperature was recorded continuously. For the determination of the nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date, i.e. 7, 14 and 28 days. The glucose induced respiration rate was determined in each sample of treated and control soils after 0 days (within 6 hours), 8, 14 and 28 days.
Statistics:	Calculation of mean values per treatment, standard deviation and coefficient of variation: Normality and homogeneity of variances were tested using R/S-test ($\alpha = 0.05$) and Cochran's test ($\alpha = 0.05$), respectively and pair-wise comparisons of treated and control values according to Student t-test / Welch t-test ($\alpha = 0.05$) were performed.
<u>Findings:</u>	
Nitrogen transformation:	No adverse effects of the formulation Ethofol 500 SC on nitrogen transformation (nitrogen transformation rate, mean content of nitrogen and mineral nitrogen) in soil could be observed at both test concentrations (2.96 and 14.19 mg prod./kg dry soil dw) during the 28 day experiment.

Table B. 9.9-1: Effects of Ethofol 500 SC on mean nitrogen and mean mineral nitrogen

Treatment	Time (days)	Mean Nitrate-N [mg/kg soil dw]	% difference to the control	Mean Mineral-N [mg/kg soil dw]	% difference to the control
Control	0	16.854	-	22.228	-
	7	4.313	-	6.289	-
	14	12.493	-	14.028	-
	28	31.657	-	33.076	-
2.96 mg/kg soil dw	0	15.854 *	-5.93	17.032 *	-23.38
	7	4.481	3.90	6.802	8.22
	14	11.591	-7.22	13.024	-7.16
	28	29.892	-5.58	31.474	-4.84
14.81 mg/kg soil dw	0	16.095 *	-4.50	17.455 *	-21.47
	7	2.409 *	-44.15	4.455 *	-29.16
	14	10.332 *	-17.30	11.441 *	-18.44
	28	28.222	-10.85	29.720	-10.15

* Statistically significant different compared to the control, Student t-test or Welch t-test in case of inhomogeneity, α 0.05

Mean nitrogen and mineral nitrogen:

Differences in the mean nitrate (NO_3^- - N) from the control of -5.58 % (test concentration 2.96 mg/kg dry soil) and -10.85% (test concentration 14.81 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Statistically significant effects on the mean nitrate content and the mean mineral nitrogen content were observed in the lower test concentration shortly after application and in the higher test concentration until 14 days after application. However, no statistically significant effects were observed at the end of the test.

Table B. 9.9-2: Effects of Ethofol 500 SC on nitrogen formation rate

Treatment	Time (days)	Nitrogen formation rate [mg/kg soil dw/d]	% difference to the control
Control	0-7	-1.79	-
	7-14	1.17	-
	14-28	1.37	-
2.96 mg/kg soil dw	0-7	-1.63	-8.94
	7-14	1.07	-8.55
	14-28	1.31	-4.38
14.81 mg/kg soil dw	0-7	-1.95	8.94
	7-14	1.13	32.26
	14-28	1.28	-18.87

Nitrogen formation rate:

Differences in the nitrogen formation rate from the control of -4.38 % (test concentration 2.96 mg/kg dry soil) and -18.87% (test concentration 14.81 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Table B. 9.9-3: Effects of Ethofol 500 SC on soil respiration

Treatment	Time (days)	Soil respiration [mg CO ₂ /kg soil dw/h]	% difference to the control
Control	0	9.654	-
	7	8.845	-
	14	8.720	-
	28	8.443	-
2.96 mg/kg soil dw	0	10.842	12.31
	7	9.074	2.58
	14	9.088	4.22
	28	8.322	-1.43
14.81 mg/kg soil dw	0	10.933 *	13.25
	7	9.347 *	5.66
	14	8.652	-0.78
	28	7.743 *	-8.29

Mean nitrogen and mineral nitrogen:

Differences in the soil respiration from the control of -1.43 % (test concentration 2.96 mg/kg dry soil) and -8.29% (test concentration 14.81 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Statistically significant effects on the soil respiration were observed on the higher test concentration only. Statistically significant effects were observed at day 0 and 7 and at the end of the test. However, the differences of soil respiration compared to the control were below 25% at the end of the test.

Conclusion:

The formulation Ethofol 500 SC had no impact on respiration activity, soil nitrate content and soil nitrate formation rate of soil micro-flora when applied up to 14.81 mg/kg soil dw.

Comment RMS:

The nitrogen transformation and soil respiration test was conducted according to the OECD test guideline 216 (2000) and 217 (2000).

According to the test guidelines (OECD 216 and 217) the study is considered valid if the coefficients of variation in the control for soil respiration and nitrate content were ≤ 15%. In this study the CV in the control were maximum 14.7% (nitrate content) and thus fulfilled the validity criteria.

Based on results of the study an EC₂₅ of > 14.81 mg/kg soil dw (corresponding to 6.47 mg ai/kg soil dw) was determined.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION**Table B.9.10-1: Summary of effects on non-target micro-organisms**

Test substance	Test parameter	Test concentration	Time	Effects (deviation from control)	Reference
Ethofumesate techn.	Nitrogen mineralisation	0.3 mg ai/kg soil dw	42 d	< 20 %	Vonk, J.W., 1988
		3.0 mg ai/kg soil dw		< 20 %	
Ethofol 500 SC	Nitrogen mineralisation	2.96 mg/kg soil dw = 1.29 mg ai/kg soil dw	28 days	-4.38	Feil, N., 2010
		14.81 mg/kg soil dw = 6.47 mg ai/kg soil		-18.87	
Metabolite NC 8493	Nitrogen mineralisation	1.20 mg/kg soil dw	28 days	-1.4%	Schulz, L., 2013b
		12.0 mg/kg soil dw		15.2%	
Metabolite NC 20645	Nitrogen mineralisation	1.38 mg/kg soil dw	28 days	6.9%	Schulz, L., 2013b
		13.8 mg/kg soil dw		6.7%	

According to the Terrestrial Guidance Document (SANCO/10329/2002) the risk is considered acceptable if the effect on nitrogen mineralisation at a recommended application rate is below 25% after 100 days.

Table B. 9.10-2: Risk assessment

Test substance	Effects < 25% at test concentration	PEC _{soil, plateau}	Risk acceptable?
Ethofol 500 SC	6.47 mg ai/kg soil dw	1.336 mg ai/kg soil dw	Yes
Metabolite 8493	12.0 mg/kg soil dw	0.291 mg/kg soil dw	Yes

The formulated active substance ethofumesate did not significantly affect the activity of the soil nitrogen transformation under test conditions at application rates up to 6.47 mg ai/kg soil dw. The initial PEC_{soil} after application in sugar and fodder beets, pre-emergence and post-emergence was calculated to be 1.336 mg ai/kg soil and 0.357 mg ai/kg soil, respectively. Thus the exposure concentration used in the tests was significantly higher than the maximal expected PEC_{soil} when applied according to the GAP.

No study with the soil metabolite NC 8493 was submitted by the notifier United Phosphorous Ltd. Since no soil microorganism toxicity endpoint is available for the metabolite the applicant proposed to assume that the metabolite is 10 times more toxic than the active substance. Based on this assumption a toxicity endpoint of 0.647 mg/kg soil dw for the metabolite NC 8493 was determined. Even under consideration of this assumption the risk to soil microorganism is considered acceptable taking into account that the initial PEC_{soil} for the metabolite is 0.078 mg/kg soil (post-emergence) and 0.291 mg/kg soil (pre-emergence).

However, nitrogen transformation studies with the soil metabolites NC 8493 and NC20645 were provided by the Task Force. Based on the results a toxicity endpoint for the metabolite NC 8493 of 12.0 mg/kg soil dw was determined.

The soil metabolite NC 20645 was found in a lysimeter study of the Task Force. Hence, studies were submitted by the Task Force addressing the risk to soil organisms from exposure to this metabolite.

However, according to the e-fate experts the metabolite is not considered relevant and hence, no risk assessment was conducted for this metabolite.

According to the results of the data provided for the active substance ethofumesate it can be assumed that the risk for soil micro-organisms is low when applied according to the GAP.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Summary of screening data

In the first EU peer review evaluation of ethofumesate a post- and pre-emergence screening test (Thürwächter, 1999) was submitted to address the risk to non-target plants. The study was conducted with three different formulations containing the active substances phenmedipham, desmedipham and ethofumesate. Screening data with a solo-formulation of ethofumesate were not submitted. The study summary from the DAR is given below.

Additionally, screening data with the technical active substance (Rosinger, 2000) were provided for the first EU approval. Please refer to Volume 3, B.9 (AS).

Thuerwaechter, F, 1999

Methods

The effects of three Betanal co-formulations with ethofumesate (Betanal OF EC 25, Betanal OF EC 23 and Betanal OF EC 28 on the terrestrial plants Avena fatua, Setaria viridis, Zea mays, Brassica napus, Chenopodium album, Stellaria media and Glycine max was investigated after pre- and post emergent application, respectively. All formulations were applied at rates corresponding to the maximum recommended 56 g phenmedipham, 9.4 g desmedipham, 28 g ethofumesate per hectare, and at rates corresponding to 50%, 25%, 12.5% and 4% of the maximum dose (the lowest concentration simulating spray drift at 1 m distance from the field according to Ganzelmeier et al, 1995). Fresh weight and dry weight determinations were performed 21 days after treatment.

Results

The most sensitive of the tested species was Stellaria media, which was affected at all treatment rates. EC₅₀ was estimated to be somewhere between 4% and 12.5% of the maximum treatment level. At the highest dose, all tested species were affected.

Comments

Since the test was performed with a co-formulation with three active substances, the results are of less value for the assessment of the effects of ethofumesate on terrestrial plants.

B.9.11.2. Testing on non-target plants

Seedling emergence and vegetative vigour studies (Bramby-Gunary, 2004ab) have been conducted with the representative formulation Ethofol 500 SC (500 g/L Ethofumesate) following the OECD test guidelines (OECD 208 A and B, 2000). These studies were not evaluated during the first EU peer-review of the active substance.

Reference:	Evaluation of the phytotoxicity of Ethofol formulation (500 g/L ethofumesate) (based on OECD guideline 208 B) vegetative vigour test terrestrial non-target plants
Author(s), year:	Bramby-Gunary, J., 2004a
Report/Doc. number:	Study no. ACE-04-075, Reference no. IDD00151
Guideline(s):	OECD 208B (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol 500 SC, Batch No.: 030R, content: 507 g ai/L
Type of test:	Vegetative vigour test
Test duration:	21 days
Test species:	6 dicotyledonous and 2 monocotyledonous plant species <i>Daucus carota</i> (carrot), <i>Cucumis sativus</i> (cucumber), <i>Avena sativa</i> (oat), <i>Allium cepa</i> (onion), <i>Brassica napus</i> (oilseed rape), <i>Pisum sativum</i> (pea), <i>Beta vulgaris</i> (sugar beet) and <i>Vicia faba</i> (field bean).
Test soil:	Mixture of loam, sand and coarse grit. The soil type was assessed as loamy sand with an organic carbon content of < 1.5%.
Applied concentrations:	Control: Deionized water Test item: 0.015625, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 L prod./ha
Replicates:	Six replicates with three (oilseed rape, pea, sugar beet, field bean, carrot and cucumber) or five (onion and oats) plants per pot for each species
Exposure route:	All test species were germinated in seed trays of compost and transplanted shortly after emergence, at growth stages between 12 and 14. Plants were grown in pots (7x7x8 cm) containing a soil mix of sand, coarse grit, loam and slow release fertiliser. Applications were made using a cabinet track sprayer. After application the pots were placed in a greenhouse.
Test conditions:	Photoperiod: ambient lighting only Temperature: 15.9 – 28.0 °C Relative humidity: 4.9 – 93.1 %
Test parameter:	Plants were assessed for survival and for phytotoxicity on Day's 14 and 21. Total foliar fresh weight (biomass per pot above the soil level) was measured

immediately after harvest at 21 days after application.

Statistics : Statistical analysis was carried out using the statistical software ARM Gylling software.

Findings:

Analytical measurements: The test concentration was confirmed by analytical verification of the highest concentration of the test solution. The mean measured value of ethofumesate ranged from 103 to 105 % of nominal and indicated that the desired application rates were achieved.

Biological results: Phytotoxic effects on the test plants were observed for all test species, apart from peas. The most sensitive species considering phytotoxic effects were identified to be cucumber, oilseed rape and field bean.

The survival of the terrestrial plants was 100% for nearly all species. At a test rate of 1.0 L prod./ha slight effects on survival (~ 5% mortality) on the dicotyledonous plant field bean was observed.

Table B. 9.11.2-1: Foliar fresh weight (percentage of untreated control)

Rate [L prod./ha]	Oilseed rape ^d	Pea ^d	Sugar beet ^d	Field bean ^d	Carrot ^d	Cucumber ^d	Onion ^m	Oat ^m
Control	-	-	-	-	-	-	-	-
0.015625	100	90	97	77	100	87	71	100
0.03125	100	96	99	74	93	81	78	98
0.0625	100	94	94	81	93	81	75	92
0.125	100	95	100	80	92	78	79	100
0.25	100	86	94	73	94	68	73	99
0.5	100	91	99	68	100	68	88	92
1.0	97	90	98	54	82	65	78	79
2.0	93	86	100	53	89	59	75	87

m...monocotyledonous, d...dicotyledonous

Taking into account adverse effects on the foliar fresh weight the most sensitive plant species was observed to be the monocotyledonous species onion and the dicotyledonous species cucumber and field bean.

Table B. 9.11.2-2: Effects of Ethofol SC on non-target plants (plant foliar fresh weight)

Test species	NOEL	LOEL	EC ₅₀
	[L prod./ha]		
Carrot ^d	2.0	> 2.0	> 2.0
Cucumber ^d	n.a.	0.015625	> 2.0
Oilseed rape ^d	2.0	> 2.0	> 2.0
Oat ^m	0.03125	0.0625	> 2.0
Onion ^m	n.a.	0.015625	> 2.0
Pea ^d	2.0	> 2.0	> 2.0
Sugar beet ^d	2.0	> 2.0	> 2.0
Field bean ^d	n.a.	0.015625	~ 2.0

m...monocotyledonous, d...dicotyledonous, n.a...not applicable

Conclusions:

The most sensitive species was observed to be the dicotyledonous species field bean with an EC_{50} of approximately 2.0 L prod./ha. The remaining terrestrial plant species are less sensitive with an EC_{50} of greater than 2.0 L prod./ha.

Comment RMS:

The vegetative vigour test was conducted according to the OECD test guideline 208 B (July 2000). In order for the test to be considered valid, the following performance criteria must be met in the negative control:

- the mean seedling growth does not exhibit visible phytotoxic effects and
- the plant survival in the vegetative vigour test is at least 90% at the end of the test.

The test is considered acceptable given the validity criteria regarding phytotoxic effects (no phytotoxic effects were observed in the control groups) and survival of the plants (95-100% survival at the end of the test). Hence, the study is considered acceptable according to the OECD guideline 208 B (July 2000).

Taking into account the current valid OECD test guideline 227 (July 2006) the following validity criteria are given:

- The seedling emergence is at least 70% (control and treatment groups)
- In the control groups the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.
- In the control groups the mean plant survival is at least 90% for the duration of the study.
- In the control groups environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

The vegetative vigour test does also meet the given validity criteria according to the OECD test guideline 227 (2006).

Even though no information on the seedling emergence in the control and treatment groups are given a sufficient emergence of seedlings can be considered taking into account the available data on plant survival (95-100%) and phytotoxic effects.

In the control groups no phytotoxic effects were observed and the mean plant survival was 100% for all tested terrestrial plants.

According to the OECD test guideline the temperature and the humidity has to be in a range of $20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ and $70\text{ \%} \pm 25\text{ \%}$ (OECD 227), respectively. In the vegetative vigour test the temperature was in a range of $15.9 - 28.0\text{ }^{\circ}\text{C}$ and the

humidity was in a range of 4.9 and 93.1 %. There is no information (raw data) to address possible effects on the test plants due to the low humidity in the greenhouse. However, under consideration of the observed effects on plant survival and plant phytotoxicity the influence of the humidity on the test plants is considered to be low.

Reference:	Evaluation of the phytotoxicity of Ethofol formulation (500 g/L ethofumesate) (based on OECD guideline 208 A) seedling emergence terrestrial non-target plants
Author(s), year:	Bramby-Gunary, J., 2004b
Report/Doc. number:	Study no. ACE-04-074, Reference no. IDD00152
Guideline(s):	OECD 208 A (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofol SC, Batch No.: 030R, 507 g ai/L
Type of test:	Seedling emergence test
Test duration:	21 days
Test species:	6 dicotyledonous and 2 monocotyledonous plant species <i>Daucus carota</i> (carrot), <i>Cucumis sativus</i> (cucumber), <i>Avena sativa</i> (oat), <i>Allium cepa</i> (onion), <i>Brassica napus</i> (oilseed rape), <i>Pisum sativum</i> (pea), <i>Beta vulgaris</i> (sugar beet) and <i>Vicia faba</i> (field bean).
Test soil:	Mixture of loam, sand and coarse grit. The soil type was assessed as loamy sand with an organic carbon content of < 1.5%.
Applied concentrations:	Control: Deionized water Test item: 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 L prod./ha
Replicates:	Six replicates with three (oilseed rape, pea, sugar beet, field bean, carrot and cucumber) or five (onion and oats) plants per pot for each species
Exposure route:	Seeds were planted in test pots and the test substance was applied on the soil surface using a cabinet track sprayer. The pots were placed in a temperature supplemented greenhouse following planting. Temperature and relative humidity were recorded continuously.
Test conditions:	Photoperiod: ambient lighting only Temperature: 16.4 – 26.9 °C Relative humidity: 44.6 – 77.7 %
Test parameter:	Plants were assessed for survival, emergence and for phytotoxicity on Day's 14

and 21. Total foliar fresh weight (biomass per pot above the soil level) was measured immediately after harvest at 21 days after application.

Statistics : Statistical analyses were carried out using the statistical software ARM Gylling software.

Findings:

Analytical measurements: The mean measured value of ethofumesate was about 107 % of nominal and indicated that the desired application rates were achieved.

Biological results: Considering the plant emergence the carrot was identified to be the most sensitive species. At the highest test concentration of 4.0 L prod./ha only 55% of the total seeds sown emerged. The percentage of emergence for the other test plants at the highest test rate ranged between 72% (pea) and 100% (sugar beet). The most phytotoxic effects were observed in the test plants oat, cucumber, field bean and carrot.

The most sensitive plant species was observed to be the dicotyledonous species cucumber, carrot and field bean and the monocotyledonous species oat. The lowest endpoint was determined to be EC₅₀ 0.328 L prod./ha based on effects of the foliar fresh weight of oat.

Table B. 9.11.2-3: Emergence (percentage of total seeds sown)

Rate [L prod./ha]	Oilseed rape ^d	Pea ^d	Sugar beet ^d	Field bean ^d	Carrot ^d	Cucumber ^d	Onion ^m	Oat ^m
Control	11	83	100	94	100	89	73	83
0.03125	27	83	100	89	77	100	90	70
0.0625	27	83	100	94	89	89	76	90
0.125	33	83	94	94	77	94	80	90
0.25	44	72	100	94	89	94	66	90
0.5	27	94	94	100	83	100	86	90
1.0	27	72	94	83	66	94	66	76
2.0	44	77	94	100	55	94	73	86
4.0	33	72	100	89	55	89	73	93

m...monocotyledonous, d...dicotyledonous

The oilseed rape failed to achieve 65% emergence in the untreated control. Hence, the results for rape were not included in the assessment and are included for information only.

Apart from the dicotyledonous species oilseed rape no adverse effects on emergence greater than 50% were observed. Hence, the EC₅₀ for all tested plant species is greater than 4.0 L prod./ha (based on seedling emergence).

Table B. 9.11.2-4: Foliar fresh weight (percentage of untreated control)

Rate [L prod./ha]	Oilseed rape ^d	Pea ^d	Sugar beet ^d	Field bean ^d	Carrot ^d	Cucumber ^d	Onion ^m	Oat ^m
Control	-	-	-	-	-	-	-	-
0.03125	29	10	85	93	100	100	97	85
0.0625	43	99	89	100	100	100	93	100
0.125	100	95	80	99	100	100	100	100
0.25	100	100	89	100	100	100	98	88
0.5	100	94	88	100	100	100	100	47
1.0	32	72	81	76	86	66	82	6
2.0	100	84	70	69	100	27	87	0
4.0	100	46	72	38	29	19	72	0

m...monocotyledonous, d...dicotyledonous

Under consideration of the effects on the foliar fresh weight the most sensitive plant species was identified to be the monocotyledonous plant oat. In addition, the dicotyledonous plants field bean, carrot and cucumber were observed to be sensitive.

Table B. 9.11.2-5: EC₅₀ values base on effects on foliar fresh weight

Test species	EC ₅₀ [L prod./ha]
Carrot ^d	~ 4.0
Cucumber ^d	2.278
Oilseed rape ^d	- ^a
Oat ^m	0.328
Onion ^m	> 4.0
Pea ^d	~ 4.0
Sugar beet ^d	> 4.0
Field bean ^d	~ 4.0

m...monocotyledonous, d...dicotyledonous

^a Oilseed rape failed to achieve 65% emergence in the untreated control.**Conclusions:**

The most sensitive species was identified to be the monocotyledonous plant oat with an EC₅₀ of 0.328 L prod./ha. Other sensitive plant species were identified to be cucumber, carrot and field beans.

Comment RMS:

The seedling emergence test was conducted according to the OECD test guideline 208 A (July 2000). In order for the test to be considered valid, the following performance criteria must be met in the negative control:

- a seedling emergence of at least 65% has to be observed and
- the mean seedling growth does not exhibit visible phytotoxic effects.

In the test a seedling emergence of > 65% was observed for almost all test plants. Only the oilseed rape failed to achieve 65% emergence in the untreated control. Hence, the results for oilseed rape were not included in the assessment and are

included for information only.

In the control groups no phytotoxic effects were observed.

The test is considered acceptable as the validity criteria are met.

Taking into account the current valid OECD test guideline 208 (July 2006) the following validity criteria are given:

- The seedling emergence in the controls is at least 70%
- In the controls the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.
- In the controls the mean plant survival is at least 90% for the duration of the study.
- In the controls environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

The seedling emergence in the control groups is at least 70% at test termination, except for oilseed rape which is not included in the assessment.

For the duration of the test no visible phytotoxic effects were observed in the controls.

The plant survival of all tested plant species was at least 90% for the duration of the test. Plant mortality (< 10%) in the control groups was observed for the plant species pea, carrot and onion.

The environmental conditions and the used test substrate are identical for a particular species.

Taking into account the validity criteria given in the OECD guideline the study is considered acceptable.

B.9.11.3. Extended laboratory studies on non-target plants

Based on the risk assessment on non-target terrestrial plants indicating an acceptable risk, further testing on non-target plants is not required.

B.9.11.4. Semi-field and field tests on non-target plants

Based on the risk assessment on non-target terrestrial plants indicating an acceptable risk, further testing on non-target plants is not required.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Seedling emergence and vegetative vigour studies have been conducted with Ethofol following OECD guideline 208 A and B (Bramby-Gunary, 2004ab). Each study included 8 species which were tested at application rates of up to 2 L prod./ha (Bramby-Gunary, 2004a) and at an application rate of up to 4 L prod./ha (Bramby-Gunary, 2004b), respectively.

The findings from these studies are summarised in the table B.9.12-1.

Table B.9.12-1: Summary of non-target plant tests performed with the formulation Ethofol 500 SC

Test organisms	Study type	Test duration	Lowest ER ₅₀ [L prod./ha]	Most sensitive species	Reference
Terrestrial non-target plants (8 species)	Vegetative vigour (Tier 2)	21 days	ER ₅₀ ~ 2.0	Field bean (foliar fresh weight)	Bramby-Gunary, J., 2004a
Terrestrial non-target plants (8 species)	Seedling emergence (Tier 2)	21 days	ER ₅₀ = 0.328	Oat (foliar fresh weight)	Bramby-Gunary, J., 2004b

The risk assessment is based on the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final, 2002).

The representative formulation is applied on sugar beet and fodder beet at a maximal seasonal application rate of 2.0 L prod./a (corresponding to 1.0 kg ai/ha). The number of applications per season is between 1 (pre-emergence) and 6 (post-emergence) applications.

The corresponding off-field predicted environmental rates (PER) for three different use patterns are presented in the table below.

Table B.9.12-2: Predicted environmental rates (PER) at 1 m distance from the edge of field

Crop	Timing of application	No. of applications	Single application rate	Drift	MAF	PER _{off-field} (at 1 m distance)
Sugar beet, fodder beet	Pre-emergence	1	2.0 L prod./ha	2.77%	1.0	0.0554 L prod./ha
	BBCH until 18	1	2.0 L prod./ha	2.77%	1.0	0.0554 L prod./ha
		3	0.666 L prod./ha	2.01%	2.7	0.0361 L prod./ha
		6	0.333 L prod./ha	1.64%	4.6	0.0251 L prod./ha

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. For a single application to sugar beet and fodder beet, 2.77% of the full application rate of 2.0 L prod./ha (corresponding to 1.0 kg ai/ha) are assumed to reach areas at 1 m from the edge of the crop, respectively. The amount of spray drift from one application reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA (2000) from spray-drift predictions of Ganzelmeier & Rautmann (2000).

The risk assessment based on a single application of the maximal application of 2.0 L prod./ha is considered to cover also the multiple applications as the PER_{off-field} considering a single application is higher than those for multiple applications.

B.9.12.1. Deterministic risk assessment

The following risk assessment is based on the findings of the seedling emergence study. The lowest endpoint obtained from this study is 0.328 L prod./ha (foliar fresh weight, ER₅₀ of oat).

According to the Terrestrial Guidance Document, the risk to non-target terrestrial plants is assessed by comparing the exposure in field margins caused by drift with the lowest ER₅₀ obtained from the non-target plant studies. An assessment factor of 5 is required in order to prove safe use.

Table B.9.12-3: Deterministic risk assessment based on the lowest ER₅₀

Distance	Drift rate	Drift reducing nozzles	PER _{off-field} [L prod./ha]	Toxicity [L prod./ha]	TER
1 m	2.77%	-	0.0554	ER ₅₀ = 0.328	5.92
		50%	0.0277		11.84
		75%	0.01385		23.68
		90%	0.00554		59.21
5 m	0.57%	-	0.0114		28.77

According to the results of the deterministic approach involving the most sensitive endpoint in the seedling emergence study the risk to non-target plants is considered acceptable at distance of 1 m. No risk mitigation measures are required to refine the risk to terrestrial plants in the off-field.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No other studies on other non-target species are required.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not required, as no additional studies were submitted addressing the risk to other terrestrial organisms.

B.9.15. REFERENCES RELIED ON

Annex point	Author(s)	Year	Title Source (where different from company) Company name, Report no., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.1.1.1		1998	Acute Oral Toxicity study with Ethofumesate 500 SC (Ethofumesate: 500 g/L) in Japanese Quails United Phosphorus Ltd., 2296/97-AOQ GLP: yes Published: no	Y	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.2.1	Hoffmann, K., Deierling, T.	2010	Toxicity of Ethofol 500 SC (HBX01) to the Aquatic Plant <i>Lemna gibba</i> in a Static Growth Inhibition Test United Phosphorus Ltd., 56172240 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.2.1		1997	Acute Toxicity of Ethofumesate 500 SC (500 g/L) against <i>Oncorhynchus mykiss</i> AgroDan, 5548 GLP: yes Published: no	Y	Y	New data for existing formulation, not previously submitted nor evaluated	AGR*	Submitted for the purpose of renewal (2014)
KCP 10.2.1	Pors, J.	1997a	The Effect of Ethofumesate 500 SC on the <i>Daphnia magna</i> acute test AgroDan, 5591 Hedeselskabet 's Laboratory, DK-8800 Viborg, Denmark GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	AGR*	Submitted for the purpose of renewal (2014)
KCP 10.2.1	Scheerbaum, D.	2013	Phytotoxicity Test with the Rooted Submerge Aquatic Macrophyte <i>Myriophyllum aquaticum</i> , Static, 7 - 10 d United Phosphorus Ltd., SMA15459 DR.U.NOACK-LABORATORIEN, Sarstedt, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.2.1	Ruymen, V.	2003	Alga growth inhibition test with Ethofumesate 500 g/L SC LISEC nv, Genk, Belgium, Study no. WE-06-391 GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.3.1.1.1	Mallikarjunappa, S.	1998	Honeybee - Acute oral Toxicity test with Ethofumesate 500 SC (Ethofumesate 500 g/L) United Phosphorus Ltd., 2295/97 Rallis Research Centre, Bangalore, India GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.3.1.1.2	Schmitzer, S.	2010	Effects of Ethofol 500 SC (HBX01) (Acute Contact Test) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory United Phosphorus Ltd., 56173035 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)

Annex point	Author(s)	Year	Title Source (where different from company) Company name, Report no., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.3.1.2	Kleebaum, K.	2014	Chronic toxicity of Ethofol 500 SC to the honeybee <i>Apis mellifera</i> L. under laboratory conditions United Phosphorus Ltd., 13 10 48 017 B BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.3.1.3	Schmitzer, S.	2012	Study on the effects of Ethofol 500 SC (HBX01) on honey bee brood (<i>Apis mellifera</i> L.) - Brood Feeding Test - United Phosphorus Ltd., 63831031 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.3.2.1	Moll, M.	2011	Effects of Ethofol 500 SC on the parasitoid <i>Aphidius rhopalosiphii</i> in the laboratory - Dose Response Test - United Phosphorus Ltd., 63551001 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.3.2.1	Schwarz, A.	2011	Effects of Ethofol 500 SC on the predatory mite <i>Typhlodromus pyri</i> in the laboratory -Dose Response Test- United Phosphorus Ltd., 19.04.2011-30.05.2011, 63552063 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.4.1.1	Lühns, U.	2011a	Effects of Ethofol 500 SC on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat United Phosphorus Ltd., 63554022 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.4.1.1	Stäbler, D.	2002	Ethofol 500 SC: Assessment of effects on reproduction and growth on <i>Eisenia fetida</i> in artificial soil United Phosphorus Ltd., 20021052/01-NREf GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.4.1.1	Witte, B.	2013	Effects of Ethofol 500 SC on reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 5% Peat (Spraying Application) (including amendment) United Phosphorus Ltd., 81041022 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)

Annex point	Author(s)	Year	Title Source (where different from company) Company name, Report no., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.4.2.1	Ganßmann, M.	2012	Effects of Ethofumesate 500 g/L SC on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat AgriChem B.V., 74803089 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCP 10.4.2.1	Lührs, U.	2011b	Effects of Ethofol 500 SC on reproduction of the collembola <i>Folsomia candida</i> in artificial soil with 5% peat United Phosphorus Ltd., 63553016 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.5	Feil, N.	2010	Effects of Ethofol 500 SC (HBX01) on the Activity of the Soil Microflora in the Laboratory United Phosphorus Ltd., 56176080 IBACON GmbH, Rossdorf, Germany GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.6.2	Bramby-Gunary, J.	2004a	Evaluation of the Phytotoxicity of Ethofol formulation (500 g/L Ethofumesate) (Based on OECD Guideline 208 B) Vegetative Vigour Test Terrestrial Non Target Plants - GLP Study United Phosphorus Ltd., ACE-04-075 Agrochemex Ltd, Manningtree, Essex, UK GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCP 10.6.2	Bramby-Gunary, J.	2004b	Evaluation of the Phytotoxicity of Ethofol formulation (500 g/L Ethofumesate) (Based on OECD Guideline 208 A) Seedling emergence Terrestrial Non Target Plants - GLP Study United Phosphorus Ltd., ACE-04-074 Agrochemex Ltd, Manningtree, Essex, UK GLP: yes Published: no	N	Y	New data for existing formulation, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)

*Studies conducted for AgroDan or AgriChem are now owned by United Phosphorus Ltd.