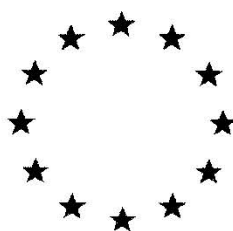


European Commission



**Draft Renewal Assessment Report prepared according to the Commission
Regulation (EU) N° 1107/2009**

ETHOFUMESATE

**Volume 3 – B.9 (PPP) – ETHOFUMESATE SC
500**

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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 Directive 91/414/EEC has been repealed by Regulation (EC) No 1107/2009 of 21 October 2009 concerning the placing of plant protection products on the market.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).

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 (study title is greyed out).

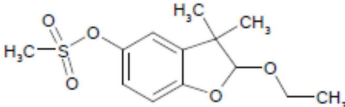
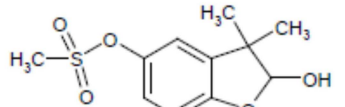
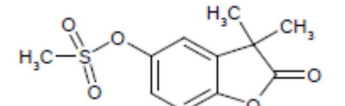
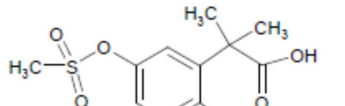
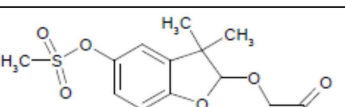
Studies which were submitted for the first EU peer-review of the active substance ethofumesate but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are 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. The herbicidal activity of the two enantiomers has been shown to be equivalent and not different from the racemic mixture. 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. 9.1.1-1: 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, AE C508493, BCS-BB94377	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate	Animals: Rat, lactating cow, laying hen Plants: Sugar beet, ryegrass, CRC Soil: Soil aerobic, soil anaerobic Water: Photolysis in water	
Ethofumesate-lactone Synonym: NC 9607, AE C509607	2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulfonate	Animals: Rat, lactating cow, laying hen Plants: Sugar beet, ryegrass, CRC Soil: Soil aerobic, soil anaerobic	
Ethofumesate-carboxylic acid Synonym: NC 20645, AE C520645, BCS-AV65501 ----- AE C639175 (potassium salt) BCS-CU88901 (sodium salt)	2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methyl propionic acid	Animals: Rat, lactating cow, laying hen Plants: Sugar beet, onion, tobacco, ryegrass, CRC Soil: Soil aerobic, soil anaerobic Water: Water/sediment, aerobic mineralization in surface water	
Ethofumesate-acetic acid Synonym: BCS-CW35117	({3,3-dimethyl-5-[(methylsulfonyl)oxy]-2,3-dihydro-1-benzofuran-2-yl}oxy)acetic acid	Water: Aerobic mineralization in surface water	

CRC...rotational crops

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. 9.1.1-2: 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, red beet	BBCH 16-18	1-3	5	0.4 – 2.0	0.2 – 1.0 ¹

¹ The maximum amount of active substance must not exceed 1.0 kg/ha every 3 year

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 re-newal of the EU peer-review. The study summaries are provided under point B.9.1.1 of Volume 3 – B.9 (AS).

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 mammals

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

Due to animal welfare reasons, no additional study with the current formulation was conducted in birds. However, a formulation study is available on rats, indicating that the formulation does not pose additional risk to

vertebrates (for further information, reference is made to point 9.1.2). Therefore, the risk assessment will be based on the active substance.

Endocrine disruption

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

Metabolites of ethofumesate

The metabolism of ethofumesate has been investigated in sugar beets and ryegrass. Main plant metabolites are NC 9607 and NC 20645 (ethofumesate-lactone and ethofumesate-carboxylic acid (including its conjugate)). Both metabolites have been identified also in animal metabolism studies and thus are considered as covered by the toxicological studies performed with the parent molecule.

From their molecular structure a specific toxicological concern would not be discernible. It is therefore concluded that these metabolites are not of higher or more critical toxicity than the parent compound and a specific risk assessment for birds would not be indicated.

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 re-newal 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 re-newal 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 re-newal 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	██████, 1980
Rabbit	Teratogenicity study	NOAEL = 300 mg ai/kg bw	██████, 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 Ethofumesate SC 500 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 > 2100 mg prod./kg bw	1989

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:

The metabolism of ethofumesate has been investigated in sugar beets and ryegrass. Main plant metabolites are NC 9607 and NC 20645 (ethofumesate-lactone and ethofumesate-carboxylic acid (including its conjugate)). Both metabolites have been identified also in animal metabolism studies and thus are considered as covered by the toxicological studies performed with the parent molecule. From their molecular structure a specific toxicological concern would not be discernible. It is therefore concluded that these metabolites are not of higher or more critical toxicity than the parent compound and a specific risk assessment for mammals would not be indicated.

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).

Ethofumesate 500 SC is intended to be applied as an herbicide in sugar-, red and fodder beets 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 risk assessment is based on the worst-case application rate of 1 x 1000 g ai/ha. This use pattern is considered to cover also the possible multiple applications, e.g. 2 x 500 g ai/ha and 3 x 333 g ai/ha.

B.9.2.1. Risk assessment for birds

With regard to the relevant scenarios small insectivorous, small omnivorous and small granivorous birds (see Table B.9.2.1-1) 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. 9.2.1-1: Relevant generic avian focal species for the Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				RUD ₉₀	RUD _m
Sugar beet	Early (spring) BBCH 10-19	Small omnivorous bird "lark"	Woodlark	24.0	10.9
	BBCH 10 – 19	Small insectivorous bird "wagtail"	Yellow wagtail	10.9	5.9
Root & stem vegetables	BBCH 10 – 39	Small granivorous bird "finch"	Linnet	24.7	11.4
	BBCH 10 – 39	Small omnivorous bird "lark"	Woodlark	24.0	10.9
	BBCH 10 - 19	Small insectivorous bird "wagtail"	Yellow wagtail	26.8	11.3

Bold values are used for the risk assessment. Shortcut values from "root & stem vegetables" were used, as they are equal or higher compared to the short values for sugar beet and therefore represent a worst-case.

Acute risk assessment for birds:

The acute risk assessment is based on the endpoints derived from acute toxicity studies with bobwhite quail (■■■■■ 1990b) and mallard duck (■■■■■ 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 LD_{50 extrapolated} of 3776 mg ai/kg bw.

Table B. 9.2.1-2: Tier 1 acute risk assessment for birds

Crop	Generic focal species	SV ₉₀	Application rate [kg ai/ha]	MAF ₉₀	DDD _A	LD ₅₀ [mg ai/kg bw]	TER _A
Sugar beet, root & stem vegetables	Small omnivorous bird “lark”	24.0	1.0	1.0	24.0	3776	157
	Small insectivorous bird “wagtail”	26.8			26.8		141
	Small granivorous bird “finch”	24.7			24.7		153

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 bobwhite quail (■■■■■ 2001) and mallard duck (■■■■■, 2000).

The acute oral LD₅₀ value used in the acute avian assessment (LD_{50 interpol.} = 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: Tier 1 long-term risk assessment for birds

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, root & stem vegetables	Small omnivorous bird “lark”	10.9	1.0	1.0	0.53	5.8	265	45.7
	Small insectivorous bird “wagtail”	11.3				6.0		44.2
	Small granivorous bird “finch”	11.4				6.0		44.2

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 16 – 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 ($K_{oc} < 500 \text{ L/kg}$).

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 ($BCF = 67 - 144$) 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)
	2.69 (pH = n.s., 20°C)	Mueller, 1990
Metabolite NC 8493	1.5 (pH = 5 – 9, 25°C)	Bogdoll & Peschke, 2012
Metabolite NC 9607	2.2 (pH = 5 – 9, 25°C)	Bogdoll & Peschke, 2012
Metabolite NC 20645	0.4 (pH 5) - 1.4 (pH = 7) - 2.4 (pH = 9), 22°C mean	Ziemer & Kloeckner, 2012
Metabolite BCS-CW35117	0.2 (pH 5) - 1.3 (pH = 7) - 1.6 (pH = 9), 23°C mean	Eyrich & Ziemer, 2013

n.s...not stated

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 (post-emergence).

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} (initial) [mg ai/kg]	1.069
BCF _{worm}	2.18
PEC _{worm} [mg ai/kg]	2.33
Daily dose [mg ai/kg bw/d]	2.45
TER	108
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 generic mammalian focal species for the Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				RUD ₉₀	RUD _m
Sugar beet	BBCH 10-19	Small insectivorous mammal “shrew”	Common shrew	7.6	4.2
	BBCH 10-39	Large herbivorous mammal “lagomorph”	Rabbit	35.1	14.3
	BBCH 10-39	Small omnivorous mammal “mouse”	Wood mouse	17.2	7.8
Root & stem vegetables	BBCH 10-19	Small insectivorous mammal “shrew”	Common shrew	7.6	4.2
	BBCH 10-39	Small omnivorous mammal “mouse”	Wood mouse	17.2	7.8

Bold values are used for the risk assessment. Shortcut values from “sugar beet” were used, as they are equal or higher compared to the short values for root & stem vegetables and therefore represent a worst-case.

Acute risk assessment for mammals:

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

Table B.9.2.2-2: Tier 1 acute risk assessment for mammals

Crop	Generic focal species	SV ₉₀	Application rate [kg ai/ha]	MAF ₉₀	DDD _A	LD ₅₀ [mg ai/kg bw]	TER _A
Sugar beet, root & stem vegetables	Small insectivorous mammal “shrew”	7.6	1.0	1.0	7.6	> 5000	> 658
	Large herbivorous mammal “lagomorph”	35.1			35.1		> 142
	Small omnivorous mammal “mouse”	17.2			17.2		> 29

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:

Table B.9.2.2-3: 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, root & stem vegetables	Small insectivorous mammal “shrew”	4.2	1.0	1.0	0.53	2.23	60.9	27.3
	Large herbivorous mammal “lagomorph”	14.3				7.58		8.0
	Small omnivorous mammal “mouse”	7.8				4.13		14.7

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

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.9.2.2-4: 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 > 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 ($BCF = 67 - 144$) 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.

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]	1.069
BCF _{worm}	2.18
PEC _{worm} [mg ai/kg]	2.33
Daily dose [mg ai/kg bw/d]	2.98
TER	20
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:

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

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 0.142 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

In addition to the acute toxicity studies with the active substance ethofumesate and its metabolites (see RAR, Volume 3, B.9. (A.S.)) studies with the EU representative formulation Ethofumesate 500 g/L SC (other name: Trammat 500 SC) were conducted with fish, aquatic invertebrates, algae and aquatic plants.

The study summaries are given below.

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

Reference:	Tramat® 500 – Determination of acute toxicity (LC₅₀) to mirror carp (96 h, semi-static) and the analysis of ethofumesate in water samples
Author(s), year:	██████████ 1989
Report/Doc. number:	Study no. A83350, Reference no. M-155619-01-1
Guideline(s):	None
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Tramat @ 500, batch no.: CR 18654/2, purity: 484.7 g ai/L
Test species:	Mirror carp (<i>Cyprinus carpio</i>)
Holding of fish :	All fish were acclimatised to laboratory conditions for at least 12 days prior to initiation of the study.
	Feeding of fish: Daily
Number of organisms:	2 replicates per treatment and control, 5 fish per replicate
Age, length, weight:	Length: 41 – 49 mm, Weight: 1.532 – 2.185 g
	Fish are of the same age, but no specific age is given in the study report
Loading	< 1.0 g/L fish loading per test vessel
Type of test:	Semi-static, 96 h, fish were transferred to freshly prepared tanks at 24 hour intervals.

Applied concentrations:

Nominal:	0 (control), 12.5, 25, 50, 100 and 200 mg prod./L
	0 (control), 5.53, 11.1, 22.1, 44.2 and 88.4 mg ai/L (based on content of ai: 44.2%)
Measured (mean):	Not given
Solvent:	None

Test conditions:

Water quality:	Charcoal-filtered dechlorinated tap water, conductivity: 0.21 – 0.24 mS, total hardness: 80 – 84 mg/L as CaCO ₃
Temperature:	21.1 – 24.8 °C

pH:	8.2 – 8.3
O ₂ content:	79 – 92% of air saturation (> 60% throughout the test)
Light regime:	Light/dark cycle of 16/8
Feeding	The fish were not fed for the period 24 hours before the initiation of the test nor throughout the duration of the test.
Methods:	25 L vessels (all glass) with 20 L test medium were used. The test substance was added directly to the vessels.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 3, 6, and 24 hours and thereafter daily up to 96 hours. At the end of the test each fish was weighed and measured. Measurements of temperature, pH, conductivity and dissolved oxygen were made daily in all treatment solutions.
Analytical measurements:	Samples of water were removed from each tank at 0, 24, 48, 72 and 96 hours. Concentrations of ethofumesate in water samples were determined using the HPLC-method.
Statistics:	The LC ₅₀ at 96 hours was determined using probit analysis.
<u>Findings:</u>	
Analytical data:	The test material dispersed well in all test tanks, being a visible suspension at 100 and 200 mg prod./L. The measured concentrations of ethofumesate were in a range of 14 – 98% of the nominal test concentrations. Hence, the results should be based on mean measured concentrations.

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

Test concentration [mg prod./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)
12.5	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
25.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
50.0	10 (1/10) ^a	10 (1/10)	10 (1/10)	10 (1/10)	10 (1/10)
100	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
200	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 64.1 mg prod./L (95% C.I. = 49 – 87 mg prod./L)					
96 h NOEC = 25 mg prod./L (based on behavioural effects and mortality)					

^a Loss of equilibrium and periods spent motionless on the floor of the test tanks.

Conclusion: No mortalities or sublethal effects were recorded at test concentrations equal and up to 25 mg prod./L. Hence, the NOEC was 25 mg prod./L. The LC₅₀ was determined to be 64.1 mg prod./L based on nominal concentrations.

<u>Comment RMS:</u>	<p>The study was conducted according to no given test guideline, but is in line with the current valid OECD test guideline 203 (1992).</p> <p>Taking into account the validity criteria given in the test guideline according to OECD (1992) the acute fish study is considered acceptable.</p> <p>The mortality in the control was below 10 % (being: 0%) and the environmental conditions (dissolved oxygen, temperature, pH,...) were maintained throughout the test.</p> <p>The LC₅₀ is based on nominal test concentrations, even though the measured concentrations were below 80% of the nominal test concentrations.</p> <p>Hence, the applicant submitted a re-evaluation of the toxicity endpoint based on mean measured concentrations.</p> <p>Over the whole exposure period mean measured concentrations of 3.4, 5.7, 12.8, 31.8, 35.7 mg ai/L were determined.</p> <p>Under consideration of the mean measured concentrations the LC₅₀ was recalculated using the software ToxRat® (based on logit analysis). The 96 h LC₅₀ was determined to be 14.43 mg ai/L based on mean measured concentrations.</p> <p>The study is considered acceptable, however the recalculated endpoint based on mean measured concentrations should be used in the risk assessment.</p>
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Reference:	The acute toxicity of Norton 50 SC (ethofumesate) to zebra fish (<i>Brachydanio rerio</i>)
Author(s), year:	██████████ 1988
Report/Doc. number:	Study no. A83338, Reference no. M-155607-01-1
Guideline(s):	OECD test guideline 203 (1984)
GLP:	Yes
Deviations:	None
Validity:	Additional information

Material and methods:

Test substance:	Norton 50 SC, batch no.: CR 18654/4, purity: 500 g ai/L
Test species:	Zebra fish (<i>Danio rerio</i> , formerly known as <i>Brachydanio rerio</i>)
Holding of fish :	All fish were acclimatised to laboratory conditions for at least 7 days prior to initiation of the study.
	Temperature: 20 ± 1 °C, dissolved oxygen ≥ 8.9 mg O ₂ /L
	Feeding of fish: Daily
Number of organisms:	10 fish per treatment and control

Age, length, weight:	Length: 3.2 cm (SD = 0.2 cm), Weight: 0.71 g (SD = 0.13 g) Fish are of the same age, but no specific age is given in the study report
Loading	0.36 g/L fish loading per test vessel
Type of test:	Semi-static, 96 h, daily renewal
Applied concentrations:	
Nominal:	0 (control), 10, 18, 32, 56 and 100 mg prod./L 0 (control), 5.0, 9.0, 16, 32 and 50 mg ai/L
Measured (mean):	Not given
Solvent:	None
Test conditions:	
Water quality:	Laboratory dechlorinated tap water, total hardness: 350 mg/L as CaCO ₃
Temperature:	21 ± 1 °C
pH:	8.3 – 8.4
O ₂ content:	8.1 – 8.9 mg O ₂ /L (> 60% throughout the test)
Light regime:	Light/dark cycle of 16/8
Feeding	The fish were not fed for the period 24 hours before the initiation of the test nor throughout the duration of the test.
Methods:	Glass vessels with 20 L test medium were used. The test substance was added directly to the vessels.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 3, 6, and 24 hours and thereafter daily up to 96 hours. At the end of the test each fish was weighed and measured. Measurements of temperature, pH, conductivity and dissolved oxygen were made daily in all treatment solutions.
Analytical measurements:	Samples of water were removed from each tank at 0, 24 and 96 hours. Concentrations of ethofumesate in water samples were determined using the HPLC-method.
Statistics:	The LC ₅₀ at 96 hours was determined by the method of Thompson & Weil (1952).
<u>Findings:</u>	
Analytical data:	The mean measured concentrations of ethofumesate were in a range of 83.6 and 99.2% of the nominal test concentrations. Hence, the results are based on nominal concentrations.

Table B. 9.3.1-2: Mortality and sub-lethal effects

Test concentration [mg prod./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)
10	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
18	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)

Test concentration [mg prod./L]	Mortality [%] (no. of dead fish / no. of treated fish)				
	3 h	24 h	48 h	72 h	96 h
32	0 (0/10) ^a	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
56	0 (0/10) ^{bcd}	0 (0/10) ^{ad}	20 (2/10) ^{bd}	20 (2/10) ^{ab}	20 (2/10) ^a
100	40 (4/10) ^d	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 68 mg prod./L (95% C.I. = 58 - 80 mg prod./L)					
96 h NOEC = 18 mg prod./L (based on behavioural effects)					

^a Lethargy, ^b Loss of equilibrium, ^c Lying on the bottom, ^d Moribund

Conclusion:

No mortalities or sublethal effects were recorded at test concentrations equal and up to 18 mg prod./L. Hence, the NOEC was 18 mg prod./L. The LC₅₀ was determined to be 68 mg prod./L based on nominal concentrations.

Comment RMS:

The study was conducted according to the OECD test guideline 202 (1984).

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.

The LC₅₀ is based on nominal test concentrations. The information on the analytical measurements given in the study report is not sufficient to calculate mean measured concentrations. According to the analytical measurements given in the study report the mean measured concentrations were in a range of 83.6% and 99.2% of nominal test concentrations. Hence, the results are based on nominal test concentrations.

The study is considered valid and acceptable for the use in the risk assessment.

Reference:	Tramat ® 500 – Determination of acute toxicity (LC₅₀) to Daphnia (48 hours, static) and the analysis of ethofumesate in water samples
Author(s), year:	Cameron, B.D., et al., 1989
Report/Doc. number:	Study no. A83348, Reference no. M-155617-01-1
Guideline(s):	None
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Tramat ® 500, batch no.: CR 18654/2, purity: 484.7 g ai/L
Test species:	Water flea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates per treatment and control, each replicate containing 5 daphnids
Age:	First instar, 6 - 24 h old
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control), 31.25, 62.5, 125, 250, 500 and 1000 mg prod./L 0 (control), 13.8, 27.6, 55.3, 110.5, 221 and 442 mg ai/L (based on ai content: 44.2%)
Mean measured:	Not given
Solvent:	None
Test conditions:	
Water quality:	Daphnia synthetic medium according to the formula for hard water recommended by the US EPA (1975), conductivity: 0.48 – 0.58 mS, total hardness: 160 mg/L as CaCO ₃
Temperature:	20.0 – 21.9 °C
pH:	8.5 (0 h), 8.3 – 8.5 (48 h)
O ₂ content:	56 – 72% of air saturation
Light regime:	16 hours light / 8 hours darkness
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, conductivity and dissolved oxygen concentrations were made at the start (0 h), at 24 and 48 h.
Analytical measurement:	Samples (duplicates) of water were removed from each tank at 0, 24, and 48 hours after test start. The concentration of ethofumesate in each was determined using the HPLC-method.
Statistics:	The LC ₅₀ at 24 and 48 hours were determined using probit analysis.

Findings:

Analytical measurements:	A white precipitate was observed at nominal test concentrations between 250 and
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1000 mg prod./L based on the low solubility of the active substance in water.

The measured concentrations were in range of 10 and 73% of the nominal test concentrations. Hence, the results should be based on mean measured concentrations.

Table B. 9.3.1-3: Effects on daphnids (*D. magna*) exposed to Trammat ® 500

Ethofumesate [mg prod./L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
31.25	0	0
62.5	0	15
125	15	85
250	20	95
500	40	100
1000	90	100
48 h EC ₅₀ = 93.2 mg prod./L (95 % C.I. = 76.3 and 112.8 mg prod./L) 48 h NOEC = 31.25 mg prod./L (based on immobilisation)		

Conclusion:

The acute toxicity of formulated ethofumesate to *Daphnia magna* has been investigated. The 48-hour EC₅₀ was calculated as 93.2 mg prod./L based on nominal concentrations.

Comment RMS:

The study was conducted according to no given test guideline, but is in line with the current valid OECD test guideline (OECD 202, 2004). The validity criteria given in the test guidelines according OECD (202, 2004) are met.

The immobilisation in the control group was below 10% (being: 0%). The dissolved oxygen concentration was observed to be greater than 60% at the test start and 24 h after test start. However, at the end of the study the dissolved oxygen concentration in the treatment groups between 62.5 and 1000 mg prod./L was determined to be below 60% of air saturation (being: 56 – 58%). This is only a slight deviation to the test guideline and considering the results of the study does not have an impact on the outcome of the study.

Based on the validity criteria the study is considered valid.

The results are based on nominal concentrations. However, the mean measured concentrations of ethofumesate were observed to be < 80% of the nominal concentrations. Hence, the results should be based on mean measured concentrations.

The RMS conducted a re-calculation of the statistical analysis based on mean measured concentrations.

The mean measured concentrations (9.3, 19.57, 40.26, 43.68, 45.3 and 47.82 mg ai/L) were determined to be between 11 and 73 % of the nominal concentrations. At the three highest test concentrations (125 – 500 mg ai/L nominal) the mean measured concentrations were nearly in the same range, between 43.7 and 47.8 mg ai/L.

Based on the mean measured concentration the toxicity endpoints were recalculated using the software ToxRat ® (based on probit analysis). The 48 h EC₅₀ was determined to be 26.8 mg ai/L (95% C.I. 22.9 – 30.2 mg ai/L) based on mean measured concentrations.

The NOEC was determined to be 19.57 mg ai/L based on effects on immobility.

The study is considered acceptable, however the recalculated endpoint based on mean measured concentrations should be used in the risk assessment.

Reference:	A study of the toxicity to algae of ethofumesate 50 SC
Author(s), year:	Knacker, T., 1989
Report/Doc. number:	Study no. A83342, Reference no. M-155611-01-1
Guideline(s):	OECD 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate 500 SC, batch no.: CR 18654/2, purity: 484.7 g ai/L
Test species:	Green algae (<i>Desmodesmus subspicatus</i> , formerly known as <i>Scenedesmus subspicatus</i>)
Number of organisms:	1 x 10 ⁴ cells/mL; 4 replicates per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (control), 0.447, 1.0, 2.236, 5.0, 11.18 and 25.0 mg prod./L
Mean measured:	Not given
Solvent:	None
Toxic reference:	Potassium dichromate (K ₂ Cr ₂ O ₇), E _r C ₅₀ = 0.9 mg/L and E _b C ₅₀ = 0.6 mg/L
Test conditions:	
Water quality:	Algal medium II (according OECD guideline), pH = 8.1
Temperature:	21 °C
pH:	7.3 – 9.8
Incubation:	Continuous illumination, universal white

Test parameters:	At the start of the test and after 18, 42, 66 and 90 h of exposure, the cell mass was measured spectrophotometrically. Temperature was measured continuously. The pH was measured at the test start and at the end of the test.
Analytical measurements:	Samples were taken from the test system and were analysed using GC/MS analyses.
Statistics:	To determine the highest concentration tested without statistically significant differences to the control (NOEC) the oneway analysis of variance (ANOVA) was applied to the data derived from the alga growth inhibition test. To calculate the EC values a probit transformation of the data was performed (Finney).

Findings:

Analytical data:	The mean measured concentrations of ethofumesate are in a range of 93 – 113% (0 h) and 95-114% (90 h) of nominal test concentrations. Hence, the results are based on nominal test concentrations.
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Table B. 9.3.1-4: Effects of Trammat 500 SC on the green alga *Desmodesmus subspicatus*

Tramat 500 SC [mg ai/L] (nominal)	Area under the growth curve		Average specific growth rates	
	Area	% inhibition relative to control	Growth rate [1/h]	% inhibition relative to the control
Control	2211.9	-	0.060267	-
0.447	2058.0	7.0	0.058294	3.3
1.0	2361.6	-6.8	0.060549	-0.5
2.236	2212.5	0.0	0.058669	2.7
5.0	1598.4 *	27.7	0.051654 *	14.3
11.18	263.1 *	88.1	0.019581 *	67.5
25.0	190.5 *	91.4	0.008485 *	85.9

* Statistically significant compared to the control, ANOVA, $p < 0.05$

Conclusion:

96 h $E_{rC_{10}}$ = 3.8 mg prod./L

96 h $E_{rC_{50}}$ = 9.7 mg prod./L

96 h E_bC_{10} = 2.6 mg prod./L

96 h E_bC_{50} = 6.7 mg prod./L

NOEC = 2.2 mg prod./L (biomass and growth rate)

based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 1984).

In general the study is in line with the stated test guideline as well as with the

current valid OECD test guideline (OECD 201, 2006).

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 (being: 23.1).

The results of the study are based on nominal test concentrations as the mean measured concentrations were determined to be in a range between 93 and 113% of the nominal concentrations.

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 the formulation ethofumesate SC 500A G (ethofumesate 500 g/L) to the aquatic macrophytes, <i>Myriophyllum spicatum</i>
Author(s), year:	Banman, C.S., 2012
Report/Doc. number:	Study no.: EBADL034-1, Reference no.: M-437702-02-1
Guideline(s):	Higher tier study based on OECD 221
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC 500A G, batch no.: ECED101300, purity: 44.3% w/w, CAS no.: 26225-79-6
Test species:	<i>Myriophyllum spicatum</i> (Eurasian water milfoil), rooted macrophytes
Number of organisms:	3 replicates per treatment groups and control groups, 5 plants per replicate (thinned to 3 shoots on day 0)
Type of test, duration:	Static, 14 days
Applied concentrations:	
Nominal:	0 (control), 0.012, 0.038, 0.122, 0.39 and 1.25 mg prod./L 0 (control), 0.0053, 0.017, 0.054, 0.17 and 0.55 mg ai/L
Measured (mean):	0 (control), 0.005, 0.014, 0.041, 0.16 and 0.60 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	UV filtered hard processed water
Sediment:	Artificial sediment according to OECD guideline 218, 20x AAP medium was used to wet the sediment (provide shoots with a fertilizer source). 5% pear, 20% kaolin and 75% quartz sand, pH = 7.55
Temperature:	19.54 – 22.98 °C
pH:	8.2 – 10.1

O ₂ content:	8.6 – 13.6 mg O ₂ /L
Light regime:	16 hours light, 8 hours dark, light intensity 10700 – 12380 lux (mean: 11968 lux)
Methods:	All plants within a replicate were planted into a single crystallization dish containing 550 g of artificial sediment. The rooted aquatic plants were submerged in 4-L beakers. Following a 7 day acclimation period, the five shoots in each replicate were thinned to three uniform appearing shoots. Remaining shoots were then exposed to the test solutions for 14 days. Following the 14 day exposure period plants were sacrificed and measured. All test vessels were contained in an environmentally controlled study area.
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 and oxygen concentration were measured at Day -7, 0, 7 and 14. Temperature was measured hourly.</p> <p>Light intensity was determined prior to start of the rooting phase.</p>
Analytical measurements:	Analytical evaluation of the test concentrations of ethofumesate and the control was carried out via LC-MS/MS on day 0, 7 and 14. Samples were taken from the sediment layer and the water layer.
Statistics:	The EC ₅₀ values were calculated based on linear interpolation. The NOEC was determined using ANOVA followed by the Dunnett's test. The Shapiro-Wilks test was used for normality and the Bartlett equality of variance test was used to test the homogeneity of variance.
<u>Findings:</u>	
Analytical data:	The mean measured concentrations in the water layer are between 76 and 109% of the nominal test concentrations. Hence, the results should be based on mean measured concentrations.
Morphological observations:	Plants in the control vessels and all treatment groups appeared normal throughout the study. At study termination plant shoots appeared normal in the controls and in the 0.012, and 0.038 mg prod./L treatment groups. In the 0.122, 0.39, and 1.25 mg prod./L treatment groups, the shoots were darker green, shorter and more crisp as compared to the controls.

Table B. 9.3.1-5: Mean yield for plant shoots, wet weights and dry weights, 14 d

Nominal concentration [mg prod./L]	Length		Wet weight		Dry weight	
	[cm]	% inhibition	[g]	% inhibition	[g]	% inhibition
Control	40.5	-	1.5531	-	0.1639	-
0.012	35.5	12.3	1.4258	8.2	0.1697	-3.5
0.038	26.3	35.2 *	1.3180	15.1	0.1519	7.3
0.122	18.7	53.8 *	1.0301	33.7 *	0.1486	9.3
0.39	17.3	57.4 *	0.9562	38.4 *	0.1329	18.9
1.25	6.0	85.1 *	0.9963	35.8 *	0.1725	-5.2

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

Table B. 9.3.1-6: Growth rate for plant shoots, wet weights and dry weight, 14 d

Nominal concentration [mg prod./L]	Length		Wet weight		Dry weight	
	[1/cm]	% inhibition	[1/g]	% inhibition	[1/g]	% inhibition
Control	0.1326	-	0.1088	-	0.0736	-
0.012	0.1290	2.7	0.1040	4.4	0.0753	-2.2
0.038	0.1119	15.6 *	0.0998	8.3	0.0701	4.7
0.122	0.0941	29.1 *	0.0857	21.2 *	0.0689	6.5
0.39	0.0936	29.4 *	0.0832	23.5 *	0.0644	12.6
1.25	0.0424	68.0 *	0.0851	21.8 *	0.0757	-2.9

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

Based on the results the following endpoints based on yield and growth rate were determined:

- Shoot length: 14 d E_yC_{50} = 0.105 mg prod./L (95% C.I. = 0.043 – 0.537 mg prod./L)
 14 d E_yC_{20} = 0.021 mg prod./L (95% C.I. = 0.0006 – 0.038 mg prod./L)
 14 d E_rC_{50} = 0.848 mg prod./L (95% C.I. = 0.6602 – 0.9427 mg prod./L)
 14 d E_rC_{20} = 0.065 mg prod./L (95% C.I. = 0.0057 – 0.1392 mg prod./L)
 14 d NOEC = 0.012 mg prod./L (yield and growth rate)
- Wet weight: 14 d E_yC_{50} > 1.25 mg prod./L
 14 d E_yC_{20} = 0.0594 mg prod./L (95% C.I. = 0.0228 – 0.2228 mg prod./L)
 14 d E_rC_{50} > 1.25 mg prod./L
 14 d E_rC_{20} = 0.1141 mg prod./L
 14 d NOEC = 0.038 mg prod./L (yield and growth rate)
- Dry weight: 14 d E_yC_{50} > 1.25 mg prod./L
 14 d E_yC_{20} > 1.25 mg prod./L
 14 d E_rC_{50} > 1.25 mg prod./L
 14 d E_rC_{20} > 1.25 mg prod./L
 14 d NOEC > 1.25 mg prod./L (yield and growth rate)

Conclusion:

The lowest E_yC_{50} and E_rC_{50} in the 14 d exposure of formulated ethofumesate to the rooted macrophytes *Myriophyllum spicatum* was shoot length. The statistical EC_{50} for this endpoint was 0.105 mg prod./L (based on yield) and 0.848 mg prod./L (based on growth rate) based on nominal test concentrations.

Comment RMS:

The study was conducted according to the OECD test guideline 221. No validity criteria are given in the OECD test guideline. The draft OECD test guideline “Water-sediment *Myriophyllum spicatum* toxicity test”, published in May 2013 and the validity criteria stated in the guideline were not considered in the study report.

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.

Based on the available information given in the study not all mentioned validity criteria can be considered. However, based on the available data it can be shown that the mean total shoot length and the mean shoot fresh weight in the control groups double during the exposure phase of the test. In addition, no visual symptoms were observed in the control plants.

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.

In addition, the study is based on nominal concentrations instead of mean measured concentrations.

The mean measured concentrations < 80% were determined at day 7 of the exposure period. At test start and at test end the mean measured concentrations were > 80%. Hence, the study director argued that the active substance is stable in water and that the results should be based on nominal concentrations.

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

Reference:	Determined of the effects of Trammat on the life-cycle of <i>Daphnia magna</i> Straus
Author(s), year:	Barber, I., 1991
Report/Doc. number:	Study no. A83364, Reference no. M-155633-01-1
Guideline(s):	OECD 202 (Part 2), 1984
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Tramat ® 500 SC, batch no.: CR 18654/8, purity: 487g ai/L
Test species:	Waterflea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates each with 10 daphnids per treatment and control group
Age:	First instar, < 24 h old
Type of test, duration:	Semi-static test, Medium renewal three times weekly , 21 days
Feeding:	Unicellular green algae (<i>Chlorella vulgaris</i>), daily
Applied concentrations:	
Nominal:	0 (control), 0.2, 0.64, 2.0, 6.32 and 20 mg prod./L
Mean measured:	Not given
Solvent:	None
Test conditions:	
Water quality:	Water, conductivity: 153.3 – 189.6 µS/cm, total hardness: 66 – 102 mg/L as CaCO ₃ , alkalinity: 65.67 – 89.66 mg/L as CaCO ₃
Temperature:	19.8 - 20.4 °C
pH:	7.91 – 8.04
O ₂ content:	91.1 – 95.3% of air saturation
Light regime:	16 hours light / 8 hours darkness
Test parameters:	The test organisms were checked daily and the effects on survival and reproduction were recorded. At the end of the exposure period samples of the surviving <i>Daphnia</i> from each test concentration were taken and body length, i.e. the length from the tip of the head to the base of the spine, measured. At the start of the test and each renewal period temperature, pH and dissolved oxygen of the fresh and used test solutions were measured in the first vessel of each test concentration.
Analytical measurements:	Samples of fresh and used solutions periodically analysed using appropriate methods to quantify the actual test concentrations.

Statistics: All data were analysed for significance at $p = 0.05$, using Oneway Analysis of Variance (ANOVA). Where significant differences between treatments were found, Tukey multiple range tests were used to compare differences between the means for each treatment.

Using these techniques the no observed effect concentration and the lowest observed effect concentration were identified.

Findings:

Analytical measurements: The mean measured concentrations were in range of 88.5 and 119.2% of nominal test concentrations. Hence, the results are based on nominal test concentrations.

Biological effects: Mortality of the test daphnids was less than 5% in all treatments. Eggs were first recorded in the brood pouch of control animals and animals exposed to Trammat® on day 5, and by day 6 eggs were visible in the brood pouch of all daphnids.

The body length of the parental daphnids on day 21, was about 4 mm.

One-way analysis of variance and Tukey multiple range tests showed that body length was significantly reduced at the highest test concentration (20.00 mg prod./L). Hence, the NOEC for growth was 6.32 mg prod./L.

At the highest test concentration (20 mg prod./L) there was substantial algal accumulation on the bottom of the test vessels compared with the control vessels. This accumulation was the results of reduced consumption of algae by Daphnia at these concentrations.

Table B. 9.3.2-1: Effects on daphnids (*Daphnia magna*) exposed to Trammat ® 500 SC at day 21

Tramat ® [mg prod./L] (nominal)	Immobilisation of adults [%]	Mean number of live offspring per vessel	Mean number of live offspring per female
Control	2.5	912.25	91.275
0.20	2.5	918.75	92.45
0.64	0.0	899.75	88.025
2.0	2.5	822.75 *	84.275
6.32	0.0	160.5 *	16.05 *
20	0.0	0.25 *	0.025 *
21 d $EC_{50} = 3.94 \pm 0.15$ mg prod./L based on reproduction 21 d $EC_{50} > 20$ mg prod./L based on immobility 21 d NOEC = 0.64 mg prod./L (based on reproduction)			

* Statistically significant compared to the control, Tukey multiple range tests, $p = 0.95$

Conclusion: Due to the distribution of the data, the statistically derived EC_{50} value for the reproduction was 1.2 mg ai/L and the EC_{50} value for the immobilisation was 13.5 mg ai/L. The NOEC based on reproduction was determined to be 0.32 mg ai/L.

The results are based on nominal concentrations.

Comment RMS:

The study was conducted according to the OECD 202 (Part II, 1984). The validity criteria given in the test guidelines are met.

The mortality in the control groups was below 20% (being: 12.5%) at the end of the test. The dissolved oxygen was greater than 60% of the air saturation throughout the test duration. The average cumulative number of young per female in the controls after three broods should be greater than 20 at a temperature of 20 ± 1 °C. The number of offspring per female was greater than 20 on day 12 and 14 of the test.

The first young should have been born in the controls after a maximum of nine days (being: 7 days).

Based on the validity criteria the study is considered valid.

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.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	██████████ 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	██████████ 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	██████████ ██████████ 1989
<i>Leuciscus idus</i> Golden orfe	Static	96 h	Mortality	n	9.3	22.0	██████████ ^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	mm	0.25	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 20643 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 times 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 time 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	Sobczyk, H., 2013

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

^a Limit test

Table B.9.4-3: Endpoints: Acute toxicity of Ethofumesate 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>Cyprinus carpio</i> Mirror carp	Semi-static	96 h	Mortality	mm	5.7	14.4	██████████, 1989
<i>Danio rerio</i> Zebra fish	Semi-static	96 h	Mortality	n	9.0	34.0	██████████, 1988 ^a
Aquatic invertebrates							
<i>Daphnia magna</i> Waterflea	Static	48 h	Immobility	n	19.57	26.8	Cameron, B.D., et al., 1989

Test organism	Test condition	Time	Endpoint	Test conc.	NOEC [mg ai/L]	EC ₅₀ /LC ₅₀ [mg ai/L]	Reference
<i>Daphnia magna</i> Waterflea	Semi-static	21 d	Reproduction	n	0.32	1.2	Barber, I., 1991
Algae							
<i>Desmodesmus subspicatus</i> Green algae	Static	72 h	Growth rate Biomass	n	2.2	9.7 6.7	Knacker, T., 1989
Aquatic macrophytes							
<i>Myriophyllum spicatum</i> Water milfoil	Static	14 d	Growth rate Yield	n	0.005	0.38 0.05	Banman, C.S., 2012

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

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.

The risk assessment was conducted considering a single application of 1000 g ai/ha on sugar-, fodder- and red beets (post-emergence). The additional proposed uses (multiple applications) are covered by the worst-case risk assessment.

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

Substance	Sugar, fodder and red beet (post-emergence), Application rate: 1 x 1.0 kg ai/ha		
	FOCUS Step 1 [µg/L]	FOCUS Step 2 [µg/L]	
		Northern EU	Southern EU
Ethofumesate	297.2	49.6	91.0
NC 8493	70.8	nc	nc
NC 20645	7.43	nc	nc
Ethofumesate acetic acid	1.36	nc	nc

nc...not calculated

For a detailed summary of the FOCUS PEC_{sw} values please refer to section B.8.

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 (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
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.2972	36.7	100

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
<i>Cyprinus carpio</i>	Ethofumesate	LC ₅₀ = 10.92	96 h	0.091	120	100

Under consideration of FOCUS Step 2 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 *Americamysis 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.

The notifier Task Force Ethofumesate proposed a geomean EC₅₀ considering those three invertebrate species.

The RMS does not agree on the geomean approach including the oyster endpoint for the calculation. Hence, the RMS calculated a geomean EC₅₀ based on the endpoints for *Daphnia magna* and *Americamysis bahia*. The geomean EC₅₀ of 12.3 mg ai/L might be used to refine the acute risk to aquatic invertebrates.

Nevertheless, the risk assessment for aquatic invertebrates is triggered by the endpoint for the oyster *Crassostrea virginica*.

Studies with the representative formulation 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, NC 20645 and ethofumesate acetic acid were conducted addressing the risk to aquatic invertebrates.

Table B. 9.4.1-3: 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
<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
Geomean EC ₅₀	Ethofumesate	EC ₅₀ = 12.3	-	0.2972	41.4	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. 9.4.1-4: 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
<i>Daphnia magna</i>	Ethofumesate	EC ₅₀ = 28.1	48 h	0.091	309	100
<i>Americamysis bahia</i>	Ethofumesate	EC ₅₀ = 5.4	96 h	0.091	59.3	100
<i>Crassostrea virginica</i>	Ethofumesate	EC ₅₀ = 1.7	96 h	0.091	18.7	100
Geomean EC ₅₀	Ethofumesate	EC ₅₀ = 12.3	-	0.091	135	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 3 – mentioned are only these organisms which failed the trigger at Step 2

FOCUS Step 3 scenario	PEC [mg/L]	TER _A values	
		<i>Americamysis bahia</i> EC ₅₀ = 5.4 mg ai/L	<i>Crassostrea virginica</i> EC ₅₀ = 1.7 mg ai/L
D3 ditch	0.0052	1038	327
D4 pond	0.0004	13500	4250
D4 stream	0.0043	1256	395
R1 pond	0.0004	13500	4250
R1 stream	0.0047	1149	362
R3 stream	0.0606	89.1	28.1
Trigger		100	100

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

Table B. 9.4.1-6: 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

FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _A values	
			<i>Americamysis bahia</i> EC ₅₀ = 5.4 mg ai/L	<i>Crassostrea virginica</i> EC ₅₀ = 1.7 mg ai/L
R3 stream	10	0.02792	193	60.9
	20	0.01462	-	116
Trigger			100	100

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 20 m 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 (Teigeler, 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
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.2972	0.52	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
<i>Danio rerio</i>	Ethofumesate	NOEC = 0.156	FFLC	0.091	1.71	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]	TER _{LT} values
		<i>Danio rerio</i> NOEC = 0.156 mg ai/L
D3 ditch	0.0052	30
D4 pond	0.0004	390
D4 stream	0.0043	36
R1 pond	0.0004	390
R1 stream	0.0047	33
R3 stream	0.0606	2.6
Trigger		10

Based on the risk assessment an acceptable risk to fish was identified. For one FOCUS scenario (i.e. 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

FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _{LT} values
			<i>Danio rerio</i> NOEC = 0.156 mg ai/L
R3 stream	10	0.02792	5.6
	20	0.01462	10.7
Trigger			10

Under consideration of risk mitigation measured, i.e. vegetated buffer strip of 20 m 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
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.2972	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
<i>Daphnia magna</i>	Ethofumesate	NOEC = 0.32	21 d	0.091	3.5	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]	TER _{LT} values
		<i>Daphnia magna</i> NOEC = 0.32 mg ai/L
D3 ditch	0.0052	62
D4 pond	0.0004	800
D4 stream	0.0043	74
R1 pond	0.0004	800
R1 stream	0.0047	68
R3 stream	0.0606	5.3
Trigger		10

Based on the risk assessment an acceptable risk to aquatic invertebrates was identified. For one FOCUS scenario (i.e. 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. 9.4.2-8: 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

FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER _{LT} values
			<i>Daphnia magna</i> NOEC = 0.32 mg ai/L
R3 stream	10	0.02792	11.5
	20	0.01462	-
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
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.2972	8.14	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
<i>Chironomus riparius</i>	Ethofumesate	NOEC = 2.42	28 d	0.091	27	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. Three different algae species (*P. subcapitata*, *Anabaena flos-aquae* and *Skeletonema costatum*) were tested with the technical active substance.

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* (E₆C₅₀ = 5.2 mg ai/L).

In addition, studies with the metabolites NC 8493, NC 20645 and ethofumesate acetic acid were submitted addressing the risk to algae from exposure to the metabolites.

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 PEC_{sw} from FOCUS Step 1

Organism	Test substance	Toxicity endpoint [mg ai/L]	Time scale	PEC [mg/L]	TER	Trigger
<i>P. subcapitata</i>	Ethofumesate	$E_y C_{50} = 9.68$	72 h	0.2972	32.6	10
<i>P. subcapitata</i>	Ethofumesate 500 SC	$E_b C_{50} = 6.7$	72 h	0.2972	22.5	10
<i>P. subcapitata</i>	NC 8493	$E_y C_{50} = 0.865$	72 h	0.07081	12.2	10
<i>D. subspicatus</i>	NC 20645	$E_y C_{50} = 8.83$	72 h	0.00743	1188	10
<i>P. subcapitata</i>	Ethofumesate acetic acid	$E_y C_{50} = 98.98$	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 the water milfoil *Myriophyllum spicatum* 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 formulated active substance was observed to be of higher toxicity to aquatic macrophytes than the technical active substance.

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
<i>Myriophyllum spicatum</i>	Ethofumesate	E _y C ₅₀ = 0.25	14 d	0.2972	0.84	10
<i>Myriophyllum spicatum</i>	Ethofumesate 500 SC	E _y C ₅₀ = 0.05	14 d	0.2972	0.17	10
<i>Myriophyllum spicatum</i>	NC 8493	E _y C ₅₀ = 0.025 ^a	14 d	0.07081	0.35	10
<i>Myriophyllum spicatum</i>	NC 20645	E _y C ₅₀ = 0.025 ^a	14 d	0.00743	3.4	10
<i>Myriophyllum spicatum</i>	Ethofumesate acetic acid	E _y C 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
<i>Myriophyllum spicatum</i>	Ethofumesate	E _y C ₅₀ = 0.25	14 d	0.091	2.7	10
<i>Myriophyllum spicatum</i>	Ethofumesate 500 SC	E _y C ₅₀ = 0.05	7 d	0.091	0.5	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

^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]	TER values	
		<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L	<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.05 mg ai/L
D3 ditch	0.0052	48	10
D4 pond	0.0004	625	125
D4 stream	0.0043	58	12
R1 pond	0.0004	625	125
R1 stream	0.0047	53	11
R3 stream	0.0606	4.1	0.83
Trigger		10	10

Based on the risk assessment a high risk to aquatic macrophytes was identified considering the FOCUS scenario R3 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

FOCUS Step 4 scenario	Vegetated buffer strips	PEC [mg/L]	TER values	
			<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L	<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.05 mg ai/L
R3 stream	10	0.02792	9.0	1.8
	20	0.01462	17	3.4
Trigger			10	10

Under consideration of risk mitigation measured, i.e. vegetated buffer strips of 20 m no acceptable risk to aquatic macrophytes was identified considering the lowest endpoint derived from the study with the formulated active substance.

However, considering multiple application rates of 2 x 500 g ai/ha the risk to aquatic macrophytes was identified to be acceptable.

Table B. 9.4.2-16: 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 values	
				<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.25 mg ai/L	<i>Myriophyllum spicatum</i> E _y C ₅₀ = 0.05 mg ai/L
2 x 500 g ai/ha	R3 stream	10	0.013342	19	3.7
		20	0.006986	-	7.2
3 x 333 g ai/ha	R3 stream	10	0.008718	29	5.7
		20	0.004565	-	11
Trigger				10	10

Based on the available data an acceptable risk to aquatic organisms taking into account risk mitigation measures was identified.

However, a high risk to aquatic macrophytes exposed to the formulated active substance considering a single application rate of 1000 g ai/ha was observed. The risk assessment is based on an endpoint based on yield.

According to the EFSA guidance document on aquatic organisms the risk assessment for algae and aquatic macrophytes should be based on the growth rate instead of the biomass/yield.

However, at the time of the submission of the dossier the EFSA guidance document was not noted and hence, the risk assessment was conducted according to the SANCO guidance document. Hence the RMS conducted the risk assessment based on the lowest endpoint, irrespective of growth rate and biomass/yield.

Under consideration of the endpoint based on growth rate (E_rC₅₀ = 0.38 mg ai/L) an acceptable risk to aquatic plants was identified at FOCUS Step 4 (10 m vegetative buffer strip).

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 route 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 Ethofumesate SC 500 were submitted by the notifier Bayer CropScience (Task Force Ethofumesate, TFE).

B.9.5.1.1. Acute toxicity to bees

Reference:	Tramat 500: Laboratory tests on the effects to honeybees (<i>Apis mellifera</i>)
Author(s), year:	Barber, I., 1995
Report/Doc. number:	Report No. A83401, Reference No. M-155669-01-1
Guideline(s):	BBA guideline 23-1
GLP:	No
Deviations:	None
Validity:	Not acceptable

Material and Methods:

Test substance:	Tramat 500
Reference:	None
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Inhalation, contact and oral toxicity test

Inhalation toxicity test:

For testing the toxicity of Tramat 500 due to inhalation, contact and wetting, the formulation was applied at the maximum recommended application concentration (i.e. 2% w/v) for one data set, while for the remaining tests the formulation was applied at twice the maximum recommended application concentration (i.e. 4% w/v).

For the inhalation test, the test cages containing honey bees are placed on the edge of an open petri dish containing test material at the concentration indicated above. The test cages have a perforated base to ensure that any volatile, gaseous components of the formulation can enter the cages. For controls, a petri dish half-filled with water is used.

Contact toxicity test:

For acute contact toxicity test a single treatment of Tramat 500, equivalent to 100 µg/bee was used.

For the contact test (contact poison, lasting contact), filter paper is soaked with the test material at the concentration indicated above, then air dried and placed in the test cages. For controls a filter paper soaked in water is used.

For the wetting test (wetting/direct spray) the bees are evenly wetted with the formulation, at twice

recommended application rate, using a fine spray. Immediately after treatment, the bees are transferred to clean test cages.

For controls, the bees are sprayed with water.

Oral toxicity test:

For the acute oral toxicity test a single treatment of Trammat 500, equivalent to 100 µg/bee was used. To assess the effect of the test material as a stomach poison the test material is mixed with 0.5% (w/w) sugar solution.

Each bee is dosed with a maximum of 100 µg of test substance.

All four tests are conducted for a minimum of 24 hours and for up to 72 hours after treatment. The number of dead bees in each cage is assessed and compared to controls.

Findings:

For the tests conducted with Trammat, applied at concentrations equivalent to the recommended application concentration, honey bee mortality was less than 15%. Therefore this indicates that at recommended application concentrations the formulation can be classified as being of low risk to honey bees.

For the tests conducted with Trammat applied at twice the recommended application concentration, honey bee mortality was also less than 15%, except for one contact toxicity test (Hohenheim, test 2) and one inhalation toxicity test (Saarbrücken, test 1).

In the case of the latter, no control mortality data is reported and so the health of the bees used in the test cannot be assessed. Furthermore, mortality was still less than 50% in both cases, and according to BBA guidelines, would not indicate that the formulation is a risk to bees.

Table B. 9.5.1.1-9.5.1-1: Effects of Trammat 500 on *Apis mellifera* following exposure (inhalation, contact, oral) to adult honey-bees (maximum application rate)

Test type	Mortality [%] / time [h]			
	Test 1		Test 2	
	C	T	C	T
Inhalation	0 / 24	7 / 24	20 / 72	5 / 72
Contact (lasting exposure)	0 / 24	0 / 24	1.5 / 24	0 / 24
Contact (wetting)	3 / 24	0 / 24	10 / 72	7 / 72
Ingestion (100 µg/bee)	0 / 24	0 / 24	0 / 24	0 / 24

C...Control, T...Treatment

Table B. 9.5.1.1-9.5.1-2: Effects of Trammat 500 on *Apis mellifera* following exposure (inhalation, contact, oral) to adult honey-bees (twice the maximum application rate)

Test type	Mortality [%] / time [h]							
	Saarbrücken				Hohenheim			
	Test 1		Test 2		Test 1		Test 2	
	C	T	C	T	C	T	C	T
Inhalation	0 / 72	0 / 72	0 / 72	0 / 72	0 / 24	0 / 24	7 / 72	7 / 72
Contact (lasting exposure)	0 / 72	0 / 72	7 / 72	7 / 72	0 / 24	0 / 24	0 / 72	20 / 72
Contact (wetting)	0 / 72	0 / 72	7 / 72	0 / 72	0 / 24	0 / 24	3 / 72	7 / 72
Ingestion (100 µg/bee)	3 / 24	0 / 24	-	40 / 24	-	-	-	-

C...Control, T...Treatment

Conclusions:

Based on these results, Trammat 500 can be classified as being of low toxicity to honey bees. This is consistent with expectations based on the results of tests with the active ingredient ethofumesate.

Comment RMS:

The study is considered not valid as the study documentation is poor and the study does not comply with the current valid test guidelines according to OECD and US EPA.

Important information regarding the used test material (batch, purity), the test method (number of bees, number of replicates), environmental conditions (temperature, humidity,...) and the statistical analysis of the results is missing. In addition no toxic reference was tested to address the sensitivity of the honeybees.

Reference:	Effects of ethofumesate SC 500 G (Acute Contact and Oral) on Honeybees (<i>Apis mellifera</i> L.) in the Laboratory
Author(s), year:	Schmitzer, S., 2011b
Report/Doc. number:	IBACON project no.: 63931035, Reference No. M-421700-01-1
Guideline(s):	OECD 213 and 214 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate SC 500 G (ETO SC 500 G), Batch no. : ECE5100755, Content : 44.1% w/w (analytical), 498.2 g/L
Reference:	Perfekthion (BAS 152 11 D), Batch no.: 90924-06, Dimethoate 414.8 g/L (analysed)
Wetting agent	Adhäsit, 100 g/L Marlopon (nominal), Batch no.: 0180201
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
Oral toxicity test:	
Applied concentrations:	Control: 50% (w/w) aqueous sucrose solution (50% tap water, 50% ready-to-use syrup) Test item: 100 µg ai/bee (nominal), 108.8 µg ai/bee (measured) Reference item: 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee (nominal) 0.06, 0.08, 0.16 and 0.32 µg dimethoate/bee (measured)
Exposure route:	Aqueous stock solutions of the test item and reference item were prepared before the ready-to-use syrup was added (ratio 1:1) and offered to the bees. The test bees were starved for 20 minutes before they were fed with the solutions. After 1 hours and 25 minutes, the feeding troughs were exchanged with clean feeders containing ready-to-use syrup and the retrieved containers re-weighed to determine the quantity of feed consumed.
Test conditions:	Temperature: 25 °C, Relative humidity: 51 - 66 %, 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.
Contact toxicity test:	
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: 100 µg ai/bee

Reference item: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee (nominal)

Test parameter: A 5.0 µL droplet of ethofumesate SC 500 G in an appropriate carrier (tap water + 0.5% Adhäsit) was administered to the thoracic surface of CO₂-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 *ad libitum*.

Test conditions: Temperature: 25 °C, Relative humidity: 51 - 66 %, 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:

Oral toxicity test: The actual consumption per bee in the oral test was 108.8 µg ai/bee. During the 4-hour assessment in the test item treatment group one honeybee was found apathetic. At the next assessment (24-hour) the bee was found dead. Effects on the survival of the honeybees see Table B.9.5.1.5-4

Table B. 9.5.1.1-9.5.1-3: Effects of ethofumesate SC 500 G on *Apis mellifera* following 48-h oral exposure in an acute toxicity test (averages from 5 replicates per dosage/control)

Nominal dose [µg ai/bee] (consumed)	Mortality (corrected mortality ^a) [%]		
	4 h	24 h	48 h
Control (tap water + sugar solution)	0.0	0.0	2.0 ^b
Treatment			
100 (108.8)	0.0 ^b	2.0	2.0
Reference item			
0.05 (0.06)	0.0 ^b	2.0	2.0
0.08 (0.08)	0.0	16.0 ^a	16.0 ^a
0.15 (0.16)	2.0 ^{a,b}	84.0	84.0
0.30 (0.32)	26.0 ^{a,b}	80.0 ^a	84.0 ^a
Test substance: 24h / 48h LD ₅₀ > 108.8 µg ai/bee			
Reference: 24 h LD ₅₀ = 0.15 µg dimethoate/bee (95 % C.I. 0.12 – 0.18 µg ai/bee)			
48 h LD ₅₀ = 0.15 µg dimethoate/bee (95 % C.I. 0.13 – 0.18 µg ai/bee)			

^a moving coordination problems

^b apathy

Contact toxicity test: No test item induced behavioural effects were observed at any time in the contact toxicity test. Effects on the survival of the honeybees see table below.

Table B. 9.5.1.1-9.5.1-4: Effects of ethofumesate SC 500 G on *Apis mellifera* following 48-h contact exposure in an acute toxicity test (averages from 5 replicates per dosage/control)

Nominal dose [$\mu\text{g ai/bee}$]	Mortality [%]		
	4 h	24 h	48 h
Control (tap water + wetting agent)	0.0	0.0	0.0
Treatment			
100	0.0	0.0	0.0
Reference item			
0.10	6.0 ^a	12.0	16.0
0.15	6.0	22.0	34.0 ^a
0.20	0.0 ^a	72.0	82.0 ^a
0.30	10.0 ^{a,b}	86.0	92.0
Test substance: 24 h / 48 h LD ₅₀ > 100 $\mu\text{g ai/bee}$ Reference: 24 h LD ₅₀ = 0.18 $\mu\text{g dimethoate/bee}$ (95 % C.I. 0.18 – 0.21 $\mu\text{g ai/bee}$) 48 h LD ₅₀ = 0.16 $\mu\text{g dimethoate/bee}$ (95 % C.I. 0.14 – 0.18 $\mu\text{g ai/bee}$)			

^a moving coordination problems^b apathyConclusions:48 h LD₅₀ > 108.8 $\mu\text{g ai/bee}$ (oral toxicity)48 h LD₅₀ > 100 $\mu\text{g ai/bee}$ (contact toxicity)Comment RMS:

The study is considered acceptable. All validity criteria according to the OECD guidelines 213 and 214 are met. The mean mortality of the controls (water) in the oral and contact toxicity test was maximal 2 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.

The 24 h LD₅₀ values of the reference item (dimethoate) in the oral (24 h LD₅₀ = 0.18 $\mu\text{g ai/bee}$) and contact (24 h LD₅₀ = 0.15 $\mu\text{g ai/bee}$) toxicity tests were within the recommended range of 0.10 – 0.35 $\mu\text{g ai/bee}$ (oral) and 0.10 – 0.30 $\mu\text{g ai/bee}$ (contact), respectively.

Some deviations to the OECD guidelines were identified. However, these deviations are not considered of relevance for the results of the acute oral and contact toxicity test.

In the oral toxicity test the bees were starved for 30 minutes only and not for 2 hours as recommended in the OECD guideline. However, this may not have an effect on the results of the study considering the amount of consumed sugar solution.

In the contact toxicity test a 5 μL droplet was used in deviation to the OECD guideline recommendation of a 1 μL droplet. This deviation is considered acceptable since a higher volume ensured a more reliable dispersion of the test item.

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).

A 10 day chronic oral toxicity study was conducted with technical ethofumesate; the corresponding summary is given in Volume 3 – B.9 (A.S.).

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

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

Reference:	Study on the Effects of Ethofumesate SC 500A G on Honey Bee Brood (<i>Apis mellifera</i> L.) – Brood Feeding Test
Author(s), year:	Schmitzer, S., 2013
Report/Doc. number:	IBACON project no.: 71411031, M-454691-01-1
Guideline(s):	Oomen <i>et al.</i> (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate SC 500A G, Batch no.: ECED101300, Content: 44.3% w/w (analytical), 500.6 g/L
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 13665 and 15300 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:	<p>Rossdorf, Darmstadt-Dieburg, Germany</p> <p>Test site: Uncultivated fields, surrounding area underlies agricultural use mainly with arable crops and meadow.</p>
Test conditions:	<p>Natural field conditions. Temperature, relative humidity and rain were recorded during the experimental time.</p> <p>Temperature (daily mean values): 8.8 – 32.9 °C (mean: 14.6 – 26.0 °C)</p> <p>Rain: 0.0 – 30.6 mm (total precipitation per day)</p> <p>Rel. humidity (daily mean values): 69.9 – 98.9%</p>
Feeding:	Natural food and water sources
Test design:	<p>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.</p> <p>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.</p> <p>Each feeding trough was weighed before introduction to the bee colonies and after uptake of the contaminated food. About 25 hours after application, the uptake of the colonies was complete.</p> <p>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.</p>
Test concentrations:	<p>Control: 1 L untreated ready-to-use syrup (Apiinvert containing 30% sucrose, 31% glucose and 39% fructose) per colony.</p> <p>Test item: 5.64 g test item (Ethofumesate SC 500A G) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to an active substance concentration of 2.5 g ethofumesate ai/L.</p> <p>Reference item: 3.0 g reference item (Insegar; 25 % fenoxycarb) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to a nominal active substance concentration of 0.75 g fenoxycarb ai/L.</p>

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 (= BFD22) 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:

Although the mean termination rate of eggs was slightly higher in the test item treatment group (28.9 %) when compared to the values of the control group (18.9 %), there was no statistically significant difference.

No effect on the development of young larvae was observed after consumption of the test item. The mean termination rate of young larvae in the test item treatment group was lower with a mean of 7.3 % compared to 14.7 % in the control group. Accordingly, this was not statistically significant compared to the control group.

There was also no effect on the development of old larvae after consumption of the test item. The mean termination rate of old larvae in the test item treatment group was 2.7 % compared to 3.3 % in the control group. Accordingly, this was not statistically significant compared to the control group.

Adult bee mortality in the test item treatment group was lower and thus not statistically significantly different when compared to the control group.

No effects of the test item on honey bee pupae and larvae were observed.

No behavioural abnormalities were observed at any time in any of the test or reference item treatment groups until test end. Also in the controls no behavioural abnormalities were noted.

The reference item treatment (Insegar) resulted in a statistically significantly increase of unsuccessful egg-, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Table B. 9.5.1.3-1: Effects of ethofumesate SC 500A G on honey bee brood

Treatment	Untreated control	Ethofumesate SC 500A G	Reference item
Rate per L sugar solution [product]	-	5.64 g prod./L	3.0 g prod./L
Rate per L sugar solution [ai]	-	2.5 g ai/L	0.75 g ai/L
Termination rate of eggs [%] ^a	18.9%	28.9%	100%
Termination rate of the young larvae [%] ^a	14.7%	7.3%	97.8% *
Termination rate of the old larvae [%] ^a	3.3%	2.7%	54.3% *
Mean brood terminate rate over all stages ^a	12.3%	13.0%	84.0% *
Mean mortality of worker bees/colony/d during pre-application phase ^b	23.1	17.1	24.3
during the entire post-application phase ^b	54.0	29.7	33.9
Mean mortality of pupae/colony/d during pre-application phase ^c	0.6	0.2	0.1
during the entire post-application phase ^c	3.6	3.2	4.0
Mean number of bees before application (range)	15300 (14760 – 16245)	13890 (8145 – 19350)	13665 (10260 – 15705)

^a Mean terminate rate of 3 colonies per treatment group^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 Ethofumesate SC 500A G does neither adversely affect honey bee colonies nor bee brood development.

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. However, the study was well conducted and is therefore considered valid and acceptable for use in the risk assessment.

Mean control mortality of the adult bees from day 0 after application to day 21 differed between 5.7 and 165.3 dead bees per colony. On a few occasions, mortality levels were unexpectedly high. This might be explained by heavy rain on those days. Sudden rain might have caught forager bees by surprise, which subsequently died in the dead bee trap.

In addition, the highest observed peak of worker bee mortality in the control groups at day 2 after the application (e.g. day 2, day 3 and day 16) was also observed in the treatment and reference groups. This might be an additional indication that the high mortalities are based on external events (e.g. rain).

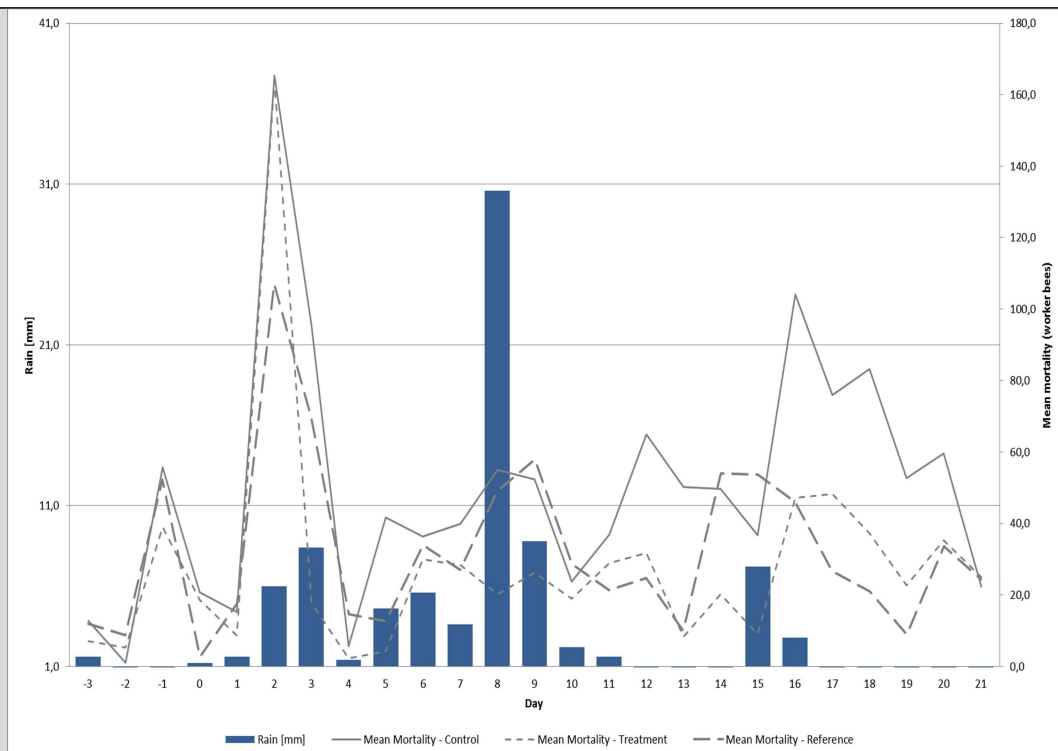


Figure B.9.5.1.3-1 : Mean mortalities of worker bees in the control, treatment and reference groups under consideration of the rainfall

As the overall mean mortality in the control group after application was within a reasonable level (54.0 dead bees/colony/day), this value can be empirically regarded to be within the range of normal mortality levels of colonies of the employed size under field conditions.

In addition, a mean of 3.6 dead pupae per colony per day were found during the 21 days post-application period. This value can be considered to represent a biologically typical number of dead pupae over a period of 21 days. The mean mortality of the controls (water) in the oral and contact toxicity test was maximal 2 % which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.

In the reference item group a high number of impacted bee brood was observed, which resulted in 84.0 % mean loss of the initial observed cells (100.0 % eggs, 97.8 % young larvae and 54.3 % old larvae stages, respectively). The termination rate of eggs was 100 % and the termination rates of young and old larvae were statistically significantly higher than the control values and are on an absolute scale sufficiently high to demonstrate the sensitivity of the test system and the validity of the test conditions.

According to Oomen *et al.* the colonies should have a size between 10 000 and 15 000 bees per colony. However, one colony used in the test (treatment group) has a size of only 8 145 bees.

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. Filed 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.9.5.2. 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 Ethofumesate 500 SC, not-peer reviewed on EU level have been performed.

Reference:	Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphi</i> (DeStephani-Perez) (Hymenoptera: Braconidae) in the laboratory
Author(s), year:	Waltersdorfer, A., 2002a
Report/Doc. number:	Report No. CW02/012, Reference No. M215537-01-1
Guideline(s):	Mead-Briggs et al., 2000 (IOBC), Candolfi et al. 2000 (ESCORT 2 recommendation)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate, 44.7% w/w (AE B049913 00 SC45 A202), batch no. BK 142
Toxic reference:	Dimethoate, 38.7% w/w (AE F020846 00 EC37 A203 = BAS 152 11 I)
Test species:	Adult female wasps <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae), < 48 h old
Type of test:	Acute contact laboratory test
Number of organisms:	4 replicates with 15 adult wasps per replicate
Treatments:	Control: deionised water Toxic reference: 0.12 g ai/ha (Dimethoate) Test item: 500 and 1000 g ai/ha; deionised water as diluent; Treatments applied with a calibrated sprayer in 200 L water/ha
Exposure route, duration:	The test units were made up of two glass plates fitted to the top and bottom sections of a steel casing perforated with holes. The holes provided, variously, ventilation (through mesh covers), an entrance aperture for introducing and feeding the wasps. After the sprayed residues had dried on the glass plates the test units were assembled with the treated surfaces of the plates facing inwards and wasps introduced. At the end of the exposure period 15 healthy females were transferred and kept individually in untreated acrylic cylinders with aphid-infested cereal plants. One day later the wasps were removed and the plants kept for 12 more days. Exposure time: 48 h, parasitisation period: 24 h, post-parasitisation period: 12 days.

Feeding:	A solution of honey in small test tubes provided as source of food.
Test conditions:	Temperature: 20 °C; relative humidity: 63 - 78 %, 16 h light, 360 - 1600 lux (mortality phase) and 12400 – 25400 lux (reproduction phase)
Test parameters:	Mortality and behavioural abnormalities were assessed 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.
Statistics:	It was not necessary to conduct statistical analysis of the mortality and reproduction data.

Findings:

In both dose rates the mortality rate was lower than in the water control. No effects on the behaviour of the wasps were observed.

There was no reduction on reproduction success relative to the control at the 500 g ai/ha rate and a reduction of 18.7% at the rate of 1000 g ai/ha.

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

Nominal application rate (g ai/ha)	Mortality (out of 60 wasps/treatment)		Parasitisation efficiency	
	Cumulative [%]	Control corrected [%] ^a	Mean number of aphid mummies per female wasp	% Reduction relative to control ^a
Control	12 (7/60)	-	8.2	-
500	5 (3/60)	- 8.3	13.8	- 68.3
1000	5 (3/60)	- 8.0	6.7	18.7
Reference item	100 (60/60)	100	-	-

^a Corrected according to Abbott

Negative values indicate a positive effect on the survival/reproduction compared to the control.

<u>Conclusion:</u>	48 h LR ₅₀ > 1000 g ai/ha 48 h ER ₅₀ > 1000 g ai/ha
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<u>Comment RMS:</u>	<p>The study was conducted according to the IOBC test guidelines (Mead-Briggs et al., 2000) and the ESCORT II recommendations (Candolfi et al., 2000).</p> <p>The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline.</p> <p>The mortality of the adult wasps in the control treatment was below 13% (being: 12%). For the fecundity assessments, wasps in the control group produced more than 5 mummies per female (being: mean 8.2 mummies per female).</p> <p>The number of wasps in the control treatment producing no mummies was not more than two wasps (being: 2 wasps).</p>
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The mortality observed in the toxic reference treatment group (100% at 0.12 g ai/ha) was in line with the IOBC test guideline.

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

Reference:	Toxicity to the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) in the laboratory
Author(s), year:	Waltersdorfer, A., 2002b
Report/Doc. number:	Report no. CW02/010, Reference no. M-215086-01-1
Guideline(s):	Blümel et al., 2000 (IOBC), Candolfi et al., 2001 (ESCORT 2 recommendation)
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate, 44.7% w/w (AE B049913 00 SC45 A202), batch no. BK 142
Toxic reference:	Dimethoate, 38.7% w/w (AE F020846 00 EC37 A203 = BAS 152 11 I)
Test species:	<i>Typhlodromus pyri</i> protonymphs, < 24 h old
Type of test:	Acute contact laboratory test
Number of organisms:	5 replicates with 20 individuals per replicate
Treatments:	Control: deionised water Toxic standard: 2.5 g ai/ha (Dimethoate) Test item: 1000 g ai/ha. The test item was sprayed in 200 L/ha on the glasses.
Exposure route, duration:	The “island method” was used for testing the effects on <i>T. pyri</i> . One unit consisted of round cover glasses floating in petri dish bottoms of glass and with an orifice in the middle. Six of these petri dishes are arranged in a stainless steel tray. They were applied to the test cover glasses using a sprayer. Prior to application, each glass was placed on a plastic petri dish lid and bottom with a small edge on the flat outside, laid with the flat side upwards. After the spray coating had dried, the cover glasses were moved from the treatment dishes into the corresponding petri dishes with the aid of a microscope needle. A small volume of deionized water was then poured slowly into the surrounding plastic vessel so that the glass arenas were lifted from the bottom and floated at approximately half the height of the dish edges. After the test units were set up the protonymphs were placed onto the glass surface. Protonymphs were exposed to dried residues for 14 d.
Feeding:	Pollen (birch + pine)

Test conditions:	Temperature: 23.5 - 26°C; relative humidity: 63 - 78 % (with a short decline to 47%), 16 h photo period
Test parameter:	<p>Mortality (the number of dead mites, together with any trapped in the glue barrier or missing) was assessed on Day 1, 3, 7, 10, 12 and 14.</p> <p>The number of eggs laid and the number of live and dead juvenile stages per female were counted and removed afterwards at day 7, 10, 12 and 14, in the control and all treatment groups.</p> <p>On day 7, 10 and 12 males were added if the sex ratio was more than 5 females:1 male.</p>
Statistics:	It was not necessary to conduct statistical analysis of the mortality and reproduction data.

Findings:

Up to day 7 of the test 93% of the mites survived in the control group, 2% died, and 5% were missing. This compared with 91% survival in the 2 l/ha rate of the test substance (5% dead, 4% missing). In the reference substance group, 19% of the mites survived to day 7, 59% died and 22% were missing.

The mean number of offspring produced per female in the control group was 9.32 (± 0.68). This compared to 7.79 (± 0.68) eggs/female in the 2 l/ha rate of the test substance.

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

Nominal application rate [g ai/ha]	Mortality after 7 days ^b (out of 100 mites/treatment)		Reproduction (days 7 - 14)	
	Cumulative [%]	Control corrected [%]	Mean no. of eggs per female mite	% Reduction relative to control
Control	7% (7/100)	-	9.32 \pm 0.68	-
1000	9% (9/100)	2.1%	7.79 \pm 0.68	16.4%*
Reference item	81% (81/100)	79.6%	-	-

* Statistically significant compared to the control, one-way analysis of variance, $p = 0.05$.

^a Corrected according to Abbott

^b Number of dead mites including escaped mites

Conclusion:

7 d LR₅₀ > 1000 g ai/ha

14 d ER₅₀ < 1000 g ai/ha

<u>Comment RMS:</u>	<p>The study was conducted according to the IOBC test guidelines (Blümel et al., 2000) and the ESCORT II recommendations (Candolfi et al., 2001).</p> <p>The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline.</p> <p>The arithmetic mean mortality (dead and escaped) in the control treatment was below 20% on day 7 after treatment application (being: 7%). The cumulative mean</p>
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number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female (being: 8.75 – 10.18 eggs/ female).

The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the reference item was 79.6% and therefore in the recommended range between 50% and 100%.

For a short period during the test the relative humidity was measured to be about 47%. However, this deviation to the study seems to have no impact on the outcome of the study.

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

Reference:	An evaluation of the side-effects of Trammat 500 SC on the staphylinid beetle (<i>Aleochara bilineata</i>)
Author(s), year:	Mead-Briggs, M., 1991
Report/Doc. number:	Report no. A83379, Reference no. M-155647-01-1
Guideline(s):	Samsøe-Petersen, 1988 (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Tramat 500 SC, Batch no.: CR 18654/01/900109, Content of ai: 501 g/L (analysed)
Test species:	<i>Aleochara bilineata</i> , adult female (1-2 weeks old)
Type of test:	Acute contact laboratory test
Number of organisms:	25 mated females per treatment group and per control group
Treatments:	Control: water Test item: 1252.5 g ai/ha (= 2.5 L prod./ha) The test item was sprayed in 500 L/ha on the glasses.
Exposure route, duration:	The test arenas comprised disposable, plastic Petri dishes which were filled with (washed and sieved) lime-free, silica sand. The dishes were filled with the dry sand and each was re-moistened distilled water. The dishes were then ready to be treated. Once the sand had been treated with either the product or with water, the test units were assembled and the beetles introduced one per dish. A small pellet of moist dog food was placed in each dish for food and this was replaced every 1-2 days for the duration of the 4-day exposure period. To prevent the insects from escaping, fine nylon gauze lids were fitted to the arenas.

Test conditions: Temperature: $22 \pm 2^{\circ}\text{C}$; relative humidity: 76 - 100 %, 16 h photo period

Test parameter: The condition of the beetles was assessed daily for four days, at which point the test was terminated.

At the end of the exposure period the number of eggs in each dish was counted.

The number of eggs hatching was monitored over the following 14 days.

Statistics: It was not necessary to conduct statistical analysis of the mortality and reproduction data.

Findings:

During the study, a single beetle died in both the control and the Tramate500 SC treatments. One beetle from the Tramate 500 SC treatment escaped from its arena.

No abnormal behaviour was seen in any of the beetles.

In both treatments, the majority of the eggs hatched within 7 days of the adults being removed. In the control, 24 beetles laid a total of 206 eggs (8.6 per insect) of which 201 (98 %) hatched. In the Tramate 500 SC treatment, 23 beetles laid a total of 292 eggs (12.7 per insect) of which 273 (93 %) hatched.

Table B. 9.5.2-3: Summary of the effects of Tramate 500 SC on the beetle *Aleochara bilineata* following exposure under laboratory conditions

Nominal application rate [g ai/ha]	Mortality after 4 days ^a (out of 25 beetles/treatment)	Reproduction	
		No. of eggs	No. of hatched eggs
Control	4% (1/25)	206	201
1252.5	8% (2/25)	292	273

^a Number of dead mites including escaped mites

Conclusion: 4 d LR₅₀ > 1252.5 g ai/ha
14 d ER₅₀ > 1252.5 g ai/ha

Comment RMS: The study was conducted according to the IOBC test guidelines (Samsøe-Peterson, 1988).

No explicit validity criteria are given in the IOBC test guideline by Samsøe-Peterson. The study was conducted in line with the stated test guideline and thus is considered valid.

Due to the age of the study and the change of the test guidelines within this period an evaluation of the study considering the current valid test guidelines is difficult. However, the RMS is of the opinion that the study was conducted in line with the test guidelines and hence, is considered valid and acceptable for the use in the risk assessment.

Reference:	A study of the acute toxicity of Trammat 500 (Norton 50 SC) to the carabid <i>Poecilus cupreus</i>
Author(s), year:	Römbke, J., 1990
Report/Doc. number:	Report no. A83354, Reference no. M-155623-01-1
Guideline(s):	Heimbach, U. (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Tramat 500 (Norton 50 SC), Content of ai: 500 g/L
Toxic reference:	Afugan (ai: Pyrazophos), Content of ai: 294 g/L E 605 forte (ai: Parathion), Content of ai: 500 g/L
Test species:	<i>Poecilus cupreus</i> , adults (> 6 weeks)
Type of test:	Acute contact laboratory test, 14 days
Number of organisms:	6 beetles (3 males and 3 females) per replicate, 5 replicates per treatment group, control group and toxic reference group
Treatments:	Control: water (400 L/ha) Test item: 4 L prod./ha Toxic reference: 2 L prod./ha (Afugan), 210 mL prod./ha (E 650 forte) The test item was sprayed in 200 – 400 L/ha on the petri dishes.
Exposure route, duration:	During the test carabid beetles were kept in plastic vessels. Each of the five test vessels was filled with 250 g quartz sand (grain size 0.1 - 0.4 mm; 99.7 % Silicium dioxide). In the centre of the transparent covers of the vessels a hole was cut, covered by a coarse net. Six beetles (3 males and 3 females) were placed in each test vessel. They were acclimatised to the test conditions for a period of three days during which time they were not fed. Moisture of approximately 70 % of the maximum water capacity was adjusted in each test vessel. Then the carabids were fed with one fly pupae per animal. Immediately afterwards the application process began. Every two to three days the beetles were fed with one fly pupa per surviving animal. On the same dates the sand was watered to compensate moisture losses.
Test conditions:	Temperature: 20 ± 2°C; relative humidity: 85 %, 16 h photo period, 500 – 1500 lux
Test parameter:	On the following days the beetles were assessed (mortality, behaviour): Day 1 (2, 4 and 6 hours), 2, 5, 8, 12 and 15.
Statistics:	It was not necessary to conduct statistical analysis of the mortality.

Findings:

Table B. 9.5.2-4: Summary of the effects of Trammat 500 on *Poecilus cupreus* following exposure under laboratory conditions

Nominal application rate	Mortality after 14 days (out of 30 beetles/treatment)
Control	0% (0/30)
Tramat 500 (4 L/ha)	3.3 (1/30) ^a
Toxic reference (Afugan, 2 L/ha)	93.3% ^b (28/30)
Toxic reference (E 605 forte, 210 mL/ha)	100% (30/30)

^a Behavioural effects (beetles were sitting in the sand) were observed. Immediately after application the beetles dug holes in which most of them remained for nearly two weeks.

^b Behavioural effects (surviving animals were laying on their back with shivering legs) were observed.

Conclusion: 14 d LR₅₀ > 4 L prod./ha

Comment RMS:

The study was conducted according to the IOBC test guideline (Heimbach, U.). According to the guideline the results are only valid if the mortality in the control group is lower than 20% and if the recapture rate of the released beetles is at least 70% in the control. These validity criteria are met in the study by Römbke.

The beetles in the treatment group were observed to dig holes and remain in the sand nearly throughout the exposure duration. This may indicate an escape reaction.

The LR₅₀ was determined to be greater than 4 L prod./ha, corresponding to 2 kg ai/ha, considering an active substance content of 500 g/L. No information on the density of the formulation is given in the study protocol, thus the relative density was not considered.

The RMS is of the opinion that the study was conducted in line with the test guidelines and hence, is considered valid and acceptable for the use in the risk assessment.

Reference:	Side-effects of Trammat 500 on the lacewing, <i>Chrysoperla carnea</i> Steph. in the laboratory
Author(s), year:	Kühner, C., 1990
Report/Doc. number:	Report no. A89489, Reference no. M-155645-01-1
Guideline(s):	Bigler, F., 1988 (IOBC)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Tramat 500, Content of ai: 500 g/L
Toxic reference:	None
Test species:	<i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae), larvae (2-3 days old)
Type of test:	Acute contact laboratory test
Number of organisms:	15 larvae per replicate, 3 replicates per treatment group and control group
Treatments:	Control: tap water (400 L/ha) Test item: 2% solution of Trammat 500 (2000 g ai/ha) The test item was sprayed in 200 – 400 L/ha on the glass plates.
Exposure route, duration:	<p>Glass plates were used as exposure units. After drying the study substance, sheets of Plexiglas perspex of the same size but with 15 recesses were fixed to the glass plates. A perspex ring was fitted into each recess using a rubber band. The internal surfaces of the rings were coated with fluon which was intended to act as a slippery barrier to prevent the larvae and their prey escaping, but to have no harmful effects on the study organisms.</p> <p>The study substance was applied using a glass atomizer driven by a compressor during the spraying process with a constant pressure. Two to three hours after the application of the study substance the glass plates were assembled into the exposure units described above.</p> <p>Then one <i>Chrysoperla</i> larva 2 - 3 days old was transferred with a fine brush into each of the perspex rings on the treated glass plate and was fed daily with approx. 30 fresh aphids (<i>Acyrtosiphon pisum</i>, <i>Aphis fabae</i>) until they pupated. The pupae resulting after 14 days of larval development were carefully loosened from the glass plate and transferred to Petri dishes until they emerged.</p> <p>The emerged imagoes were transferred after sexing into egg-laying boxes, with lids replaced by gauze. The females used the gauze for egg-laying. It was renewed twice a week. Egg-laying performance was observed over a period of approx. 4 weeks from the start of egg-laying. The imagoes were supplied regularly with fresh water and an artificial food (consisting of honey, brewer's yeast and water) during the fecundity test.</p>
Test conditions:	Mortality: Temperature: 22 - 25°C (day) and 16 – 18 °C (night); relative humidity:

50 ± 10 %, 16 h photo period, 3000 lux

Fecundity: Temperature: 24°C; relative humidity: 80 %, 16 h photo period

Statistics:

It was not necessary to conduct statistical analysis of the mortality.

Findings:

Table B. 9.5.2-5: Summary of the effects of Trammat 500 on larvae of *Chrysoperla carnea* following exposure under laboratory conditions

Nominal application rate	Control	Tramat 500
Mortality		
∑ larvae	45	45
∑ dead larvae	2	1
∑ escaped or squashed larvae	3	1
∑ pupae	40	43
∑ imagoes not emerged or died after emerging	4	5
∑ imagoes emerged	36	38
Mean mortality [%]	14%	13.8%
Corrected pre-imaginal mortality [%] ^a	-	- 0.23
Fecundity		
∑ imagoes	36	38
∑ females	16	21
∑ males	20	17
∑ eggs	2890	5097
Egg-laying performance [%]	100%	134.3%
Corrected egg-laying performance [%]	-	- 34.3
Reproduction factor	-	1.34

^a Mortality corrected according to Scheinder-Orelli's formula

Conclusion:

ER₅₀ > 4 L prod./ha (= 2000 h ai/ha)

Comment RMS:

The study was conducted according to the IOBC test guideline (Bigler, F., 1988.). According to the guideline no explicit validity criteria are given. The study is in line with the IOBC test guideline and hence is considered valid.

According to the IOBC test guideline by Bigler (2000) the following validity criteria are given:

- The maximum acceptable cumulative mortality (dead larvae and pupae and adults dying during emergence or not successfully moulted) should be ≤ 20%.
- The fecundity (mean number of eggs per female per day) should be ≥ 15.
- The fertility (mean hatching rate) should be ≥ 70%.

Due to the age of the study and the change of the test guidelines within this period an evaluation of the study considering the current valid test guidelines is difficult. However, the RMS is of the opinion that the study was conducted in line with the test guidelines and hence, is considered valid and acceptable for the use in the risk assessment.

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 Ethofumesate SC 500 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 post-emergence application (single and splitting applications) on sugar, fodder and red beet. 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	
Ethofumesate 500 SC	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	> 108.8 µg ai/bee	Schmitzer, S., 2011b
	Acute contact		> 100 µg ai/bee	

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
Ethofumesate SC 500	Oral	1 x 1000	48 h LD ₅₀ > 108.8	< 9.2
	Contact		48 h LD ₅₀ > 100	< 10

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

The chronic study was designed as a limit test by exposing adult honey-bees for 10 consecutive days to a concentration of nominally 120 mg ai/kg in aqueous sugar solution. As the active substance is only moderately soluble in water (39 – 57 mg/L at pH 3 – 11 and at 20 – 30 °C), the test was conducted by using technical ethofumesate with 3 % acetone to enhance the solubility. The nominal test concentration as such equals 2 – 3 x the water solubility of ethofumesate.

No adverse lethal-, sub-lethal, behavioural or delayed effects were observed by exposing adult honeybees for ten consecutive days exclusively to sugar solution, containing 120 mg ethofumesate/kg (43.56 µg ai/bee), corresponding to 4.4 µg ai/bee/day.

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:

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

With

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

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
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

The ETR_{chronic adult oral} 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:

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

With

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

Ef... Exposure factor

twa... 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	twa	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.

Risk assessment for honeybee brood:

Table B. 9.6.1-6: Summary of effects of formulated ethofumesate (500 g/L, SC formulation) on honeybees and honeybee brood

Test substance	Exposure route	Results	Reference
Ethofumesate 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 typically present in the spray tank (2500 ppm)	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.

This particular study was conducted by mixing formulated ethofumesate via ethofumesate SC 500 into sugar solution and the tested concentration corresponded to a typical concentration ethofumesate present in the spray tank. The actual test concentration of ethofumesate was 2500 mg/L. The administration of 1 litre sugar solution per colony, containing 2500 ppm ethofumesate 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 Ethofumesate Task Force 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* were conducted.

Table B. 9.6.2-1: 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>Aphidius rhopalosiphi</i> (adults)	contact with dried residues on treated glass plates	Ethofumesate 44.7% w/w	500 1000	Corrected mortality / Reproduction	- 8.3 / - 68.3 - 8.0 / 18.7	Waltersdorfer, A., 2002a
				48 h LR ₅₀ > 1000 g ai/ha 48 h ER ₅₀ > 1000 g ai/ha		
<i>Typhlodromus pyri</i> (protonymphs)	contact with dried residues on treated glass plates	Ethofumesate 44.7% w/w	1000	Corrected mortality / Reproduction	2.1 / 16.4 *	Waltersdorfer, A., 2002b
				7 days LR ₅₀ > 1000 g ai/ha 14 d ER ₅₀ < 1000 g ai/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, fodder and red-beet (post-emergence) is a single rate of 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)

Test species	Application rate [g ai/ha]	MAF	Drift [%]	LR ₅₀ [g ai/ha]	HQ _{in-field}	HQ _{off-field}
<i>Aphidius rhopalosiphi</i>	1000	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 Ethofumesate 500 SC were submitted by the notifier for the re-newal of the EU approval of ethofumesate.

New studies on other soil macro-organisms (*Hypoaspis aculeifer*, *Folsomia candida*) were submitted with the 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. For details please refer to section B.8.

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 Ethofumesate 500 SC are given below.

B.9.7.1. Earthworms

For the first EU approval of the active substance ethofumesate acute earthworm studies were submitted addressing the risk to earthworms. According to the current data requirements for plant protection products (Regulation No 284/2013) acute toxicity studies are no longer required. Nevertheless, the study summaries from the DAR are included in the RAR as additional information.

Kühner, 1991a

Methods

The acute toxicity of Trammat 500 (500 g/L ethofumesate) to the earthworm, Eisenia foetida was investigated in an artificial soil under laboratory conditions. Earthworms were introduced to soils treated with Trammat at six different rates: 0, 100, 178, 316, 562 and 1000 mg/kg dry soil. Four replicates with ten adult worms were used for each treatment. The worms were incubated for 14 days at a temperature of 20 °C and constant light. After 7 and 14 days of exposure the worms were examined and counted, and the weight of the worms were recorded at the beginning and the end of the test.

Results

A mortality of 83% was recorded at the highest test concentration. No other mortalities were observed. A dose related decrease in weight (>10% compared to the control) was observed at 178 mg/kg and above. The 14 days

LC₅₀-value was not possible to determine, but was in the range 500 - 1000 mg/kg, based on nominal concentrations.

Comments

The study was stated to have been conducted in compliance with GLP, and in accordance with the OECD guideline 207.

For the first EU approval of the active substance one earthworm reproduction study with the formulated active substance has been evaluated in the Addendum to the DAR (2000) on ethofumesate. In addition, a new earthworm reproduction study with the representative formulation Ethofumesate 500 SC was submitted addressing the risk to soil organisms.

Reference:	Effects on growth and reproduction on earthworms (<i>Eisenia fetida</i>), Ethofumesate ; water miscible suspension ; 500 g/L
Author(s), year:	Sowing, P. and Gosch, H., 1999
Report/Doc. number:	Report No. C003978, Reference No. M-187147-01-1
Guideline(s):	BBA VI, 2-2 (1994)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate (water miscible suspension concentrate) 500 g/L, Batch no.: AE B049913 00 SC45 A103, 43.7% (w/w)
Test species:	Earthworm <i>Eisenia fetida</i> (<i>Eisenia fetida andrei</i> , det. Bouché 1982)
Number of organisms:	4 replicates per treatment control, each with 10 individuals.
Weight, age:	Mean: 0.365 – 0.446 g/worm, adults with clitellum > 7.5 months
Type of test, duration:	Laboratory sub-lethal test, 8 weeks
Applied concentrations:	Nominal: 0 (control), 1.5, 3.0 and 15 L test substance/ha corresponding to
Solvent:	Reagent grade water, no additional solvent was used.
Toxic standard:	Carbendazim (water miscible suspension concentrate) 360 g/L, Batch no.: AE F017411 00 SC42 A204, 41.6% (w/w)

Test conditions:

Test substrate:	Artificial soil, 10 % sphagnum peat, 20 % kaolin clay, 69 % industrial sand, 1% cow manure, moisture was measured at the beginning and the end of the study
Substrate/test vessel:	619 g dry weight/test container (2.8 L)
Temperature:	20 ± 2 °C
Air humidity:	40 – 90%
Light regime:	16 hours light (55 µE/m ² /s) / 8 hours dark

Feeding:	The worms were fed at day 7, 14 and 21 after application with 5 g dried and pulverised cow manure. Additionally, the test substrate was blended with 5 g cow manure at day 0 and day 28.
Test parameters:	<p>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.</p> <p>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)</p>
Statistics:	NOEC was determined by statistical analysis, employing Analysis of Variance, General Linear Models, Duncan's Multiple Range Test Procedures (SAS, 1989)
<u>Findings:</u>	
Water content:	<p>Day 0: 27.13% based on wet weight</p> <p>Day 56: 26.65% based on wet weight</p>
pH:	5.9 (day 0), 5.6 (day 56)
Effects test item:	<p>No statistically significant adverse effects compared to the control were observed regarding the body weight change of adults and the mean number of offspring.</p> <p>No mortalities of adults (after 28 days) and offspring (after 56 days) as well as no symptoms of intoxications were observed.</p>

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

	Control	Positive control	Ethofumesate 500 SC [L/ha]		
Exposure [L form./ha]		-	1.5	3.0	15
Mortality of adult earthworms [%] after 28 d	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [g] (Standard deviation)	0.15 (0.012)	0.13 (0.012)	0.14 (0.022)	0.17 (0.025)	0.17 (0.025)
Mean change of body weight of the adults from day 0 to day 28 [%]	35	33	32	47	44
Mean number of offspring per treatment group after 56 d (Standard deviation)	70 (11.5)	15 * (2.2)	65 (8.1)	68 (10.1)	67 (7.4)

<u>Conclusion:</u>	NOEC _{mortality} = 15 L prod./ha
	NOEC _{body weight} = 15 L prod./ha
	NOEC _{reproduction} = 15 L prod./ha

<u>Comment RMS:</u>	<p>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% (actual: 0%). The reduction of body weight of the adults in the control was $\leq 20\%$ (actual increase of body weight). The number of juveniles per control replicate was greater than 30 (actual: 53-77 juveniles per replicate). The coefficient of variation of the mean of juveniles in the control was $\leq 50\%$.</p> <p>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 (≥ 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.</p> <p>In addition, the study is well</p> <p>Based on the results of the study the NOEC was determined to be the highest test concentration, 15 L prod./ha, corresponding to 25 mg ai/kg soil dw (see Review Report for ethofumesate, SANCO/6503/VI/99-final, 2002).</p>
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Reference:	Ethofumesate SC 500 G: Effects on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil
Author(s), year:	Lühns, U., 2011
Report/Doc. number:	Report No. 64121022, Reference No. M-409272-01-1
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	- The water content of the soil at experimental end was $> 60\%$ of the total water holding capacity (maximum 62.3%). The food added during the experiment is moistened with deionised water causing an increase of the water content. The deviation was only slight and is considered as minor because the guideline recommendation of no standing or free water appearing when the soil was compressed was fulfilled.
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC 500 G (ETO SC 500 G), Batch no.: ECE5100755, 498.2 g/L (analysed), 44.1% w/w
Test species:	Earthworm <i>Eisenia fetida andrei</i> (Savigny, 1826)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, each with 10 individuals.

Weight, age:	Mean: 306 - 600 mg/worm, adults with clitellum, approximately 11 to 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), 62.5, 125, 250, 500 and 1000 mg prod./kg soil dw corresponding to 27.6, 55.1, 110, 221 and 441 mg ai/kg soil dw
Solvent:	None
Toxic standard:	Luxan Carbendazim 500 FC, tested at concentrations of 2.3, 3.0, 4.1, 5.6 and 7.5 mg ai/kg soil dw

Test conditions:

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: 20.7 – 23.0% (equivalent to 51.8 – 57.5% of WHC) Test end: 22.3 – 24.9% (equivalent of 55.8 – 62.3% of WHC)
pH:	Test start: 6.2 – 6.3 Test end: 6.4
Feeding:	Finely ground cattle manure was used as food; 10 g food/kg dry soil was mixed into the artificial soil 1 day before the start of the study. 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:	Body weight change and reproduction data were tested for normal distribution and homogeneity of variance using the Kolmogorov-Smirnov test and the Cochran's test. Because data of body weight changes and reproduction were normally distributed and homogeneous, the Williams t-test was used (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$). The EC ₅₀ for reproduction was calculated by Probit Analysis (Finney 1971). 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
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abnormalities were observed and all worms burrowed into soil within 15 minutes after introduction.

Table B. 9.7.1-2: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	Ethofumesate 500 SC [mg prod./kg soil dw]				
Exposure	-	62.5	125	250	500	1000
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] (± SD)	247 (22)	256 (32)	215 (30)	175 * (30)	122 * (23)	60 * (61)
Mean change of body weight of the adults from day 0 to day 28 [%] (± SD)	55.6 (6.3)	57.9 (9.5)	48.5 (7.6)	39.6 * (8.5)	27.5 * (5.8)	14.0 * (14.2)
Mean number of offspring per treatment group after 56 d (± SD)	308 (42)	296 (27)	248 ** (28)	169 ** (38)	61 ** (14)	10 ** (5)
Reproduction compared to control [%]	-	95.6	80.2 **	54.6 **	19.6 **	3.1 **

SD... Standard Deviation

* 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) there were statistically significant effects on reproduction at a concentration of 1.0 mg ai/kg soil dw and higher; the EC₅₀ for reproduction was calculated as 1.21 mg ai/kg soil dw.

Conclusion:

NOEC (adult mortality) = 1000 mg prod./kg soil dw

NOEC (body weight) = 125 mg prod./kg soil dw

NOEC (reproduction) = 62.5 mg prod./kg soil dw

EC₅₀ (reproduction) = 260.5 mg prod./kg soil dw (95% C.I. 236.2 – 287.2 mg prod./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 249 - 364 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 13.6%).

Based on results of the study a NOEC of 62.5 mg prod./kg soil dw (= 27.6 mg ai/kg soil dw) based on statistically significant effects on the reproduction was determined.

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

According to the data requirements on active substances (Regulation 283/2013) and formulation (Regulation 284/2013) the risk to soil dwelling organisms has to be addressed (1) if a risk to non-target arthropods was identified or (2) if the product is applied to the bare soil (pre-emergence).

Under consideration of the intended uses on beets (BBCH 16 – 18) the risk to soil meso- and macrofauna from exposure to the active substance and its major soil metabolites has to be addressed. Even though, no pre-emergence use is intended the soil cover of sugar, fodder and red beets at BBCH 16 to 18 is low. Therefore, the risk to soil dwelling organisms has to be considered.

Hence, laboratory studies with the soil organisms *Folsomia candida* and *Hypoaspis aculeifer* were submitted by the Task Force Ethofumesate.

Reference:	Ethofumesate SC 500 G: Influence on the reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil
Author(s), year:	Frommholz, U., 2011
Report/Doc. number:	Report No. E 314 4242-0, Reference No. M-420052-01-1
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC 500 G (ETO SC 500 G), Batch No.: ECE5100755, 498.2 g/L (analysed), 44.1% w/w
Test species:	Collembola <i>Folsomia candida</i>
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, 11-12 days old
Type of test, duration:	Laboratory sub-lethal test, 28 days
Applied concentrations:	Nominal: 0 (control), 100, 178, 316, 562 and 1000 mg prod./kg soil dw corresponding to 44.1, 78.5, 139, 248 and 441 mg ai/kg soil dw
Solvent:	None
Toxic standard:	Boric acid, tested at concentrations 44, 67, 100, 150 and 225 mg ai/kg soil dw

Test conditions:

Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	30 g wet weight/test container
Temperature:	20 ± 2 °C
Light regime:	16 hours light (555 - 577 lx) / 8 hours dark

Water content:	Test start: 21.78 – 22.97% (equivalent to 45.93 – 49.18% of WHC) Test end: 20.49 – 22.73% (equivalent of 42.52 – 48.43% of WHC)
pH:	Test start: 5.58 – 5.65 Test end: 5.52 – 5.54
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:	Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test ($\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values. The $LC_{10, 20, 50}$ values were determined by Probit analysis. 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	Ethofumesate SC 500 G [mg prod./kg soil dw]				
Exposure	-	100	178	316	562	1000
Mortality of adult Collembola [%] after 28 d	11.3	12.5	10.0	5.0	7.5	7.5
Mean number of juveniles per treatment group after 28 d (\pm SD)	1342.8 (68.9)	1341.3 (139.4)	1186.8 * (224.1)	837.5 * (123)	330.3 * (100.1)	311.5 * (95.2)
Reproduction compared to control [%]	-	99.9	88.4 *	62.4 *	24.6 *	23.2 *

SD...Standard Deviation

* Statistically significantly different compared to the control (William's T-test, one-sided smaller, $\alpha = 0.05$)

Boric acid showed an EC_{50} of 91 mg test item/kg soil dw (95% C.I. 80 – 104 mg test item/kg soil dw). The NOEC based on reproduction was calculated to be 44 mg test item/kg soil dw. This shows that the test organisms are sufficiently sensitive.

Conclusion:

NOEC (mortality) = 1000 mg prod./kg soil dw (= 441 mg ai/kg bw/d)

NOEC (reproduction) = 100 mg prod./kg soil dw (= 44.1 mg ai/kg soil dw)

EC_{10} (reproduction) = 149 mg prod./kg soil dw (95% C.I. 20 – 241 mg prod./kg soil dw) (= 65.7 mg ai/kg soil dw)

<u>Comment RMS:</u>	<p>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: 11.3%). The mean number of juveniles per control replicate was greater than 100 (being 1249 - 1396 juveniles per replicate). The coefficient of variation of reproduction in the control was $\leq 30\%$ (being: 5.1%).</p> <p>Based on results of the study a NOEC of 100 mg prod./kg soil dw (= 44.1 mg ai/kg soil dw) based on effects on reproduction was determined.</p>
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Reference:	Ethofumesate SC 500 G: Influence on mortality and reproduction on the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil
Author(s), year:	Kratz, M.-A., 2011
Report/Doc. number:	Report No. E 428 4243-7, Reference No. M-417391-01-1
Guideline(s):	OECD 226 (2008)
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC 500 G (ETO SC 500 G), Batch no.: ECE5100755, 498.2 g/L (analysed), 44.1% w/w
Test species:	Predatory mites, <i>Hypoaspis aculeifer</i>
Number of organisms:	8 replicates per control and treatment group, 1 additional replicate for control and treatment for measurement of pH value and moisture of the artificial soil, each with 10 female adults.
Life stage, age:	Adult females, 28 days after start of egg laying
Type of test, duration:	Laboratory sub-lethal limit test, 14 days
Applied concentrations:	Nominal: 0 (control) and 1000 mg prod./kg soil dw corresponding to 441 mg ai/kg soil dw
Solvent:	None
Toxic standard:	Dimethoate tested at concentrations 0.99, 1.78, 3.156, 5.517 and 9.853 mg ai/kg soil dw.

Test conditions:

Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate
Substrate/test vessel:	20 g dry weight/test container
Temperature:	20.0 \pm 2 °C

Light regime:	16 hours light (617 - 743 lx) / 8 hours dark
Water content:	Test start: 21.78 – 22.88% (equivalent to 45.93 – 48.94% of WHC) Test end: 22.30 – 23.52% (equivalent of 47.34 – 50.74% of WHC)
pH:	Test start: 5.58 – 5.64 Test end: 5.62 – 5.67
Feeding:	The mites were fed with cheese mites (<i>Tyrophagus putrescentiae</i>) at test start, 3, 8 and 10 days after test start.
Test parameters:	pH and water content were determined at test start and test end. Mortality of adults, differences in morphology, behavioural effects and number of juveniles were assessed after 14 days
Statistics:	The mean number of surviving adult, female <i>Hypoaspis aculeifer</i> for control and treatment were determined. Determination of the percent mortality for control and treatment were calculated. Determination of mean number of juveniles for control and treatment and the percent values for treatment in comparison to the untreated control were calculated. For normal distribution and homogeneity of variance using Kolmogoroff-Smirnov Test and Cochran-Test ($\alpha = 0.05$), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore Student t-test (one-sided smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

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

	Control	Ethofumesate 500 g/L SC [mg prod./kg soil dw]
Exposure	-	1000
Mortality of adult mites [%] after 14 d	7.5	2.5
Mean number of juveniles per treatment group after 14 d (\pm SD)	338.5 (61.4)	394.4 (33.2)
Reproduction compared to control [%]	-	116.5

SD... Standard Deviation

Dimethoate showed a LC_{50} of 4.051 mg ai/kg soil dw and a $NOEC_{\text{reproduction}}$ of 3.156 mg ai/kg soil dw. The results of the test show that the test organisms are sufficiently sensitive.

Conclusion: NOEC (mortality, reproduction) = 1000 mg prod./kg soil dw (= 441 mg ai/kg bw/d)

<u>Comment RMS:</u>	<p>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: 7.5%). The mean number of juveniles per control replicate was greater than 50 (being: 220 - 409 juveniles per replicate). The coefficient of variation of reproduction in the control was $\leq 30\%$ (being: 18.1%).</p> <p>Based on results of the study a NOEC of 1000 mg prod./kg soil dw based on the highest tested concentrations was determined.</p>
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In addition to the studies on earthworms and other soil macro-organisms (*Folsomia candida* and *Hypoaspis aculeifer*) a litter bag study was submitted addressing possible adverse effects on the soil litter degradation.

The study was not evaluated for the first EU approval of the active substance ethofumesate.

Reference:	Ethofumesate SC45 (AE B049913 00 SC45 A203): Effects on soil litter degradation
Author(s), year:	Lechelt-Kunze, C., 2003
Report/Doc. number:	Report No. E 427 2323-3, Reference No. M-219686-01-1
Guideline(s):	This study was conducted according to the "Minutes of a meeting on the requirement of data according to EU Directive 91/414/EEC, Annex III, point 10.6.2" from Kula C. and Guske S., BBA, Germany, March 2001 and according to recommendations of the EPFES Workshop, Lisbon, Portugal, April 2002
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC45, Batch no.: ACBC 0625, Content of ethofumesate: 43.8% w/w (analysed)
Location:	Bayer Experimental Farm Höfchen, Burscheid, Germany
Replicates:	6 replicates (field plots) per control and treatment group, plot size: 144 m ² (12 m x 12 m), 48 litter bags per replicate
Type of test:	Litter bag field test
Applied concentrations:	Application of soil plateau concentration: 75 g ai/ha (= 171 g test item/ha) corresponding to a plateau soil concentration of 50 µg ai/kg soil Application of annual application rate: 1000 g ai/ha (= 2283 g test item/ha)
Toxic standard:	None

Test conditions:

Soil characterisation: Silt loam soil (4.4% sand, 75.9% silt and 19.7% clay), pH = 5.9, C_{ORG} = 0.997%, WHC = 56.9 g H₂O/100 g soil dw

Litter bags: Litter bags (polyester, 22 x 12 cm, mesh size 8 mm) were filled with 4 g dry wheat straw.

Test method: The soil plateau concentration (50 µg ai/kg soil) was applied as Ethofumesate SC45 in a volume of 300 L water/ha. By careful harrowing the test item was incorporated into the upper 10 cm soil layer. One day after the application soil samples were taken. 14 days after the application untreated perennial ryegrass (*Lolium perenne*) was sown onto all plots with a seed rate of 45 kg/ha. In addition the litter bags were buried at a depth of 5 cm in the centre of the plots. The distance between the litter bags in the plots was approximately 15 cm in each direction. 8 litter bags per sampling time and 6 projected sampling times led to a total of 576 litter bags.

On the same time the annual application rate (1000 g ai/ha) was applied as Ethofumesate SC45 in a volume of 300 L water/ha. Soil samples were randomly taken after application of the annual rate and one day after irrigation.

Test parameters: Soil samples (20 soil cores and one sample per treatment plot) were randomly taken one day after the application.

28 days, 56 days, 108 days, 183 days and 274 days after burying of the litter bags 8 litter bags per plot and sampling time were randomly selected. The content of each litter bag was mortared, sieved and dried at a temperature of 35°C. The degree of degradation was determined taking into account the difference of weight of straw at the start and the end of the test.

Statistics: In order to determine whether the results reveal statistically significant differences, the %-degraded-straw data of the two variants (untreated control and treatments with Ethofumesate SC45) were analysed with the program ToxRat®, Version 2.07 from ToxRat Solutions GmbH, Naheweg 15, 52477 Alsdorf, Germany.

Cochran's C test revealed homogeneity of variance of the %-degraded-straw data of the two variants (untreated control and treatments with Ethofumesate SC45) at a 95.0 % confidence level for the 0-28 day data, the 0-56 day data, the 0-108 day data, the 0-183 day data and the 0 - 274 day data.

All data were normally distributed as tested by Kolmogoroff-Smimow Test. The following Student-t test (two sided) revealed no significant differences between the treatment and the control at a 95 % confidence level for all data

Findings:

Analytical results: The application of the estimated plateau concentration of Ethofumesate resulted in soil residues of 42.9 µg Ethofumesate/kg dry soil, which is 85.8 % of the nominal amount of 50 µg/kg. The application of the annual rate of Ethofumesate SC45

resulted in soil residues of 769 µg Ethofumesate/kg dry soil, corresponding to 107 % of the nominal amount directly after the spray application. Two days after application of the annual rate and one day after irrigation still 723 µg Ethofumesate/kg dry soil (101% of the nominal amount) were determined.

Table B. 9.7.2-3: Degradation of straw in litter bags

	Mean straw degradation [g] (± SD)				
	0 - 28 d	0 - 56 d	0 - 108 d	0 - 183 d	0 - 274 d
Control group	0.92 (0.06)	1.37 (1.2)	1.61 (0.12)	1.83 (0.08)	2.86 (0.18)
Treatment group	0.92 (0.07)	1.44 (0.08)	1.64 (0.13)	1.90 (0.11)	3.00 (3.18)
	Mean straw degradation [%] (± SD)				
	0 - 28 d	0 - 56 d	0 - 108 d	0 - 183 d	0 - 274 d
Control group	23.04 (1.52)	34.15 (2.95)	40.29 (2.97)	45.64 (1.9)	71.62 (4.47)
Treatment group	23.06 (1.69)	36.03 (2.1)	40.88 (3.32)	47.62 (2.78)	74.92 (4.6)
	Percentage of control [%]				
	100	106	101	104	105

SD... Standard Deviation

Conclusion:

From the results of this study it can be concluded that soil residues of Ethofumesate SC45 to be anticipated after long-term use (plateau concentration) plus spray application of Ethofumesate SC45 (= the annual application rate) have no influence on organic matter breakdown, after 1, 2, 3, 6 and 9 months.

Comment RMS:

The test is considered valid if at least 60 % soil litter degradation has occurred at the end of the study in the control plots. Degradation of 71.62 % (see Table 11) straw in untreated control was reached 9 months (247 days) after introduction of litter bags into soil.

A coefficient of variation of 40 % for soil litter degradation in the control plots is recommended for those data generated within the first 6 months of the study. In the present study coefficients of variation were calculated to be 6.60 % (one month), 8.64 % (two months), 7.36 % (three months) and 4.16 % (six months).

Since both recommended validity criteria are met, this study is considered to be valid.

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

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

A summary of the toxicity of the formulation Ethofumesate 500 SC, the active substance and its soil metabolites to earthworms and other soil macro-organisms is provided below.

Table B. 9.7.2-1: Summary of effects on soil meso- and macrofauna

Species	Substance	Endpoint	Reference
<i>Eisenia fetida</i>	Metabolite NC8493	56 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012a
		56 d NOEC = 16 mg/kg soil dw	Lühns, U., 2011
	Metabolite NC 20645	56 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012b
	Ethofumesate 500 SC	56 d NOEC = 25 mg ai/kg soil dw	Sowing, P. & Gosch, H.
	Ethofumesate 500 SC	56 d NOEC = 27.6 mg ai/kg soil dw	Lühns, U., 2011
<i>Folsomia candida</i>	Metabolite NC8493	28 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2012c
		28 d NOEC = 556 mg/kg soil dw	Friedrich, S., 2013a
	Metabolite NC 20645	28 d NOEC = 100 mg/kg soil dw	Friedrich, S., 2013b
	Ethofumesate 500 SC	28 d NOEC = 44.1 mg ai/kg soil dw	Frommholz, U., 2011
<i>Hypoaspis aculeifer</i>	Metabolite NC8493	14 d NOEC = 309 mg/kg soil dw	Schulz, L., 2013
	Ethofumesate 500 SC	14 d NOEC = 441 mg ai/kg soil dw	Kratz, M.-A., 2011
<p>Litter bag test: Test item was the herbicide Ethofumesate SC45 (code: AE B049913 00 SC45 A203, analysed content of ai 43.8 % w/w)</p> <p>Six plots in a field (Germany) were treated with Ethofumesate SC45. Six plots served as untreated control plots. An amount of 75 g ai/ha (= 171 g prod./ha), corresponding to a plateau concentration of Ethofumesate of 0.05 mg ai/kg soil, was applied in a volume of 300 L water/ha to the treatment plots. By careful harrowing the test item was incorporated into the upper 10 cm soil layer. About 2 weeks later untreated perennial ryegrass (<i>Lolium perenne</i>) was sown onto all plots. The seed rate was 45 kg/ha. Directly after sowing 48 litter bags (12 cm x 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. The same day an amount of 1000 g ai/ha (= 2283 g prod./ha), the calculated annual application rate of ethofumesate, was applied in a volume of 300 L water/ha to the treatment plots.</p> <p>The application of the estimated plateau concentration of ethofumesate resulted in soil residues of 42.9 µg ai/kg dry soil, which is 85.8 % of the nominal amount of 50 µg/kg. The application of the annual rate of Ethofumesate SC45 resulted in soil residues of 769 µg ai/kg dry soil, corresponding to 107 % of the nominal amount directly after the spray application.</p> <p>At no sampling time (28, 56, 108, 183 and 274 days after introduction of litterbags into the soil), a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with Ethofumesate SC45.</p> <p>Degradation of ≥ 60 % straw in untreated control was reached 9 months (274 days) after introduction of litter bags into soil and the study was terminated. The recommended coefficient of variation of 40 % for soil litter degradation in the control plots for those data generated within the first 6 months of the study was fulfilled.</p> <p style="text-align: right;">(Lechelt-Kunze, C., 2003)</p>			

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 (20% interception) and was calculated to be 1.069 mg ai/kg soil (see fate section B.8). The other uses (multiple applications) are covered by the risk assessment for the single post-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. 9.7.2-2: 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, red beet and fodder beets (post-emergence) 1 x 1 kg ai/ha	Ethofumesate 500 SC	NOAEC = 12.5 ai *	1.069	11.7	5
	Metabolite NC 8493	NOEC = 16.0	0.233	68.7	5
<i>Folsomia candida</i>					
Sugar beets, red beet and fodder beets (post-emergence) 1 x 1 kg ai/ha	Ethofumesate 500 SC	NOEC = 22.05 ai *	1.069	20.6	5
	Metabolite NC 8493	NOEC = 100	0.233	429	5
<i>Hypoaspis aculeifer</i>					
Sugar beets, red beet and fodder beets (post-emergence) 1 x 1 kg ai/ha	Ethofumesate 500 SC	NOEC = 220.5 ai *	1.069	206	5
	Metabolite NC 8493	NOEC = 309	0.233	1326	5

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

Based on the risk assessment an acceptable long-term risk to earthworms and other soil macro-organisms from exposure to the formulated active substance and its metabolite was identified. The TER_{LT} value for the worst-case post-emergence application (1 x 1 kg ai/ha) was above the trigger of 5.

Overall, the risk to soil macro- and mesofauna is considered low and no further information is required addressing the risk to soil organisms.

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 Ethofumesate 500 SC was submitted. A summary of the study is provided below.

Studies on nitrogen mineralisation with the relevant soil metabolite NC 8493 were provided by the Task Force Ethofumesate addressing the risk to soil micro-organisms.

The notifier also submitted a study with the metabolite NC 20643. The study was evaluated and summarised (see RAR, Volume 3 – B.9. (A.S.) but the results of the study were not used in the risk assessment as the metabolite NC 20643 is not considered a ecotoxicological relevant soil metabolite.

Reference:	Ethofumesate SC 500 G: Effects on the activity of soil microflora (nitrogen transformation test)
Author(s), year:	Schulz, L., 2011
Report/Doc. number:	Report No.: 11 10 48 059 N, Reference No.: M-419266-01-1
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate SC 500 G (ETO SC 500 G), Batch No.: ECE5100755, Content of active substance: 498.2 g/L, 44.1% w/w (analysed)
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 42 days
Applied concentrations:	0 (control), 3.01 and 30.11 mg test item/kg soil dw (corresponding to 1.3 and 13.3 mg ai/kg soil dw)
Solvent/vehicle:	None
Toxic standard:	Dinoterb
Test substrate:	Agriculturally utilised soil (loamy sand), removed to a depth of 20 cm, from a field located in Canitz, Germany. No application of fertilisers and plant protection products since 2003 and 1990, respectively. C _{ORG} 1.39 %, pH: 6.4, Humus content: 2.39%, Carbon content of microbial

	biomass: 33.45 mg C/100 g soil dw (corresponding to 2.41 % of C _{ORG})
	Total nitrogen content: 0.13%
	Water holding capacity (WHC): 36.67 g/100 g soil dw
	Texture according to DIN 11277: 10.2 % clay, 38.1 % silt, 51.7 % sand
	0.5% (i.e. 1.0 g/200 g soil dw) lucerne meal
Substrate/test vessel:	200 g soil dw/500 mL wide mouth glass flasks (treatment and control groups, 3 replicates per treatment)
Incubation:	19.1 – 21.1°C, darkness
Water content	Test start: 15.65 – 16.21 g/100 g soil dw (> 40% of WHC) Test end: 15.28 – 15.85 g/100 g soil dw (> 40% of WHC)
pH:	Test start: 6.2 Test end: 6.2 – 6.3
Test parameters:	The nitrogen transformation was determined on day 0 (after approximately 3 hours), and at intervals of 7, 14, 28 and 42 days after application. Samples (10 g soil dw) were extracted with 50 mL 1M KCl, mixed on a rotator at 150 rpm for 60 minutes, centrifuged and stored deep-frozen prior to analysis at 20 ± 5 °C. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used.
Statistics:	Statistical evaluation of the test results (2-sided Student-t-test for homogeneous variances at 5 % significance level, 2-sided Welch-t-test for inhomogeneous variances at 5 % significance level) was performed.
<u>Findings:</u>	
Nitrogen transformation:	The test item Ethofumesate SC 500 G caused temporary stimulations of the daily nitrate rate at the tested concentrations of 3.01 mg/kg and 30.11 mg/kg dry soil until the time intervals 7-14 and 14-28 days after application, respectively. However, no adverse effects of Ethofumesate SC 500 G on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 42 days after application (time interval 28-42). Only negligible differences to control of -16.0 % (test concentration 3.01 mg/kg dry soil) and -14.3 % (test concentration 30.11 mg/kg dry soil) were measured at the end of the 42-day incubation period (time interval 28-42).

Table B.9.9-1: Effects of Ethofumesate SC 500 G on nitrogen transformation (mean values \pm SD)

Treatment	Time (days)	Mean Nitrate-N [mg/kg soil dw/d] (\pm SD)	% difference to the control [%]
Control	0-7	1.39 (0.14)	-
	7-14	0.66 (0.10)	-
	14-28	0.61 (0.00)	-
	28-42	0.82 (0.03)	-
3.01 mg/kg soil dw	0-7	1.82 (0.05)	+ 31.6 *
	7-14	1.03 (0.07)	+ 56.5 *
	14-28	0.64 (0.04)	+ 4.7 #
	28-42	0.69 (0.15)	- 16.0
30.11 mg/kg soil dw	0-7	2.16 (0.06)	+ 56.0 *
	7-14	1.17 (0.16)	+ 78.3 *
	14-28	0.83 (0.09)	+ 35.9 #
	28-42	0.70 (0.12)	- 14.3

* Statistically significantly different to control, Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$

No statistical analyses could be performed due to mathematical reasons

In a separate test the toxic standard dinoterb caused an effect of + 42.0 %, + 68.1 % and + 92.3 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Conclusion:

Ethofumesate SC 500 G caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as $\text{NO}_3\text{-N}$ production) at the end of the 42-day incubation period. The study was performed in a field soil at concentrations up to 30.11 mg test item/kg soil, which is equivalent up to an application rate of 20 L test item/ha.

Comment RMS:

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

According to the test guideline the study is considered valid if the coefficients of variation in the control for $\text{NO}_3\text{-N}$ were $\leq 15\%$. In this study the CV in the control were maximum 2.9% and thus fulfilled the validity criteria.

Based on results of the study a EC_{25} of > 30.11 mg prod./kg soil dw was determined.

In addition to the laboratory studies literature data on soil microbial communities were submitted by the notifier. A summary of the studies is given below.

Reference:	Fertilization can modify the non-target effects of pesticides on soil microbial communities.
Author(s), year:	Ruiz-Romera et al., 2012
Report/Doc. number:	Reference No. M-458656-01-1
Guideline(s):	ISO 16072, 2002. Soil Quality e Laboratory Methods for Determination of Microbial Soil Respiration. ISO 17155, 2002. Soil Quality e Determination of Abundance and Activity of Soil Microflora Using Respiration Curves
GLP:	No

Executive summary:

A three-month laboratory mesocosm experiment was performed to unravel interactions between pesticides (difenoconazole: fungicide, deltamethrin: insecticide, ethofumesate: herbicide) and fertilizers (NPK synthetic fertilizer, compost) regarding the potential non-target effects of pesticides on soil microbial communities. To this aim, pesticides and fertilizers were applied to soil at a rate of 5 mg active ingredient/kg DW soil and 185 mg N/kg DW soil, respectively. Soil sampling was done after 0, 7, 30, 60 and 90 days of incubation in order to determine pesticide degradation rates and microbial properties: enzyme activities, basal respiration, substrate-induced respiration, potentially mineralizable N, nitrification rate and denitrification potential. By the end of the incubation, ethofumesate in non-fertilized soils was degraded by 93%, with a half-life of 29 days. NPK fertilization led to a 26% increase in ethofumesate half-life in soil. A short-term antagonistic effect between NPK fertilization and ethofumesate presence was found regarding their inhibitory effect on potentially mineralizable N. In compost-fertilized soils, ethofumesate counteracted the stimulatory effect of compost on denitrification potential.

<u>Comment RMS:</u>	A statistically significant increase of the respiratory quotient Q_R in ethofumesate treated soils was only observed on day 7, while there was no significant difference at the later sampling moments. Similarly, a weak reduction (14%) in mineralizable nitrogen (N_{min}) was found in the ethofumesate treatment group on day 7 only. The statistically significant reduction in N-NO ₂ values on days 7, 60 and 90 after application was $\leq 25\%$ at days 60 and 90, which indicates a low risk to microbial activity according to the requirements of Regulation No. 1107/2009. N-NO ₂ values were obtained by measuring bar graphs in the results section and calculating the reduction in the ethofumesate treatment group compared to the untreated control. The reduction in the treated-soil quality index (T-
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SQI) of 9.6% in ethofumesate treated soils also lies below 25%. All comparisons were made between the non-fertilized ethofumesate treatment group and the non-fertilized control. In conclusion, no unacceptable risk to soil microbial communities is to be expected by the application of ethofumesate.

Reference:	Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil.
Author(s), year:	Ruiz-Romera et al., 2012
Report/Doc. number:	Reference No. M-462303-01-1
Guideline(s):	ISO 16072, 2002. Soil Quality e Laboratory Methods for Determination of Microbial Soil Respiration. ISO 17155, 2002. Soil Quality e Determination of Abundance and Activity of Soil Microflora Using Respiration Curves.
GLP:	No

Executive summary:

An experiment was performed to study non-target effects of inter alia ethofumesate (herbicide) on microbial parameters in a clay-loam soil. Pesticides were applied as commercial formulations to soil samples at different concentrations (5, 50 and 500 mg/kg dws) and then incubated under laboratory conditions for 3 months. Throughout the incubation period, microbial parameters were determined at days 7, 30, 60 and 90. At 5 mg/kg dws, ethofumesate did not cause significant changes in soil microbial parameters. In contrast, at 500 mg/kg dws, pesticide application decreased overall soil microbial activity, negatively affecting the activity of soil enzymes. Similarly, at 500 mg/kg dws, ethofumesate caused a pesticide-induced stress on soil microbial communities, as reflected by the respiratory quotient. Besides, ethofumesate at 50 and 500 mg/kg dws resulted in lower values of denitrification potential. It was concluded that, although pesticide concentration had a somewhat inconsistent and erratic effect on soil microbial parameters, pesticide application at 500 mg/kg dws did have an impact on many of the microbial parameters studied here.

<u>Comment RMS:</u>	At 5 mg ai/kg no adverse effect on soil microbial communities was observed. This concentration is about five times as high as the PEC _{soil} value of ethofumesate based on the recommended field application rate. The tested concentrations of 50 and 500 mg ethofumesate/kg are more than 50 and more than 500 times higher than PEC _{soil} values realistically occurring in the field. Therefore, no unacceptable risk to soil microbial communities is to be expected by the application of ethofumesate according to the proposed used pattern.
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B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The toxicity of ethofumesate on soil micro-organisms is summarised below.

Table B.9.10-1: Summary of effects on non-target micro-organisms (nitrogen transformation)

Test substance	Test concentration	Time	Effects (deviation from control)	Reference
Ethofumesate techn.	0.3 mg ai/kg soil dw	42 d	< 20 %	Vonk, J.W., 1988
	3.0 mg ai/kg soil dw		< 20 %	
Ethofumesate 500 SC	3.01 mg prod./kg soil dw	42 d	- 16.0 %	Schulz, L., 2011
	30.1 mg prod./kg soil dw		- 14.3 %	
Metabolite 8493	1.20 mg/kg soil dw	28 d	- 1.4 %	Schulz, L., 2013a
	12.0 mg/kg soil dw		+ 15.2 %	
Metabolite NC 20645	1.38 mg/kg soil dw	28 d	+ 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, accumulation}	Risk acceptable?
Ethofumesate 500 SC	13.0 mg ai/kg soil dw	1.069 mg ai/kg soil dw	Yes
Metabolite 8493	12.0 mg/kg soil dw	0.233 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 13 mg ai/kg soil dw. The initial PEC_{soil} after application in sugar, red and fodder beets, post-emergence was calculated to be 1.069 mg ai/kg soil. Thus the exposure concentration used in the tests was approximately 10 times higher than the maximal expected PEC_{soil} when applied according to the GAP.

Based on the results a toxicity endpoint for the metabolite NC 8493 of 12.0 mg/kg soil dw was determined. Under consideration of an initial PEC_{soil} of 0.233 mg/kg soil dw the risk to soil micro-organisms from exposure to the metabolite can be considered acceptable.

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 were 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). Phytotoxicity, 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.

AgrEvo, 2000*Comment*

A summary sheet with screening data on non-target fauna including insects, diseases, molluscs, endoparasites, bacteria and nematodes was submitted by AgrEvo. No serious effects were identified for any of the tested species.

B.9.11.2. Testing on non-target plants

Seedling emergence and vegetative vigour studies have been conducted with the representative formulation Ethofumesate SC 500 following the EPA guideline 123-1 or the OECD test guidelines.

Reference:	Effect on Vegetative Vigor of Non-Target Terrestrial Plants (Tier II) AE B049913: Suspension Concentrate (43.8% w/w)
Author(s), year:	Christ, M.T. & Abedi, J., 2003a
Report/Doc. number:	Report No. 02XB28225, Reference No. M-241176-01-1
Guideline(s):	US EPA 123-1 (1982)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Norton® SC (AE B049913 00 SC45 A203), Batch No.: ACBC 0625, 43.8% w/w
Type of test:	Vegetative vigour test
Test duration:	21 days
Test species:	6 dicotyledonous and 4 monocotyledonous plant species <i>Daucus carota</i> (carrot), <i>Zea mays</i> (corn), <i>Cucumis sativus</i> (cucumber), <i>Lactuca sativa</i> (lettuce), <i>Avena sativa</i> (oat), <i>Allium cepa</i> (onion), <i>Raphanus sativus</i> (radish), <i>Glycine max</i> (soybean), <i>Lycopersicon esculentum</i> (tomato) and <i>Triticum aestivum</i> (wheat)
Test soil:	Mixture of natural topsoil and sand (2:1) to achieve a sandy loam substrate with an organic matter content of < 2%.
Applied concentrations:	Control: Deionized water Test item: 0.32, 0.63, 1.25, 2.5, 5.0 and 10 L prod./ha (carrot, corn, cucumber, onion, lettuce, radish and soybean) 0.16, 0.32, 0.63, 1.25, 2.5 and 5.0 L prod./ha (wheat, oat and tomato)
Replicates:	Six replicates with five plants per pot for each species
Exposure route:	Seeds were planted in test pots consisted of 15.2 cm diameter with bottom drainage holes. Test pots were filled with test soil and seeds were planted to a seed specific depth (1.3 – 2.5 cm dependent on the seed). The pots were placed in a temperature and light supplemented greenhouse following planting. Temperature, relative humidity, and light intensity were recorded at two positions in the greenhouse. For all species, seedling age at test initiation ranged from one to three weeks following planting (2-4 leaf stage) and test duration was 21 days following application of the test substance.

Test conditions:	Spray treatments were applied once each to each species at test initiation with an Allen Track sprayer set at a nominal spray volume of 200 L/ha. Following spray application all pots were placed in a temperature supplemented greenhouse.
	Photoperiod: 15 hour light and 9 hour dark
	Light intensity: 179.9 – 361.9 $\mu\text{mol}/\text{m}^2/\text{s}$
	Temperature: 24.0 – 26.7 °C
Test parameter:	Relative humidity: 21.5 – 71.1 %
	Plants were assessed for survival and rate for phytotoxicity on Day's 7, 14 and 21. Individual plant lengths were determined on Day's 7, 14 and 21 while individual plant dry weights were determined at study termination.
Analytical measurements :	Spray stock solutions of each treatment were analysed for the quantification of ethofumesate by High Performance Liquid Chromatography (HPLC) with Ultraviolet Detection (UV).
Statistics :	Descriptive statistics (mean, standard deviation, and coefficient of variation) were calculated for each treatment and for each test parameter (shoot length and shoot dry weight). The EC_{25} and EC_{50} (with the 95% confidence intervals) were calculated following the non-linear regression equation based on Bruce and Versteeg.
	The NOEC and LOEC were identified using hypothesis testing methodology.
	All Day 21 data were subjected to a Shapiro-Wilk's Test to assess departures from a normal distribution and a Bartlett's Test to determine homogeneity of variance. To assess treatment effects for homoscedastic and normally distributed data, a Dunnett's one-way analysis of variance (ANOVA) and multiple means comparison procedure for equal replicates was used to determine those exposure concentrations exhibiting responses significantly different ($P < 0.05$) than the control group. In addition, a William's Test was used since it is more sensitive to a response due to increasing concentrations of toxicant.

Findings:

Analytical measurements: The mean measured value of ethofumesate ranged from 94 to 98 % of nominal and indicated that the desired application rates were achieved.

Biological results: The most sensitive plant species was observed to be the monocotyledonous species wheat and the dicotyledonous species tomato and soybean. The lowest endpoint was determined to be EC_{50} 1.16 L prod./ha based on effects of the shoot dry weight of tomatoes.

Table B. 9.11.2-1: EC_{25} , EC_{50} and NOEC values

Test species	Shoot length		Shoot dry weight		NOEC
	EC ₂₅ (95% CI)	EC ₅₀ (95% CI)	EC ₂₅ (95% CI)	EC ₅₀ (95% CI)	
	[L prod./ha]				
Carrot ^d	> 10 (na)	> 10 (na)	> 10 (na)	> 10 (na)	10

Test species	Shoot length		Shoot dry weight		NOEC
Corn ^m	> 10 (na)	> 10 (na)	> 10 (na)	> 10 (na)	10
Cucumber ^d	0.97 (0.54-1.43)	> 10 (na)	0.89 (0.28-2.86)	7.66 (3.77-15.53)	0.04 ^a
Lettuce ^d	4.76 (2.67-8.46)	> 10 (na)	2.04 (0.64-6.49)	> 10 (na)	1.25
Oat ^m	4.48 (na)	> 10 (na)	2.18 (1.42-3.35)	> 10 (na)	0.63
Onion ^m	> 10 (na)	> 10 (na)	8.85 (nc)	> 10 (na)	5.0
Radish ^d	> 10 (na)	> 10 (na)	0.52 (nc)	> 10 (na)	0.06 ^a
Soybean ^d	0.33 (0.27-0.47)	1.93 (1.30-3.26)	0.86 (0.37-1.99)	7.31 (4.43-12.07)	0.04 ^a
Tomato ^d	1.85 (1.14-3.02)	> 10 (na)	0.45 (0.21-0.98)	1.16 (0.70-1.91)	0.32
Wheat ^m	1.92 (1.30-2.83)	> 10 (na)	0.48 (0.27-0.84)	1.24 (0.86-1.77)	0.32

na...not applicable, nc...not calculated, m...monocotyledonous, d...dicotyledonous

^a Based on calculated EC₅ values

Conclusions:

The most sensitive species were tomato, wheat and soybean with 21 d EC₅₀ values of 1.16, 1.24 and 1.93 L prod./ha, respectively. The lowest observed effect concentration (LOEC) for soybean, radish and cucumber was 0.32 L prod./ha and the no observed effect concentration (NOEC) for soybean and cucumber was 0.04 L prod./ha.

Comment RMS:

The vegetative vigour test was conducted according to the US EPA guideline 123-1 (1982). According to the US EPA test guideline no validity criteria are given, neither for the version used to in the study protocol nor for the current valid test guideline OPPTS 850.4250 (1996). Taking into account the OECD guideline (OECD 227) the following validity criteria are given:

- The seedling emergence is at least 70%
- In the controls the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.
- 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.

For the duration of the test no visible phytotoxic effects were observed in the controls. The plant survival of all tested plant species was 100% for the duration of the test. The environmental conditions and the used test substrate are identical for a particular species.

No information on the seedling emergence in the control and treatment groups is given.

The following deviations to the study protocol were observed:

- The 2 to 4 specified leaf stage in the protocol deviated for three species. The

radish and corn had a leaf stage ranging from 4 to 5 leaves while carrot had a leaf stage of 1 to 2 leaves.

- The light photoperiod deviated from the protocol of 16 hours light to 8 hours dark. The light to dark interval for the study was based on 15 hours light to 9 hours dark due to a malfunction of the supplemental lighting. On three days photoperiod deviation also occurred where the lights were operational for 17, 22, and 14 hours, respectively.

The deviations are not considered to have an impact on the outcome of the study results. Taking into account the validity criteria given in the OECD guideline the study is considered acceptable.

Reference:	Effect on Seedling Emergence of Non-Target Terrestrial Plants (Tier II) AE B049913: Suspension Concentrate (43.8% w/w)
Author(s), year:	Christ, M.T. & Abedi, J., 2003b
Report/Doc. number:	Report No. 02XB28224, Reference No. M-241177-01-1
Guideline(s):	US EPA 123-1 (1982)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Norton® SC (AE B049913 00 SC45 A203), Batch No.: ACBC 0625, 43.8% w/w
Type of test:	Seedling emergence test
Test duration:	21 days
Test species:	6 dicotyledonous and 4 monocotyledonous plant species <i>Daucus carota</i> (carrot), <i>Zea mays</i> (corn), <i>Cucumis sativus</i> (cucumber), <i>Lactuca sativa</i> (lettuce), <i>Avena sativa</i> (oat), <i>Allium cepa</i> (onion), <i>Raphanus sativus</i> (radish), <i>Glycine max</i> (soybean), <i>Lycopersicon esculentum</i> (tomato) and <i>Triticum aestivum</i> (wheat)
Test soil:	Mixture of natural topsoil and sand (2:1) to achieve a sandy loam substrate with an organic matter content of < 2%.
Applied concentrations:	Control: Deionized water Test item: 0.16, 0.32, 0.63, 1.25, 2.5 and 5.0 L prod./ha (carrot, radish and tomato) 0.19, 0.38, 0.75, 1.25, 3.0 and 6.0 L prod./ha (corn and oat) 0.08, 0.16, 0.32, 0.63, 1.25 and 2.5 L prod./ha (cucumber) 0.04, 0.08, 0.16, 0.32, 0.63 and 1.25 L prod./ha (lettuce) 0.38, 0.63, 1.25, 2.5, 5.0 and 10 L prod./ha (onion and soybean)

	0.02, 0.04, 0.08, 0.16, 0.32 and 0.63 L prod./ha (wheat)
Replicates:	Six replicates with five plants per pot for each species (30 seeds planted per treatment)
Exposure route:	Seeds were planted in test pots consisted of 15.2 cm diameter with bottom drainage holes. Test pots were filled with test soil and seeds were planted to a seed specific depth (1.3 – 2.5 cm dependent on the seed). The pots were placed in a temperature and light supplemented greenhouse following planting. Temperature, relative humidity, and light intensity were recorded at two positions in the greenhouse.
	Spray treatments were applied once each to each species at test initiation with an Allen Track sprayer set at a nominal spray volume of 200 L/ha. Following spray application all pots were placed in a temperature supplemented greenhouse.
Test conditions:	Photoperiod: 15 hour light and 9 hour dark Light intensity: 171.5 – 408.8 $\mu\text{mol}/\text{m}^2/\text{s}$ Temperature: 22.6 – 28.2 °C Relative humidity: 13.5 – 79.0 %
Test parameter:	Plants were assessed for survival (number of initial plants/plants survived at test termination), emergence and rate for phytotoxicity on Day's 7, 14 and 21. Individual plant lengths were determined on Day's 7, 14 and 21 while individual plant dry weights were determined at study termination.
Analytical measurements :	Spray stock solutions of each treatment were analysed for the quantification of ethofumesate by High Performance Liquid Chromatography (HPLC) with Ultraviolet Detection (UV).
Statistics :	Descriptive statistics (mean, standard deviation, and coefficient of variation) were calculated for each treatment and for each test parameter (shoot length and shoot dry weight). The EC_{25} and EC_{50} (with the 95% confidence intervals) were calculated following the non-linear regression equation based on Bruce and Versteeg. The NOEC and LOEC were identified using hypothesis testing methodology. All Day 21 data were subjected to a Shapiro-Wilk's Test to assess departures from a normal distribution and a Bartlett's Test to determine homogeneity of variance. To assess treatment effects for homoscedastic and normally distributed data, a Dunnett's one-way analysis of variance (ANOVA) and multiple means comparison procedure for equal replicates was used to determine those exposure concentrations exhibiting responses significantly different ($P < 0.05$) than the control group. In addition, a William's Test was used since it is more sensitive to a response due to increasing concentrations of toxicant.

Findings:

Analytical measurements: The mean measured value of ethofumesate ranged from 87 to 102 % of nominal and indicated that the desired application rates were achieved.

Biological results: The most sensitive plant species was observed to be the dicotyledonous species lettuce, tomato and cucumber. The lowest endpoint was determined to be EC₅₀ 0.70 L prod./ha based on effects of the shoot dry weight of lettuce.

Table B. 9.11.2-2: EC₂₅, EC₅₀ and NOEC values [L prod./ha]

Test species	Shoot length		Shoot dry weight		NOEC
	EC ₂₅ (95% CI)	EC ₅₀ (95% CI)	EC ₂₅ (95% CI)	EC ₅₀ (95% CI)	
	[L prod./ha]				
Carrot ^d	> 5.0 (na)	> 5.0 (na)	> 5.0 (na)	> 5.0 (na)	5.0
Corn ^m	> 6.0 (na)	> 6.0 (na)	> 6.0 (na)	> 6.0 (na)	6.0
Cucumber ^d	0.97 (0.90-1.0)	1.39 (1.23-1.53)	0.86 (0.79-0.89)	1.11 (1.05-1.14)	0.63
Lettuce ^d	0.56 (0.40-0.78)	> 1.25 (na)	0.35 (0.20-0.63)	0.70 (0.50-0.98)	0.16
Oat ^m	1.84 (0.90-3.74)	> 6.0 (na)	0.47 (0.22-1.0)	1.38 (0.86-2.22)	0.38
Onion ^m	> 10 (na)	> 10 (na)	> 10 (na)	> 10 (na)	1.25
Radish ^d	1.27 (0.55-2.97)	> 5.0 (na)	0.63 (0.22-1.75)	> 5.0 (na)	0.32
Soybean ^d	3.32 (1.67-6.62)	> 10 (na)	2.14 (0.74-6.17)	> 10 (na)	0.38
Tomato ^d	1.01 (0.52-1.96)	2.89 (2.01-4.15)	0.80 (0.34-0.87)	1.05 (0.61-1.10)	0.63
Wheat ^m	0.45 (0.36-0.56)	> 0.63 (na)	0.31 (0.21-0.45)	> 0.63 (na)	0.16

na...not applicable, nc...not calculated, m...monocotyledonous, d...dicotyledonous

Conclusions:

The most sensitive species were lettuce, cucumber and tomato with 21 d EC₅₀ values of 0.70, 1.11 and 1.05 L prod./ha, respectively. The lowest observed effect concentration (LOEC) was for lettuce and wheat (0.32 L prod./ha) and the lowest no observed effect concentration (NOEC) was for lettuce and wheat (0.16 L prod./ha).

Comment RMS:

The seedling emergence test was conducted according to the US EPA guideline 123-1 (1982). According to the US EPA test guideline no validity criteria are given, neither for the version used to in the study protocol nor for the current valid test guideline OPPTS 850.4250 (1996). Taking into account the OECD guideline (OECD 208) 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, 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%.

For the duration of the test visible phytotoxic effects were observed in the controls. Phytotoxic effects were observed for corn (in total 2 plants from different replicates show moderate to severe effects on height inhibition), cucumber (in total 3 plants from different replicates show severe effects on height inhibition), lettuce (in total 1 plant shows moderate effects on height inhibition), radish (death of one entire plant, necrosis), wheat (in total 1 plant shows moderate effects on height inhibition) and tomato (death of one entire plant, necrosis).

The plant survival of all tested plant species was at least 90% for the duration of the test. The environmental conditions and the used test substrate are identical for a particular species.

The following deviations to the study protocol were observed:

- The treatment of 0.38 L/ha was applied to onion and soybean as opposed to the 0.32 L/ha treatment. The treatment of 1.25 was applied to oat as opposed to the 1.5 treatment.
- The light photoperiod deviated from the protocol of 16 hours light to 8 hours dark. The light to dark interval for the study was based on 15 hours light to 9 hours dark due to a malfunction of the supplemental lighting.
- The greenhouse supplemental lighting was not in operation from December 5 to 11 due to a power outage as the result of an ice storm. In addition, the GemLink data acquisition system did not record data for temperature, humidity, and light levels on these dates as a result of the power outage. The mechanical controls for operation of the greenhouse heating system remained in effect by generator power. Lettuce was the only species affected by the power failure.

In general, the deviations are not considered to have an impact on the outcome of the study results. However, based on the results for lettuce (no growth of some seedlings in the control and all treatment groups was observed) the problems with the supplemental lighting might have been an effect on the growth of the seedling. However, the seedling emergence was greater than 70%.

Taking into account the validity criteria given in the OECD guideline the validity of the study is questionable (phytotoxicity observed in the control). However, the study was not conducted according to the OECD guideline but to the US EPA guideline. No validity criteria are stated in the US EPA test guideline. In addition, only a few (max. 2 plants out of 30) plants show effects (growth inhibition) in the controls.

Hence, the RMS is of the opinion that the study is acceptable and should be used for the risk assessment.

Reference:	Ethofumesate SC 500 g/L – Effect on the seedling emergence and growth of ten species of non-target terrestrial plants (Tier 2)
Author(s), year:	Gosch, H., 2009
Report/Doc. number:	Report No. SE 09/051, Reference No. M-358944-01-1
Guideline(s):	OECD 208
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate SC 500, Batch No. ECAB001127, Purity (analysed): 44.6% w/w, 503.5 g/L
Type of test:	Seedling emergence test
Test duration:	14 days
Test species:	6 dicotyledonous and 4 monocotyledonous plant species <i>Zea mays</i> (corn), <i>Allium cepa</i> (onion), <i>Triticum aestivum</i> (wheat), <i>Lolium perenne</i> (ryegrass), <i>Glycine max</i> (soybean), <i>Brassica napus</i> (oilseed rape), <i>Brassica rapa</i> (turnip), <i>Fagopyrum esculentum</i> (buckwheat), <i>Helianthus annuus</i> (sunflower) and <i>Pisum sativum</i> (pea)
Test soil:	Mixture of 90% standard soil (silt loam) and 10% washed sand (39.7% sand, 17.2% clay and 43.1% silt) with an organic carbon content of 0.81% C.
Applied concentrations:	Control: Deionized water Test item: 0.078, 0.156, 0.313, 0.625 and 1.25 L prod/ha (wheat, ryegrass and sunflower) 0.156, 0.313, 0.625, 1.25 and 2.5 L prod./ha (buckwheat) 0.313, 0.625, 1.25, 2.5 and 5.0 L prod./ha (oilseed rape, turnip, soybean, pea, onion and corn)
Replicates:	Eight replicates with five plants per pot for each species, in total 40 seeds per treatment.
Exposure route:	Seeds were planted in test pots consisted of 10.5 cm diameter with bottom drainage holes. Test pots were filled with test soil and seeds were introduced manually in the soil. The seeds were pressed into the soil surface and completely invisible covered with a layer of the used soil. Following spray application (on the soil surface) all pots were placed in a temperature supplemented greenhouse. Spray treatments were applied once each to each species at test initiation at a

nominal spray volume of 200 L/ha using a spray chamber. The pots were placed in a temperature and light supplemented greenhouse following planting. Temperature, relative humidity, and light intensity were recorded continuously by thermo-hydrographs.

Test conditions:

Photoperiod: at least 16 h light

Light intensity: natural daylight supplemented by artificial lighting

Temperature: 23.0 ± 8 °C at day and 18.0 ± 8 °C at night

Relative humidity: 70 ± 30 %

Test parameter:

Plants were assessed for survival, emergence and phytotoxicity on Day's 7 and 14. Shoot dry weight was determined at the final assessment.

Statistics :

The effect levels (ER/LR₂₅ and ER/LR₅₀) as well as the NOEC were calculated using ToxRat (v. 2.09)

Findings:

Analytical measurements: The mean measured test concentration of ethofumesate in the highest application rate was 99.1% of nominal.

Biological results: The most sensitive plant species was observed to be the dicotyledonous species buckwheat and the monocotyledonous species wheat, ryegrass and corn. The lowest endpoint was determined to be EC₅₀ 0.101 L prod./ha based on effects of the shoot dry weight of wheat.

Table B. 9.11.2-3: Effects on emergence, survival and shoot dry weight

Test species	Emergence			Survival			Shoot dry weight		
	NOER	LR ₂₅	LR ₅₀	NOER	LR ₂₅	LR ₅₀	NOER	ER ₂₅	ER ₅₀
	[L prod./ha]								
Buckwheat ^d	2.5	> 2.5	> 2.5	1.25	> 1.25	> 1.25	< 0.156	0.119	0.347
Oilseed rape ^d	5.0	> 5.0	> 5.0	5.0	> 5.0	> 5.0	< 0.313	0.629	2.171
Pea ^d	5.0	> 5.0	> 5.0	2.5	> 5.0	> 5.0	0.313	0.895	2.412
Soybean ^d	5.0	> 5.0	> 5.0	2.5	4.462	> 5.0	0.625	1.401	3.249
Sunflower ^d	1.25	> 1.25	> 1.25	1.25	> 1.25	> 1.25	1.25	> 1.25	> 1.25
Turnip ^d	2.5	> 5.0	> 5.0	5.0	> 5.0	> 5.0	< 0.313	0.552	1.788
Corn ^m	5.0	> 5.0	> 5.0	2.5	2.7	4.15	0.313	0.459	0.842
Onion ^m	0.625	0.483	1.847	0.625	4.505	> 5.0	0.625	1.637	> 5.0
Ryegrass ^m	1.25	> 1.25	> 1.25	0.078	0.231	0.433	0.078	0.154	0.542
Wheat ^m	0.625	> 1.25	> 1.25	< 0.078	0.108	0.142	< 0.078	0.0654	0.101

na...not applicable, nc...not calculated, m...monocotyledonous, d...dicotyledonous

Conclusions:

The most sensitive species was observed to be the monocotyledonous species wheat with the lowest ER₅₀ of 0.101 L prod./ha based on effects on the shoot dry weight.

Comment RMS:

The seedling emergence test was conducted according to the OECD guideline 208.

Based on the validity criteria according to the OECD guideline the study is considered acceptable.

- 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, 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.

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

Based on the available data on non-target plants no further data are considered necessary. Hence, no extended laboratory studies have been conducted.

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

Based on the available data on non-target plants no further data are considered necessary. Hence, no semi-field or field studies have been conducted.

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

Seedling emergence and vegetative vigour studies have been conducted with Ethofumesate SC 500 following EPA guideline 123-1 (Christ & Abedi, 2003ab) and OECD testing guideline 208 (Gosch, 2009). Each study included 10 species which were tested at application rates of up to 10 L product/ha (Christ & Abedi, 2003ab) and at an application rate of up to 5 L product/ha (Gosch, 2009), respectively.

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

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

Test organisms	Study type	Test duration	Lowest ER ₅₀ [L prod./ha]	Most sensitive species	Reference
Terrestrial non-target plants (10 species)	Vegetative vigour (Tier 2)	21 days	ER ₅₀ = 1.16	Tomato (shoot dry weight)	Christ, M.T. & Abedi, J., 2003a
Terrestrial non-target plants (10 species)	Seedling emergence (Tier 2)	21 days	ER ₅₀ = 0.70	Lettuce (shoot dry weight)	Christ, M.T. & Abedi, J., 2003b
Terrestrial non-target plants (10 species)	Seedling emergence (Tier 2)	21 days	ER ₅₀ = 0.101	Wheat (shoot dry weight)	Gosch, H. 2009

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, fodder beet and red 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 and 3 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 rate	MAF	PER _{off-field} (at 1 m distance)
Sugar beet, fodder beet, red beet	BBCH 16 – 18	1	2.0 L prod./ha	2.77%	1.0	0.0554 L prod./ha
		2	1.0 L prod./ha	2.38%	1.7	0.0405 L prod./ha
		3	0.666 L prod./ha	2.01%	2.3	0.0308 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, fodder beet and red 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 0.101 L prod./ha (shoot dry weight, ER_{50} of wheat).

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 ER_{50} obtained from the non-target plant studies. An assessment factor of 5 is required in order to prove safe use.

Table B. 9.12.1-1: Deterministic risk assessment based on the lowest ER_{50}

Distance	Drift rate	Drift reducing nozzles	$PER_{off-field}$ [L prod./ha]	Toxicity [L prod./ha]	TER
1 m	2.77%	-	0.0554	$ER_{50} = 0.101$	1.82
		50%	0.0277		3.65
		75%	0.01385		7.30
		90%	0.00554		18.25
5 m	0.57%	-	0.0114		8.87

According to the results of the deterministic approach involving the most sensitive endpoint in the seedling emergence study, the trigger of 5 at 1 m distance is only exceeded if nozzles with at least 75% drift reduction are used. Considering a distance of 5 m, no drift reducing nozzles are necessary.

The results of the deterministic risk assessment for seedling emergence indicate the necessity of mitigation measures. However, as an alternative approach a probabilistic risk assessment has been conducted.

B.9.12.2. Probabilistic risk assessment

The HC_5 (the concentration below which less than 5% of the species will be harmed above the ER_{50} level) was calculated from the data set of ER_{50} growth inhibition levels. The EU guidance document for terrestrial ecotoxicology states: 'If the ED_{50} for less than 5% of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable. Thus, the HR_5 itself ($TER = 1$) can be regarded to be protective.

The HC_5 calculation ($ETX^{2.0}$ by rivm) is based on the ER_{50} values for shoot dry weight derived from two seedling emergence studies (Christ & Abedi, 2003, Gosch, 2009). The two seedling emergence tests were conducted with two different formulations, Ethofumesate SC 500 (Gosch, 2009) and Ethofumesate SC 45 (Christ

& Abedi, 2003). However, taking into account the comparable composition of the two preparations it is considered acceptable to use the endpoints of both studies for the calculation of a HC₅ value.

The endpoints used for the HC₅ calculation are based on the most sensitive parameter, the shoot dry weight. The tested terrestrial plants were observed to be less sensitive considering other parameters like emergence, shoot length and survival. Additionally, the terrestrial plants show a higher sensitivity towards pre-emergence application (seedling emergence) than post-emergence applications (vegetative vigour).

For some terrestrial plant species no ER₅₀ could be calculated, hence “greater than” values were excluded from the calculation of a HC₅ value. A summary of all ER₅₀ values included in the HC₅ calculation are given in the Table B.9.12.2-1.

Table B. 9.12.2-1: Summary of definitive ER₅₀-values used for the HC₅ calculation

Test design	Test species	Endpoint		Reference
		ER ₅₀ [L prod./ha]	Parameter	
Tier 2, seedling emergence test (US EPA 123-1)	Cucumber	1.11	Shoot dry weight	Christ & Abedi, 2003b
	Lettuce	0.70		
	Oat	1.38		
	Tomato	1.05		
Tier 2, seedling emergence test (OECD 208)	Buckwheat	0.347	Shoot dry weight	Gosch, 2009
	Oilseed rape	2.171		
	Pea	2.412		
	Soybean	3.249		
	Turnip	1.788		
	Corn	0.842		
	Ryegrass	0.542		
	Wheat	0.101		

The data were tested for their goodness of fit considering three different tests for normality, the Anderson-Darling test, the Kolmogorov-Smirnov test and the Cramer von Mises test. Based on the results of all three tests the toxicity data are considered to be normally distributed (see Figure B.9.12.2-1).

The HC₅ value was calculated to be 0.1882 L prod./ha, with a lower and upper limit of 0.0691 and 0.3437 L prod./ha, respectively.

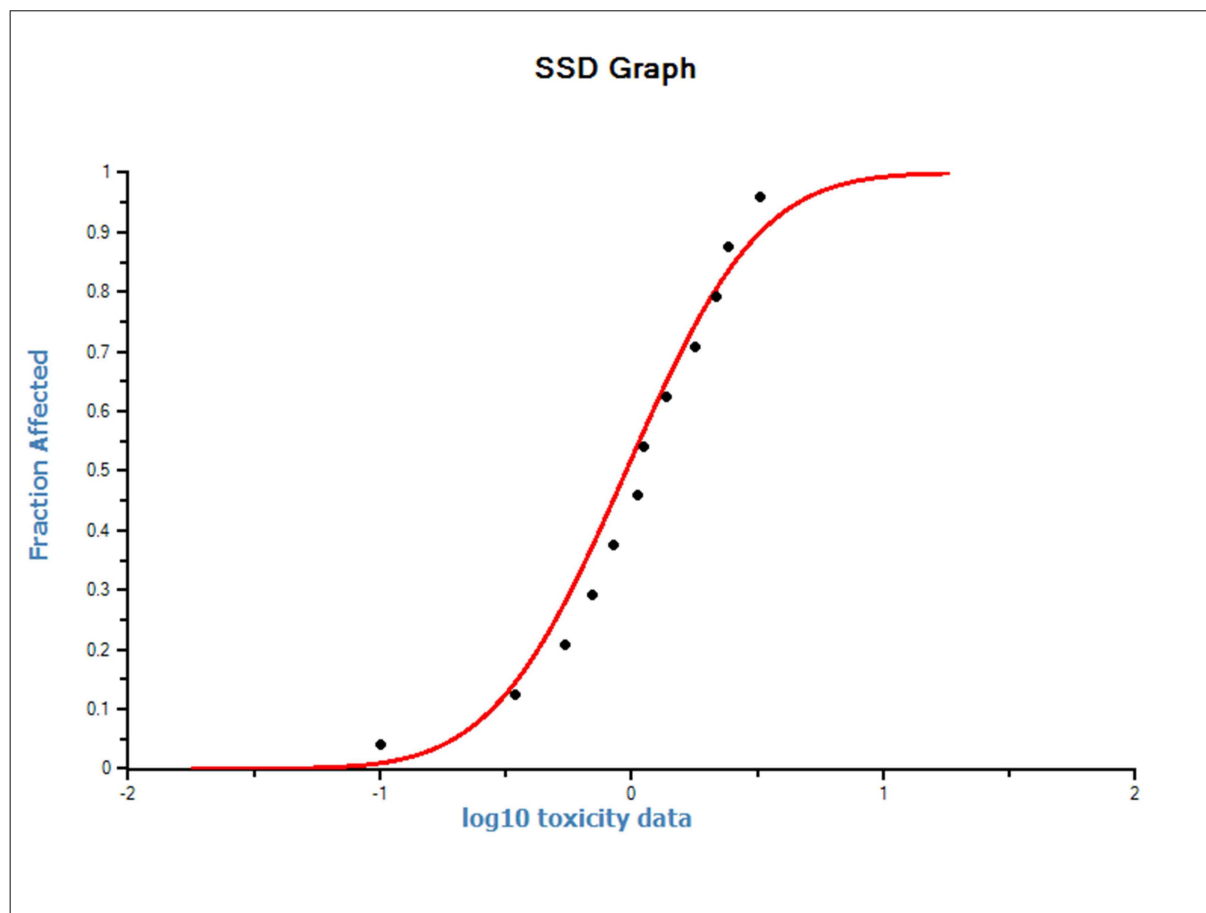


Figure B. 9.12.2-1: Species Sensitivity Graph

The probabilistic risk assessment is based on the HC₅ value (median) of 0.1882 L prod./ha. Even though the most sensitive species (wheat, EC₅₀ = 0.101 L prod./ha) is not covered by the HC₅ value a safety factor of 1 should be applied.

Table B.9.12.2-2: Probabilistic risk assessment based on the median HC₅

Distance	Drift rate	Drift reducing nozzles	PER _{off-field} [L prod./ha]	Toxicity [L prod./ha]	TER
1 m	2.77%	-	0.0554	HC ₅ = 0.1882	3.40
		50%	0.0277		6.79
		75%	0.01385		13.59
		90%	0.00554		33.97
5 m	0.57%	-	0.0114		16.51

According to the results of the probabilistic risk assessment based on the median HC₅ value and the trigger of 1 the risk to non-target plants is identified to be acceptable at a distance of 1 m. The results of the probabilistic risk assessment for seedling emergence indicate the no risk mitigation measures are required to address the risk to non-target plants.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)**Scientific peer-reviewed literature**



From searching peer-reviewed literature published over the last 10 years prior to submission three adequate and reliable publications on monitoring data concerning potential adverse effects of the active substance to non-target aquatic organisms were obtained.

The publications by Berenzen et al. (2005; M-458568-01-1, KCA 8.9 / 01, also filed in KCA 7.5), Bereswill et al. (2013; M-462597-02-1, KCA 8.9 / 02, also filed in KCA 7.5.) and Liess & Von der Ohe (2005; M-458575-01-1, KCA 8.9 / 03, also filed in KCA 7.5) are exposure monitoring studies including monitoring of aquatic macroinvertebrate communities. Amongst various selected pesticides, ethofumesate residues were analytically determined in streams and toxicity of measured concentrations was assessed using the toxic unit approach (TU). For this calculation, acute *Daphnia magna* endpoints from secondary sources were used; the endpoints correspond to the EU-agreed endpoint according to EFSA review report SANCO/6503/VI/99-final (2002). Reported TU values for ethofumesate were in all cases lower than 0.01, indicating an acceptable risk when applying a standard assessment factor of 100 according to the principles of a first-tier acute risk assessment under Regulation (EU) No 1107/2009. For the ecotoxicological risk assessment concerning ethofumesate use as a plant protection product, the existing guidance under Regulation (EU) No 1107/2009 is applied. Thus, the articles are not considered to impact the risk assessment as presented.

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

Please refer to B.9.13.

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 Section 10	Barber, I.	1999	Ethofumesate suspension concentrate 500 g per L - Ecotoxicology bridging statement - AE B049913 00 SC45 A2 AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report No.: C005358, Edition Number: M-191910-01-1 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCP 10.2.1	Banman, C. S.	2012	Toxicity of the formulation ethofumesate SC 500A G (ethofumesate 500 g/L) to the aquatic macrophyte, <i>Myriophyllum spicatum</i> Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: <u>M-437702-02-1</u> , Edition Number: <u>M-437702-02-1</u> Date: 2012-08-29 ...Amended: 2013-05-22 GLP/GEP: yes, unpublished	N	Y	Required for risk assessment as <i>Myriophyllum spicatum</i> most sensitive aquatic species in studies on the active substance	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.2.1		1989	TRAMAT (R) 500 - DETERMINATION OF ACUTE TOXICITY (LC50) TO MIRROR CARP (96 HOURS, SEMI- STATIC) AND THE ANALYSIS OF ETHOFUMESATE IN WATER SAMPLES  Bayer CropScience, Report No.: A83350, Report includes Trial Nos.: 140422 77B Edition Number: M-155619-01-1 Date: 1989-10-12 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.2.1	Cameron, B. D., Caley, C. Y., Chapleo, S., McKenzie, J., McGuire, G. M.	1989	TRAMAT (R) 500 - DETERMINATION OF ACUTE TOXICITY (LC50) TO DAPHNIA (48 HOURS STATIC) AND THE ANALYSIS OF ETHOFUMESATE IN WATER SAMPLES Inveresk Research Int. Ltd., Tranent, Scotland Bayer CropScience, Report No.: A83348, Report includes Trial Nos.: 140443 68B Edition Number: M-155617-01-1	N	N	-	Bayer CropScience	In the DAR (1998)

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
			Date: 1989-10-12 GLP/GEP: yes, unpublished					
KCP 10.2.1	[REDACTED]	1988	THE ACUTE TOXICITY OF NORTON 50 SC (ETHOFUMESATE) TO ZEBRA FISH (<i>Brachydanio rerio</i>) [REDACTED] Bayer CropScience, Report No.: A83338, Report includes Trial Nos.: 64B Edition Number: M-155607-01-1 Date: 1988-12-22 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.2.1	Knacker, T.	1989	A STUDY OF THE TOXICITY TO ALGAE OF ETHOFUMESATE 50 SC Battelle-Institut e.V., Frankfurt am Main, Germany Bayer CropScience, Report No.: A83342, Report includes Trial Nos.: 71B BE-EA-12-89-01-ALG-2- Edition Number: M-155611-01-1 Date: 1989-08-03 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.2.2	Barber, I.	1991	DETERMINATION OF THE EFFECTS OF TRAMAT ON THE LIFE CYCLE OF <i>Daphnia magna</i> STRAUS Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83364, Report includes Trial Nos.: 81B Edition Number: M-155633-01-1 Date: 1991-08-09 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.3.1.1.1	Schmitzer, S.	2011	Effects of ethofumesate SC 500 G (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 63931035, Edition Number: M-421700-01-1 Date: 2011-12-15 GLP/GEP: yes, unpublished ...also filed: KCP 10.3.1.1.2 /02	N	Y	Required due to deficiencies in previous studies	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.3.1.1.2	Barber, I.	1995	ETHOFUMESATE SUSPENSION CONCENTRATE 500 G/L CR 18654 TRAMAT 500 LABORATORY TESTS ON THE EFFECT TO HONEYBEES (<i>Apis mellifera</i>)	N	N	-	Bayer CropScience	In the DAR (1998)

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
			Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A83401, Edition Number: M-155669-01-1 Date: 1995-03-08 GLP/GEP: no, unpublished ...also filed: KCP 10.3.1.1.1 /01					
KCP 10.3.1.1.2	Schmitzer, S.	2011	Effects of ethofumesate SC 500 G (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 63931035, Edition Number: M-421700-01-1 Date: 2011-12-15 GLP/GEP: yes, unpublished ...also filed: KCP 10.3.1.1.1 /02	N	Y	Required due to deficiencies in previous studies	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.3.1.1.3	Schmitzer, S.	2013	Study on the effects of ethofumesate SC 500A G on honey bee brood (<i>Apis mellifera</i> L.) - Brood feeding test - Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report no.: 71411031, Edition Number: M-454691-01-1 Date: 2013-05-07 GLP/GEP: yes, unpublished	N	Y	New regulatory requirement	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.3.2.1	Kuehner, C.	1990	Side effects of TRAMAT 500 on the lacewing <i>Chrysoperla carnea</i> STEPH. in the laboratory IFU Umweltanalytik GmbH; Bayer CropScience, Report No.: A89489, Report includes Trial Nos.: 026/02-Cc Edition Number: M-155645-01-2 Date: 1990-08-03 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.3.2.1	Mead-Briggs, M. A.	1991	AN EVALUATION OF THE SIDE-EFFECTS OF TRAMAT 500 SC ON THE STAPHYLINID BEETLE (<i>Aleochara bilineata</i>) University of Southampton, Agrochemicals Evaluation Unit AEU, Southampton, United Kingdom Bayer CropScience, Report No.: A83379, Report includes Trial Nos.: ENVIR 92b Edition Number: M-155647-01-1 Date: 1991-12-23 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)

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 10.3.2.1	Roembke, J.	1990	A STUDY OF THE ACUTE TOXICITY OF TRAMAT 500 (NORTON 50 SC) TO THE CARABID <i>Poecilus cupreus</i> Battelle-Institut e.V., Frankfurt am Main, Germany Bayer CropScience, Report No.: A83354, Report includes Trial Nos.: BE-E-05-89-2-CAK3 Edition Number: M-155623-01-1 Date: 1990-03-26 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCP 10.3.2.1	Waltersdorfer, A.	2002	Toxicity to the parasitoid wasp <i>Aphidius rhopalosiphii</i> (DeStephani-Perez) (Hymenoptera: Braconidae) in the laboratory Ethofumesate water miscible suspension concentrate, 500 g/L Code: AE B049913 00 SC45 A202 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C028582, Edition Number: M-215537-01-1 Date: 2002-12-10 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCP 10.3.2.1	Waltersdorfer, A.	2002	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, Phytoseiidae) in the laboratory Ethofumesate water miscible suspension concentrate 500 g/L Code: AE B049913 00 SC45 A202 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C023481, Edition Number: M-215086-01-1 Date: 2002-07-03 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCP 10.4.1	Kuhner, C.	1991	Acute Toxicity of TRAMAT 500 to the Compost Worm <i>Eisenia foetida</i> (Artificial Soil Test) IFU Umweltanalytik GmbH; Bayer CropScience, Report No.: A89488, Report includes Trial Nos.: 026/01-Ef Edition Number: M-155632-01-2 Date: 1991-01-14 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 10.4.1.1	Sowig, P.; Gosch, H.	1999	Effects on growth and reproduction of earthworms (<i>Eisenia foetida</i>) Ethofumesate water miscible suspension concentrate 500 g/L Code: AE B049913 00 SC45 A103 Hoechst Schering AgrEvo	N	N	-	Bayer CropScience	In the Addendum to the DAR (2000)

Annex point	Author(s)	Year	Title Source (<i>where different from company</i>) Company name, Report No., Date, GLP status (<i>where relevant</i>), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			GmbH, Frankfurt am Main, Germany Bayer CropScience, Report no.: C003978, Edition Number: M-187147-01-1 Date: 1999-07-30 GLP/GEP: yes, unpublished					
KCP 10.4.1.1	Luehrs, U.	2011	Ethofumesate SC 500 G: Effects on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 64121022, Edition Number: M-409272-01-1 Date: 2011-06-14 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment. Old study does not fulfil the new data requirements.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.4.2.1	Frommholz, U.	2011	Ethofumesate SC 500 G: Influence on the reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil Bayer CropScience, Report No.: FRM-COLL-129/11, Edition Number: M-420052-01-1 Date: 2011-12-15 GLP/GEP: yes, unpublished	N	Y	New regulatory requirement	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.4.2.1	Kratz, M.A.	2011	Ethofumesate SC 500 G: Influence on mortality and reproduction on the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil Bayer CropScience, Report No.: KRA-HR-54/11, Edition Number: M-417391-01-1 Date: 2011-11-18 GLP/GEP: yes, unpublished	N	Y	New regulatory requirement	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 10.4.2.2	Lechelt-Kunze, C.	2003	Ethofumesate SC45 (AE B049913 00 SC45 A203): Effects on soil litter degradation Bayer CropScience, Report no.: C035996, Report includes Trial Nos.: E4272323-4 Edition Number: M-219686-01-1 Date: 2003-08-25 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCP 10.5	Ruiz-Romera, E.; Munoz-Leoz, B.; Garbisu, C.; Antiguedad, I.	2012	Fertilization can modify the non-target effects of pesticides on soil microbial communities. Journal: Soil Biol. Biochem., Pages: 125-134, Year: 2012, Report No.: M-458656-01-1, Edition Number: M-458656-01-1 Date: 2012-12-31 GLP/GEP: no, published	N	N	-		Submitted for the purpose of renewal (2014)
KCP 10.5	Ruiz-	2013	Non-target effects of three	N	N	-		Submitted for

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	Romera, E.; Antiguada, I.; Garbisu, C.; Munoz-Leoz, B.; Charcosset, J.; Sanchez-Perez, J.		formulated pesticides on microbially-mediated processes in a clay-loam soil. Journal: Sci. Total Environ., Volume: 449, Pages: 345-354, Year: 2013, Report No.: M-462303-01-1, Edition Number: M-462303-01-1 Date: 2013-05-14 GLP/GEP: no, published					the purpose of renewal (2014)
KCP 10.5	Schulz, L.	2011	Ethofumesate SC 500 G: Effects on the activity of soil microflora (nitrogen transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 11 10 48 059 N, Edition Number: M-419266-01-1 Date: 2011-12-07 GLP/GEP: yes, unpublished	N	Y	Available studies did not cover the intended application rates for the representative formulation.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCP 10.6.1	Thuerwaechter, F.	1999	Effect of three betanal formulations on non target terrestrial plants Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C005554, Edition Number: M-192273-01-1 Date: 1999-10-01 GLP/GEP: no, unpublished	N	N		Bayer CropScience	In the DAR (1998)
KCP 10.6.2	Christ, M. T.	2003	Effect on Vegetative Vigor of Non-Target Terrestrial Plants (Tier II); AE B049913; Suspension Concentrate (43.8% w/w) Bayer CropScience LP, RTP, NC, USA Bayer CropScience, Report No.: B004218, Report includes Trial Nos.: 02XB28225 Edition Number: M-241176-01-1 EPA MRID No.: 45874701 Date: 2003-02-28 GLP/GEP: yes, unpublished	N	Y	Required for herbicides according to the data requirements for chemical preparations (284/2013) under 1107/2009	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCP 10.6.2	Christ, M. T.	2003	Effect on Seedling Emergence of Non-Target Terrestrial Plants (Tier II); AE B049913; Suspension Concentrate (43.8% w/w) Bayer CropScience LP, RTP, NC, USA Bayer CropScience, Report No.: B004219, Report includes Trial Nos.: 02XB28224 Edition Number: M-241177-01-1 EPA MRID No.: 45874702 Date: 2003-02-28	N	Y	Required for herbicides according to the data requirements for chemical preparations (284/2013) under 1107/2009	Bayer CropScience	Submitted for the purpose of renewal (2014)

Annex point	Author(s)	Year	Title Source (<i>where different from company</i>) Company name, Report No., Date, GLP status (<i>where relevant</i>), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			GLP/GEP: yes, unpublished					
KCP 10.6.2	Gosch, H.	2009	Ethofumesate SC 500 g/L: Effect on the seedling emergence and growth of ten species of non-target terrestrial plants (Tier 2) Bayer CropScience, Report No.: SE09/051, Edition Number: M-358944-01-1 Date: 2009-11-19 GLP/GEP: yes, unpublished	N	Y	Conducted to obtain definitive endpoints in addition to seedling emergence study from 2003 (M-241177-01-1)	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)