

European Commission



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

ETHOFUMESATE

Volume 3 – B.9 (AS)

Rapporteur Member State: Austria
Co-Rapporteur Member State: Denmark

Version History

When	What
1998	Initial DAR
May 1999	Addendum 1 (prior to ECCO 81)
May 2000	Addendum 2 and 3
December 2000	Addendum 8
2015/01	DRAR

Table of contents

B.9. ECOTOXICOLOGY DATA	4
B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES	6
B.9.1.1. Effects on birds.....	6
B.9.1.2. Effects on terrestrial vertebrates other than birds	25
B.9.1.3. Active substance bioconcentration in prey of birds and mammals.....	26
B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)	26
B.9.1.5. Potential for endocrine disruption	26
B.9.2. EFFECT ON AQUATIC ORGANISMS	28
B.9.2.1. Acute toxicity to fish	28
B.9.2.2. Long-term and chronic toxicity to fish	50
B.9.2.3. Potential for endocrine disruption	63
B.9.2.4. Bioconcentration in fish	64
B.9.2.5. Acute toxicity to aquatic invertebrates	66
B.9.2.6. Long-term and chronic toxicity to aquatic invertebrates	82
B.9.2.7. Effects on algal growth.....	101
B.9.2.8. Effects on aquatic macrophytes.....	131
B.9.2.9. Further testing on aquatic organisms.....	139
B.9.3. EFFECTS ON ARTHROPODS	143
B.9.3.1. Effects on bees.....	143
B.9.3.2. Effects on non-target arthropods other than bees	155
B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA	156
B.9.4.1. Earthworm – sub-lethal effects.....	156
B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms).....	165
B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION	173
B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS	181
B.9.6.1. Summary of screening data	181
B.9.6.2. Testing on non-target plants	186
B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA).....	187
B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT.....	187
B.9.9. MONITORING DATA	189
B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER.....	192
B.9.11. REFERENCES RELIED ON.....	193

B.9. ECOTOXICOLOGY DATA

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 Directive 91/414/EEC has been repealed by Regulation (EC) no 1107/2009 of 21 October 2009 concerning the placing of plant protection products on the market.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

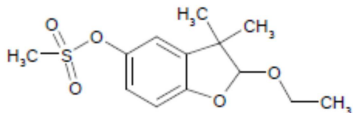
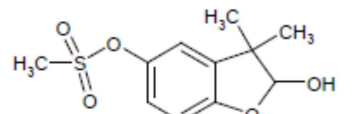
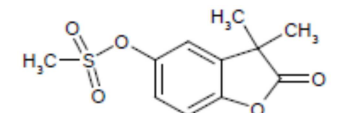
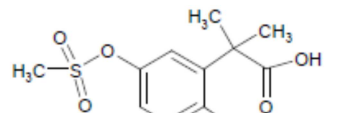
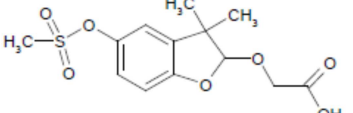
Studies which were submitted for the first EU peer-review of the active substance ethofumesate but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are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

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

No additional studies were submitted for the renewal of the active substance Ethofumesate. The studies submitted for the first EU approval of the active substance are summarised below. In addition, the studies were evaluated according to the representative test guidelines.

B.9.1.1.1. Acute oral toxicity to birds

Reference:	The acute oral toxicity (LD₅₀) of ethofumesate to the mallard duck
Author(s), year:	1990a
Report/Doc. number:	Study no. A87610, Reference no. M-161543-01-1
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD ₅₀), October 1982
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate, batch : PO4402/2, purity : 97%
Test species:	Mallard duck (<i>Anas platyrhynchos</i>)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	935 – 1185 g bodyweight, > 16 weeks old
Type of test:	Acute oral toxicity
Applied concentrations:	0 (corn oil vehicle only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 5 mL/kg body weight
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a Ch 14 plastic catheter, diluent: corn oil
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: 15-17°C, relative humidity: 75 ± 4.4% (SD), lighting: 7h light and 17h darkness. Feed (standard HRC layer diet) was provided ad libitum during acclimation and during the test, except of an overnight starvation period of at least 15 hours prior to testing.

Test parameter:

Observations:	Mortalities, bird health and clinical observations were observed daily. Individual bodyweights were observed on Days -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods :
---------------	---

Days -14 to -8, -7 to -1, 1 to 7 and 8 to 14.

Birds which died during the study were examined post-mortem. At test termination, post-mortem examination was carried out on ten birds from the highest surviving dose group.

Statistics: There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD₅₀. The LD₅₀ value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities: No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.

Clinical signs: All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed consumption: Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table B. 9.1.1-1: Mortality and growth (bodyweights and food consumption) of mallard ducks following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
			-14 to -7	-7 to 0	0 to 7	7 to 14	-14 to -8	-7 to -1	1 to 7	8 to 14
Control	0	♂	45	-70	26	15	80	51	83	74
	0	♀	-11	-22	79	-34	69	66	106	71
500	0	♂	1	-68	63	-2	71	60	103	71
	0	♀	12	-37	42	-19	80	61	97	89
1000	0	♂	-16	-54	37	-18	86	89	103	83
	0	♀	10	-46	45	8	91	94	86	97
2000	0	♂	-46	-51	16	11	94	86	91	117
	0	♀	-6	-35	38	-15	66	66	86	86

Conclusion: LD₅₀ > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

Comment RMS: The study was conducted according to an US EPA test guideline (1982). The study is in general agreement with the current valid test guidelines, e.g. US EPA guideline (OPPTS 850.2100, April 1996) and OECD guideline (OECD 223, July 2010). The control mortality was determined to be below 10% (being: 0%).

The RMS is of the opinion that the study is valid and should be used for the risk assessment.

Reference:	The acute oral toxicity (LD₅₀) of NC 8438 to the mallard duck
Author(s), year:	████████████████████ 1977a
Report/Doc. number:	Study no. A83331, Reference no. M-155600-01-1
Guideline(s):	US EPA guidelines, June 1975 (including some modification as stated in the draft US EPA guideline, April 1977)
GLP:	No
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	NC 8438 (technical ethofumesate), batch : CR. 4805/3, purity : 97%
Test species:	Mallard duck (<i>Anas platyrhynchos</i>)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	845 - 1335 g bodyweight, > 16 weeks old
Type of test:	Acute oral toxicity
Applied concentrations:	0 (corn oil vehicle only), 1227, 2458, 3445 and 3552 mg ai/kg bw, dosage volume: dosage volume: 10 mL/bird
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a Ch 14 plastic catheter, diluent: corn oil
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: 16°C, relative humidity: 60-65% Feed was provided ad libitum during acclimation and during the test, except of a starvation period of at least 15 hours prior to testing.

Test parameter:

Observations:	Mortalities, bird health and clinical observations were observed daily. Individual bodyweights were observed on Days -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 3, 7 and 14 (post-treatment period). Group mean food consumption was measured weekly. At test termination, post-mortem examination was carried out on five birds in each group.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD ₅₀ . The LD ₅₀ value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities:	no mortalities occurred in the control group and in the treatment groups.
Clinical signs:	All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test. In one bird of the control group a hard yellow

necrotic area on the dorsal lobe of the pancreas was observed. Additionally, in a bird of a treatment group (2458 mg ai/kg) a white tumor-like lesion on the ventral surface of the liver was observed.

Body weight, feed consumption:

Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table B. 9.1.1-2: Mortality and growth (bodyweights and food consumption) of mallard ducks following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
		-14 to -0	0 to 7	7 to 14	0 to 14	-14 to -8	-7 to 0	0 to 7	8 to 14
Control	0	18	-33	15	-18	80	104	86	123
1227	0	29	-1	19	18	97	131	91	127
2458	0	60	-8	24	16	130	117	107	111
3445	0	91	-26	-11	-37	123	106	111	83
3552	0	51	1	18	19	103	100	103	89

Conclusion:

LD₅₀ > 3552 mg ai/kg bw, NOEL = 3552 mg ai/kg bw

Comment RMS:

The study was conducted according to an US EPA test guideline (1975). The study is in general agreement with the current valid test guidelines, e.g. US EPA guideline (OPPTS 850.2100, April 1996) and OECD guideline (OECD 223, July 2010) with some deviations. Some information on the test methods (e.g. lighting regime, ...) and the test organisms (age, medication) are missing. In addition, the study was not conducted according to GLP.

However, the control mortality was determined to be below 10% (being: 0%) which is in line with the validity criteria according OECD 223.

Based on the results of the study the identified deviations do not seem have an adverse impact on the mortality, food consumption and body weight of the birds. Hence, the study is considered acceptable.

Reference:	The acute oral toxicity (LD₅₀) of ethofumesate to the bobwhite quail
Author(s), year:	1990b
Report/Doc. number:	Study no. A87612, Reference no. M-161547-01-1
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD ₅₀), October 1982
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate, batch : PO4402/2, purity : 97%
Test species:	Bobwhite quail (<i>Colinus virginianus</i>)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	174-215 g bodyweight, > 16 weeks old
Type of test:	Acute oral toxicity
Applied concentrations:	0 (corn oil vehicle only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a Ch plastic catheter, diluent: corn oil
Time of exposure:	One single application, monitoring during 21 days
Test conditions:	Test temperature: 16-19°C, relative humidity: 75 ± 5.0% (SD), lighting: 7h light and 17h darkness. Feed (standard HRC layer diet) was provided ad libitum during acclimation and during the test, except of an overnight starvation period of at least 15 hours prior to testing.

Test parameter:

Observations:	Mortalities, bird health and clinical observations were observed daily. Individual bodyweights were observed on Days -21, -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods : Days -21 to -15, -14 to -8, -7 to -1, 1 to 7 and 8 to 14. Birds which died during the study were examined post-mortem. At test termination, post-mortem examination was carried out on ten birds from the highest surviving dose group.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD ₅₀ . The LD ₅₀ value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities:	No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.
Clinical signs:	All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.
Body weight, feed consumption:	Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table B. 9.1.1-3: Growth (bodyweights and food consumption) of bobwhite quails following acute oral exposure (gavage)

Test substance [mg ai/kg]	Sex	Mean bodyweight change [g/bird]					Mean food consumption [g/bird/d]				
		-21 to -14	-14 to -7	-7 to 0	0 to 7	7 to 14	-21 to -15	-14 to -8	-7 to -1	1 to 7	8 to 14
Control	♂	5	1	-2	6	1	16	20	16	17	16
	♀	4	4	-2	5	1	17	19	18	19	19
500	♂	0	2	0	3	1	17	18	18	19	17
	♀	2	3	-2	4	2	16	16	16	16	16
1000	♂	5	2	-2	2	3	20	22	21	23	19
	♀	2	4	-2	4	2	18	19	15	20	20
2000	♂	0	4	-3	4	3	18	18	16	17	18
	♀	1	3	-2	4	2	19	19	17	21	20

Conclusion: LD₅₀ > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

<u>Comment RMS:</u>	<p>The study was conducted according to an US EPA test guideline (1982). The study is in general agreement with the current valid test guidelines, e.g. US EPA guideline (OPPTS 850.2100, April 1996) and OECD guideline (OECD 223, July 2010). The control mortality was determined to be below 10% (being 0%) which is in line with the validity criteria according OECD 223.</p> <p>Hence, the study is considered acceptable.</p>
---------------------	---

Reference:	The acute oral toxicity (LD₅₀) of NC 8438 to the bobwhite quail
Author(s), year:	████████████████████ 1977b
Report/Doc. number:	Study no. A83330, Reference no.: M-155599-01-1
Guideline(s):	US EPA guidelines, June 1975 (including some modification as stated in the draft US EPA guideline, April 1977)
GLP:	No
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	NC 8438 (technical ethofumesate), batch : CR. 4805/3, purity : 97%
Test species:	Bobwhite quail (<i>Colinus virginianus</i>)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	170-217 g bodyweight
Type of test:	Acute oral toxicity
Applied concentrations:	0 (corn oil vehicle only), 2781, 5474 and 8743 (two replicates) mg ai/kg bw, dosage volume: dosage volume: 4.0 mL/bird
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a Ch 14 plastic catheter, diluent: corn oil
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: 25°C, relative humidity: 55-60% Feed was provided ad libitum during acclimation and during the test, except of a starvation period of at least 15 hours prior to testing.

Test parameter:

Observations:	Mortalities, bird health and clinical observations were observed daily. Individual bodyweights were observed on Days -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 3, 7 and 14 (post-treatment period). Group mean food consumption was measured weekly. At test termination, post-mortem examination was carried out on five birds in each group.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD ₅₀ . The LD ₅₀ value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities:	No mortalities occurred in the control group and in the treatment groups.
Clinical signs:	All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed
consumption:

Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table B. 9.1.1-4: Mortality and growth (bodyweights and food consumption) of bobwhite quails following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
		-14 to -0	0 to 7	7 to 14	0 to 14	-14 to -8	-7 to 0	0 to 7	8 to 14
Control	0	-1	1	6	7	13	16	16	15
2781	0	-3	-6	1	-5	14	16	15	15
5474	0	-4	-6	3	-3	14	18	15	16
8743 (R ₁)	0	-4	-5	3	-2	13	20	19	17
8743 (R ₂)	0	-5	-4	5	1	13	15	15	16

R...replicate

Conclusion:

LD₅₀ > 8743 mg ai/kg bw, NOEL = 8743 mg ai/kg bw

Comment RMS:

The study was conducted according to an US EPA test guideline (1975). The study is in general agreement with the current valid test guidelines, e.g. US EPA guideline (OPPTS 850.2100, April 1996) and OECD guideline (OECD 223, July 2010) with some deviations. Some information on the test methods (e.g. lighting regime, ...) and the test organisms (age, medication) are missing. In addition, the study was not conducted according to GLP.

However, the control mortality was determined to be below 10% (being 0%) which is in line with the validity criteria according OECD 223.

Based on the results of the study the identified deviations do not seem have an adverse impact on the mortality, food consumption and body weight of the birds. Hence, the study is considered acceptable.

Reference:	Acute oral toxicity study (limit study) with ethofumesate in Japanese quails
Author(s), year:	██████████ 1992
Report/Doc. number:	Project no. 352060/34
Guideline(s):	OECD guideline no. 401, February 1987 and EED directive 84-449, Annex V (adopted)
GLP:	Yes (self-certified)
Deviations:	<ul style="list-style-type: none"> - At autopsy, it appeared that one female was in fact a male. Consequently, the study was carried out with 6 males and 4 females, instead of 5 males and 5 females. - On some occasions, the relative humidity exceeded the highest level of 70% mentioned in the guidelines. - Since two animals were picked by the other animals in the cage, they were housed individually.
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate, batch : 03/06/92, purity : 98%
Test species:	Japanese quail (<i>Coturnix japonica</i>)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	156-210 g bodyweight, > 6 weeks old
Type of test:	Acute oral toxicity (limit test)
Applied concentrations:	2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation directly into the crop or proventriculus of each bird using a flexible oral gavage, diluent: corn oil
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	<p>Test temperature: $22 \pm 3^{\circ}\text{C}$, relative humidity: $32-83 \pm 5.0\%$ (SD), lighting: 12h light and 12h darkness.</p> <p>Feed (standard chicken diet) was provided ad libitum during acclimation and during the test, except of an overnight starvation period prior to testing.</p>

Test parameter:

Observations:	<p>Mortalities, bird health and clinical observations were observed daily. Individual bodyweights were observed immediately prior to dosing and on Days 3, 7 and 14 (post-treatment period).</p> <p>At test termination, post-mortem examination was carried out.</p>
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform the calculation of an LD_{50} . The LD_{50} value was determined to be greater than the highest dosage tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

Findings:

Mortalities:	No mortalities occurred in the 2000 mg ai/kg treatment group.
Clinical signs:	All control birds and all birds in the treatment group were normal in appearance and behaviour throughout the test.
Body weight, feed consumption:	There were no treatment-related effects on body weight and feed consumption in the treatment group.

Table B. 9.1.1-5: Mortality and growth (bodyweights and food consumption) of Japanese quails following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight [g]			
			Day 0	Day 3	Day 7	Day 14
2000	0	♂	166	163	176	179
	0	♀	199	203	214	215

Conclusion: LD₅₀ > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

<u>Comment RMS:</u>	The study was conducted according to no given avian test guideline but to a guideline developed for mammals (rodents). The study was conducted without a control group. However, controls are required to monitor the health and husbandry of the test animals and to ensure that the results are not compromised. Hence, the study is not considered valid.
---------------------	--

B.9.1.1.2. Short-term dietary toxicity to birds

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) no short-term dietary toxicity test are required to address the risk to birds. No new short-term dietary toxicity studies were submitted for the re-newal of the active substance ethofumesate. The results of the short-term dietary studies summarised in the DAR (2000) are given below.

[REDACTED], 1991a

Methods

The dietary toxicity (LC₅₀) of ethofumesate (purity 98%) to the mallard duck, *Anas platyrhynchos*, was studied in four groups of ten birds (regardless of sex) given a diet with a nominal concentration of 650, 1300, 2600 or 5200 mg ethofumesate/kg food. Two control groups of ten birds each were offered basal diet alone. The birds were 9 days of age at the beginning of the test. The test diets were given for a period of five days and were thereafter replaced with a basal diet for an additional observation period of three days.

Results

No mortalities occurred during the study and no signs of toxicity or treatment related effects on food consumption or body weight were observed. No macroscopic abnormalities were detected. NOEC > 5200 mg/kg food. LC50 > 5200 mg/kg food.

Comments

Conducted in compliance with GLP and the US EPA FIFRA 71-2 guideline.

[REDACTED], 1990a

Methods

*The dietary toxicity (LC50) of ethofumesate (purity 97%) to the mallard duck, *Anas platyrhynchos*, was studied in six groups of ten birds (regardless of sex) given a diet with a nominal concentration of 163, 325, 650, 1300, 2600 or 5200 mg ethofumesate/kg food. Three control groups of ten birds each were offered basal diet alone. The birds were 10 days of age at time of introduction of the test diet. The test diets were given for a period of five days and were thereafter replaced with a basal diet for an additional observation period of three days.*

Results

no mortalities occurred during the study. All birds remained in good health and no signs of toxicity or treatment related effects on food consumption or body weight were observed. no macroscopic abnormalities were detected. NOEC > 5200 mg/kg food. LC50 > 5200 mg/kg food.

Comments

Conducted in compliance with GLP and the US EPA FIFRA 71-2 guideline.

[REDACTED], 1991b

Methods

*The dietary toxicity (LC50) of ethofumesate (purity 98%) to the bobwhite quail, *Colinus virginianus*, was studied in groups of ten birds (regardless of sex) given a diet with a nominal concentration of 650, 1300, 2600 or 5200 mg ethofumesate/kg food. Two control groups of ten birds each were offered basal diet only. The birds were 12 days of age at time of introduction of the test diet. The test diets were given for a period of five days and were thereafter replaced with a basal diet for an additional observation period of three days.*

Results

There were no mortalities during the study. All birds remained in good health and there were no signs of toxicity or treatment related effects on food consumption or body weight. No macroscopic abnormalities were detected. NOEC > 5200 mg/kg food. LC50 > 5200 mg/kg food.

Comments

Conducted in compliance with GLP and the US EPA FIFRA 71-2 guideline.

██████████, 1990b

Methods

*The dietary toxicity (LC50) of ethofumesate (purity 97%) to the bobwhite quail, *Colinus virginianus*, was studied in groups of ten birds (regardless of sex) given a diet with a nominal concentration of 163, 325, 650, 1300, 2600 or 5200 mg ethofumesate/kg food. Three control groups of ten birds each were offered basal diet only. The birds were 14 days of age at time of introduction of the test diet. The test diets were given for a period of five days and were thereafter replaced with a basal diet for an additional observation period of three days.*

Results

One bird in the highest dose group (5200 mg/kg) was found dead on day 4. There were no other mortalities during the study. All surviving birds appeared to be in good health and there were no treatment related effects on food consumption or body weight. no macroscopic abnormalities were detected. NOEC 2600 mg/kg food. LC50 > 5200 mg/kg food.

Comments

Conducted in compliance with GLP and the US EPA FIFRA 71-2 guideline.

██████████ 1994a

Short term toxicity, 8 d study on birds. Reference to the Agrochemicals Handbook etc. Acute oral LD50 for mallard duck >3550, bowwhite quail > 8750 mg/kg. Five-day dietary LC50 for mallard duck and bowwhite quail > 1000 mg/kg diet.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

One additional study (■■■■■, 2001) was performed, which was not submitted during the first EU peer-review process. The study was requested during the first EU peer-review process by the ECCO-Peer-Review Meeting.

Reference:	Bobwhite quail Dietary reproduction study – Ethofumesate (Code AE B049913 00 1D97 0002)
Author(s), year:	■■■■■ 2001
Report/Doc. number:	Study no. C013708, Reference no. M-205119-01-1
Guideline(s):	FIFRA Subdivision E, Section 71-4, OECD Guideline 206 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance	Ethofumesate technical (AE B049913 00 1D97 0002), Batch no.: TM99000756, Purity 97.7%, CAS no.: 26225-79-6
Test species:	Bobwhite quail (<i>Colinus virginianus</i>)
Number of organisms:	16 pens with one male and one female per group
Weight, age:	Mean body weight between 206 g (males) and 207 g (females) at test initiation, 7 months
Type of test:	Reproductive toxicity
Applied concentrations:	Control (untreated diet), 50, 225, 1000 and 3000 ppm ai
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Type of application:	Test substance mixed in the diet, prepared weekly
Phases of the study:	Acclimation (pre-treatment): week -2 to -1; Pre-egg laying phase: week 1 - 7; Induction phase: week 8 – 10; Egg-laying phase: week 11- 20
Time of exposure:	20 weeks

Test conditions:

Temperature / relative humidity:	Adult housing: 22 ± 1 °C / 50 – 80% Egg storing: 12 - 16 °C / 50 – 70 % Incubation: 37.5 ± 0.2 °C / 60 – 70 % Hatching: ~ 37.0 °C / 80 %
Lightning:	Weeks 1-7: 8L:16D Week 8 onwards: photoperiod increased to 17 hours of light per day and was maintained at that length until the adult birds were euthanized. Illuminance: 200 - 300 lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during

acclimation and testing.

Test parameter:

Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained of all mortalities and clinical observations.
Adult Body weight:	Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination. Offspring body weights were determined at hatching and at 14 days of age for surviving ducklings.
Adult feed consumption:	Feed consumption for each pen was measured weekly throughout the test. Attempts were made to minimize feed wastage.
Egg parameter:	Records were maintained for each pen and each week of the numbers of eggs that were laid, cracked, abnormal, set and hatched and the numbers of viable and live three-week embryos. Eggshell thickness was recorded for each pen and every two weeks.
Hatchling parameter:	Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week.
Necropsy:	Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the end of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas and necropsied.
Statistics:	Parameters were evaluated statistically by comparison with the control at the level of significance $p < 0.05$.

Findings:

Analytical results:	The achieved concentrations, stability and homogeneous distribution of the test substance in the diet for 35 days at room temperature were confirmed as acceptable, i.e. in the range of 80 % and 120 % of nominal (being 90 – 113 % of nominal).
Biological effects:	<p>Behaviour and general health was unaffected by the test substance in all groups up to and including 3000 ppm. no clinical signs and no mortality attributable to the test substance were observed.</p> <p>No significant treatment related differences from the controls were observed with regard to body weight or feed consumption in any of the treatment groups. The estimated test substance intakes for mallard ducks during the test were 6.7, 31.5, 140 and ca 406 mg ai/kg bw/d for the 50, 225, 1000 and 3000 ppm treatment groups, respectively. no treatment related necropsy findings were recorded. The main gross pathological observations found in the control and treatment groups were food lesions, ovary regressing/regressed and small testes (< 4.0 cm).</p> <p>No treatment related effects were seen on egg production, egg-shell thickness,</p>

embryo viability, hatching or off-spring body weight and survival were observed during the study.

Table B 9.1.1.3-1: Group body weight, food consumption and test substance intake

Parameter	Control	50 ppm	225 ppm	1000 ppm	3000 ppm
Mean male bw (treatment phase) [g] (% of control)	222.2	215.1 (-3.2%)	219.1 (-1.4%)	219.9 (-1.0%)	210.4 (-5.3%)
Mean female bw (treatment phase) [g] (% of control)	225.4	225.9 (0.2%)	224.4 (-0.4%)	225.0 (-0.2%)	221.7 (-1.6%)
Group mean food consumption [g/bird/d] (% of control)	19.1	18.5 (- 3.1%)	18.5 (-3.1%)	19.0 (- 0.5%)	19.1 (0.0%)
Test substance intake [mg ai/kg bw/d]	-	4.2	18.7	85.0	264.0

* Significantly different from the control at $p < 0.05$

Table B 9.1.1.3-2 : Summary of reproductive results in the bobwhite quail

Reproductive parameter	Test concentration [mg ai/kg diet]				
	control	50	225	1000	3000
Number of replicates	16	16	16	16	16
Egg parameters					
Total eggs laid	807	886	801	915	829
Eggs laid/hen	50.4	55.4	50.1	57.2	51.8
Eggs normal	49.8	55.1	49.5	57.1	52.6
Eggs cracked	8	4	7	1	1
Eggs cracked/eggs laid [%]	1.00	0.50	0.81	0.13	0.13
Egg weight [g]	9.93	10.09	10.05	9.99	9.89
Mean eggshell thickness measurements [mm]	0.2095	0.2126	0.2096	0.2094	0.2146
Incubation data					
Hatchlings	586	633	637	670	666
Fertile eggs / incubated eggs [%]	84.9	84.6	95.5	86.7	95.7
Live 3-week embryos / fertile eggs [%]	94.3	98.6	94.8	97.8	98.6
Hatchlings / live 3-week embryos [%]	93.5	93.9	94.5	94.1	93.1
Hatchlings / fertile eggs [%]	88.3	92.7	89.7	92.0	91.7
Hatchlings/incubated eggs [%]	75.1	78.5	86.1	79.7	87.9
Chicks (F₁) parameters					
14-day old survivors/hen	29.1	33.6	35.6	35.6	37.5
14-day old survivors / incubated eggs [%]	59.7	66.4	76.5	67.1	79.2
14-day old survivors / chicks hatched [%]	78.4	84.3	87.6	84.7	90.3
14-day old survivors / fertile eggs [%]	69.7	78.3	79.4	78.3	82.7
14-day old survivors	466	537	570	570	600
Mean hatchling bodyweight [g]	7.1	7.3	7.2	7.2	7.0
Mean 14-day old survivor bodyweight [g]	25.8	27.1	25.7	26.5	26.5

Conclusion: Dietary administration for 20 weeks of up to 3000 ppm Ethofumesate had no effect on the growth or reproductive performance of Bobwhite quail. The no Observed Effect Level (NOEL) was 3000 ppm.

Comment RMS: The bird reproduction study was conducted according to the OECD test guideline 206 (1984). Based on the validity criteria stated in the guideline the study was considered acceptable. The mortality of adults in the control was below 10% at the end of the test (being: 0%). The average number of 14-day old survivors per hen in the controls was at least 12 for mallard ducks (being: 29.1). The average egg shell thickness for the control group was at least 0.19 mm (being: 0.2095 mm).

Based on the results of the study the NOEC was determined to be the highest test concentration, 3000 ppm. Based on an average daily feed consumption of 19.1 g/bird/d and an average body weight of 216.1 g a daily dose of 265 mg ai/kg bw/d was determined (according SANCO/4145/2000-final).

Reference:	Ethofumesate, Code: AE B049913 00 1D97 0002: Mallard duck dietary reproduction study
Author(s), year:	██████████ 2000
Report/Doc. number:	Study no. C008193, Reference no. M-197270-01-1
Guideline(s):	FIFRA Subdivision E, Section 71-4, OECD Guideline 206 (1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance Ethofumesate technical (AE B049913 00 1D97 0002), Batch no.: CR19291/02/940701, Purity 97.7%, CAS no.: 26225-79-6

Test species: Mallard duck (*Anas platyrhynchos*)

Number of organisms: 16 pens with one male and one female per group,

Weight, age: 874 - 1291 g at test initiation, 17 weeks

Type of test: Reproductive toxicity

Applied concentrations: Control (untreated diet), 50, 225, 1000 and 3000 ppm ai

Analytics: Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.

Type of application: Test substance mixed in the diet, prepared weekly

Phases of the study: Acclimation (pre-treatment): 3 weeks; Pre-photostimulation: 8 weeks; Pre-egg laying (with photostimulation): 0 weeks; Egg-laying: 12 weeks; Post-adult

	termination (final incubation, hatching, and 14-day offspring rearing period): 6 weeks
Time of exposure:	20 weeks
Test conditions:	
Temperature / relative humidity:	Adult housing: 20.4 ± 1.1 °C / 37 ± 14 % Egg storing: 13.2 ± 0.2 °C / 68 ± 11 % Incubation: 37.5 ± 0.0 °C / 60.0% Hatching: 37.2 ± 0.0 °C / 77.0 % Offspring management: 23.7 ± 1.0 °C / 46 ± 12 %
Lightning:	Weeks 1-7: 8L:16D Week 8 onwards: photoperiod increased to 17 hours of light per day and was maintained at that length until the adult birds were euthanized. Illuminance: 161 ± 69 lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet contained at least 27% protein and 2.5% crude fat, and no more than 5% crude fibre. Additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the basal diet for the adults.
<u>Test parameter:</u>	
Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained of all mortalities and clinical observations.
Adult Body weight:	Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination. Offspring body weights were determined at hatching and at 14 days of age for surviving ducklings.
Adult feed consumption:	Feed consumption for each pen was measured weekly throughout the test. Attempts were made to minimize feed wastage.
Egg parameter:	Records were maintained for each pen and each week of the numbers of eggs that were laid, cracked, abnormal, set and hatched and the numbers of viable and live three-week embryos. Eggshell thickness was recorded for each pen and week as appropriate.
Hatchling parameter:	Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week.
Necropsy:	Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the end of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas and necropsied.
Statistics:	Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental

group, except adult body weight where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine transformation.

Findings:

Analytical results: Samples collected during the test to measure the achieved test substance concentrations for the 50, 225, 1000 and 3000 ppm diets had percent of nominal values in the range of 86.7% to 110.8% at the time of preparation. Analysis of diet samples collected on Day 8 for stability assessment gave results of greater than 92% of the Day 0 value for the 50, 225, 1000 and 3000 ppm test concentrations, respectively.

Biological effects: No mortalities occurred and no clinical signs of toxicity were observed among adult birds in any of the treatment groups or control. no significant treatment related differences from the controls were observed with regard to body weight or feed consumption in any of the treatment groups. The estimated test substance intakes for mallard ducks during the test were 6.7, 31.5, 140 and ca 406 mg ai/kg bw/d for the 50, 225, 1000 and 3000 ppm treatment groups, respectively. no treatment related necropsy findings were recorded. The main gross pathological observations found in the control and treatment groups were food lesions, ovary regressing/regressed and small testes (< 4.0 cm).

no treatment related effects were seen on egg production, egg-shell thickness, embryo viability, hatching or off-spring body weight and survival were observed during the study.

Table B 9.1.1.3-3: Mean group body weight from a mallard duck dietary reproduction study

Test concentration [ppm]	Sex	Group mean body weight [g]					
		Week 0	Week 2	Week 4	Week 6	Week 8	Test end
Control	Male	1147	1130	1154	1155	1150	1201
	Female	1058	1068	1096	1092	1089	1163
50	Male	1136	1132	1150	1135	1127	1192
	Female	1009	1014	1045	1030	1025	1124
225	Male	1123	1101	1090	1107	1112	1191
	Female	1025	999	996 *	1013	1005 *	1095
1000	Male	1137	1147	1142	1155	1145	1197
	Female	1017	1040	1052	1066	1060	1202
3000	Male	1106	1113	1112	1117	1117	1157
	Female	996	1012	1031	1035	1015	1089

* Significantly different from the control at $p < 0.05$

Table B 9.1.1.3-4 : Summary of reproductive results in the mallard duck

Reproductive parameter	Test concentration [mg ai/kg diet]				
	control	50	225	1000	3000
Number of replicates	16	16	16	16	16
Total eggs laid ^a	709	810	646	848	821
Eggs cracked	30	16	6	14	13
Eggs set	605	715	567	757	735
Viable embryos	578	658	522	662	601
Live 3-week embryos	567	652	517	649	596
Hatchlings	473	555	457	539	531
14-day old survivors	466	553	456	537	526
Eggs laid/hen	44	51	40	53	51
Eggs laid/hen/day	0.52	0.59	0.47	0.62	0.60
14-day old survivors/hen	29	35	29	34	33
Mean eggshell thickness measurements [mm]	0.379	0.389	0.381	0.383	0.391
Mean hatchling bodyweight [g]	36	37	37	37	36
Mean 14-day old survivor bodyweight [g]	267	268	249	255	251
Eggs laid/maximum laid [%]	57	65	52	68	66
Eggs cracked/eggs laid [%]	4	2	1 *	2	2
Viable embryos/eggs set [%]	96	93	89	88	84
Live 3-week embryos/viable embryos [%]	98	98	99	98	99
Hatchlings/live 3-week embryos [%]	84	85	89	83	89
14-day old survivors/hatchlings [%]	99	100	100	99	99
Hatchlings/eggs set [%]	79	79	79	72	74
14-day old survivors/eggs set [%]	78	79	79	71	73
Hatchlings/maximum set [%]	42	50	41	48	47
14-day old survivors/maximum set [%]	42	49	41	48	47

* Significantly different from the control at $p < 0.05$

Conclusion:

Dietary administration of up to 3000 ppm technical ethofumesate to mallard ducks for 20 weeks had no effect on their growth or reproductive performance.

The no observed effect concentration for mallard ducks treated with ethofumesate in the diet during this reproduction study was 3000 ppm, the highest concentration tested (equivalent to an achieved daily intake of 406 mg ai/kg body weight/day).

Comment RMS:

The bird reproduction study was conducted according to the OECD test guideline 206 (1984). Based on the validity criteria stated in the guideline the study was considered acceptable. The mortality of adults in the control was below 10% at the end of the test (being: 0%). The average number of 14-day old survivors per hen in the controls was at least 14 for mallard ducks (being: 29). The average egg shell thickness for the control group was at least 0.34 mm (being: 0.379 mm). However, it should be considered the age of the birds at the beginning of the test (17 weeks) was not in the range as proposed according to the OECD guideline (9 – 12 months).

Based on the results of the study the NOEC was determined to be the highest test concentration, 3000 ppm. Based on an average daily feed consumption of 145.5 g/bird/d and an average body weight of 1075 g a daily dose of 406 mg ai/kg bw/d was determined (according SANCO/4145/2000-final).

1994b

Reference to the Agrochemicals Handbook etc. "As the results of our a.m. study and the results of the enclosed literature are both >1000 mg/kg, it is not necessary to carry the following tests on ethofumesate tech. out: short term toxicity test, reproduction test."

Comments

The reproductive toxicity of the active ingredient to birds must be investigated unless it can be justified that continued or repeated exposure of adults is unlikely to occur.

B.9.1.2. Effects on terrestrial vertebrates other than birds

For detailed information please see part B.6 of this RAR

B.9.1.2.1. Acute oral toxicity to mammals

No additional studies were submitted for the re-newal of the active substance. A summary of the toxicity of ethofumesate to mammals is given in table B.9.1.2.1-1.

Table B 9.1.2.1-1: Acute toxicity of ethofumesate to mammals

Test species	Test design	Ecotoxicological endpoints	Reference
Rat	Acute, oral	LD ₅₀ > 2000 mg ai/kg bw	██████, 1992
		LD ₅₀ > 7500 mg ai/kg bw	██████, 1991
		LD ₅₀ > 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

The endpoint from the acute oral study with rats ([REDACTED] 1992) for the active substance ethofumesate is > 2000 mg ai/kg bw. No mortalities occurred at this dose and this endpoint is used for risk assessment.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

No additional studies were submitted for the re-newal of the active substance. A summary of the toxicity of ethofumesate to mammals is given in table B.9.1.2.2-1.

Table B 9.1.2.2-1: Long-term toxicity of ethofumesate to mammals

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	2-generation reproduction	NOAEC = 1000 ppm NOAEL = 60.9 mg ai/kg bw/d ¹	██████, 1993 ██████████, 2013
	2-generation reproduction	NOAEC = 3000 ppm NOAEL _{female} = 256 mg ai/kg bw/d	██████ 1990
	3-generation reproduction	NOAEC = 1000 ppm NOAEL _{male} = 78 mg ai/kg bw/d	██████ at al., 1980
Rabbit	Teratogenicity study	NOAEL = 300 mg ai/kg bw	██████ et al., 1986

Bold values were used for the risk assessment

¹ 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 P₀, 21 day survival index in P₀) and the reproduction (↓ mean litter size in P₀, ↑ pre-implantation loss in P₀ 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 P₀ generation males and in the P₁ generation males, mainly at the beginning of the study).

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

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

The log P_{OW} values of the active substance ethofumesate and its metabolites BCS-CU88901, BCS-CW35117, AE C509607 and AE C508493 are 2.7 (Bright and Stalker, 1990), -1.4 (Ziemer and Kloeckner, 2012), -1.3 (Eyrich and Ziemer, 2013), 2.2 (Bogdoll and Peschke, 2012) and 1.5 (Bogdoll and Peschke, 2012), respectively.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Based on the available information on birds and mammals and the conducted literature search no risk for reptiles and amphibians is indicated.

B.9.1.5. Potential for endocrine disruption

Wild mammals

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.

Birds

The population relevant effects of ethofumesate on birds were studied in reproductive toxicity studies on bobwhite quail 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 mg ai/kg bw. 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.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test exists, this test was developed to evaluate the potential effect on the thyroid system, and not to measure population relevant effects. Therefore no further studies can be suggested at this time for these groups of organisms.

B.9.2. EFFECT ON AQUATIC ORGANISMS

In order to complete the aquatic risk assessment and to address the new data requirements for active substances (Regulation 283/2013) and plant protection products (Regulation 284/2013) according to Regulation (EC) no. 1107/2009, additional studies were performed. In addition, tests on marine species, which were no data requirement according to the Regulation 91/414/EEC and hence were not evaluated during the first EU peer-review of the active substance are evaluated and summarised as well.

Studies submitted during the first EU peer-review of the active substance were evaluated according to the current valid test guidelines and are summarised below.

B.9.2.1. Acute toxicity to fish

Active substance:

Reference:	The acute toxicity of [¹⁴ C]-ethofumesate to rainbow trout (<i>Oncorhynchus mykiss</i>) under static conditions
Author(s), year:	██████████, 1991a
Report/Doc. number:	Study no. A83375, Reference no. M-155643-01-1
Guideline(s):	OECD test guideline 203 (1984), US EPA guideline (1985)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, batch no. : CFQ 6191, purity : 97.78%, radiolabelled
Test species:	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Holding of fish :	Test medium: Dilution water Environmental conditions: temperature 12°C ± 2°C, photoperiod 16 h light and 8 h dark, light intensity approximately 1500 lux Feeding of fish: Twice daily ad libitum, weekends only once per day
Number of organisms:	10 fish per controls and test concentrations
Age, length, weight:	12-14 weeks, 57.1 mm (average length), 1.9617 g (average weight)
Loading	1.3078 g/L fish loading per test vessel
Type of test:	Static

Applied concentrations:

Nominal:	0 (control and solvent control), 2.36, 4.72, 9.44, 18.88 and 37.76 mg ai/L
Measured (mean):	- (control and solvent control), 1.96, 4.10, 8.05, 15.96 and 33.28 mg a.s/L

Solvent:	DMF (0.5 mL/L)
<u>Test conditions:</u>	
Water quality:	Dilution water, hardness: 76.33 mg /L as CaCO ₃ , alkalinity: 63.66 mg/L as CaCO ₃
Conductivity:	145 µs/cm
Temperature:	12.0 – 13.2 °C
pH:	7.12 – 7.48
O ₂ content:	64 – 92% (mean), test start: 97%, test end: 46 – 57%
Light regime:	Light/dark cycle of 16/8, light intensity approximately 1500 lux
Feeding	The fish were not fed during the 96 hours study period.
Methods:	<p>The test was carried out in glass aquaria of ca. 28 litre capacity with internal dimensions of 450 mm x 250 mm x 250 mm (length x width x depth).</p> <p>At the initiation of the study ten fish were allocated at random to each test vessel.</p>
Test parameters:	<p>All test vessels were monitored for mortality and sub-lethal effects after 3, 14, 24, 48, 72 and 96 hours.</p> <p>Measurements of temperature, pH, conductivity and dissolved oxygen were made in all treatment solutions at the start and end of the test and after 24, 48, and 72 hours.</p> <p>In addition, temperature was continuously measured in the control vessel.</p>
Analytical measurements:	<p>At the start and the end of the test samples of the stock and test solutions were taken for quantitative and qualitative analysis.</p> <p>Quantitative measurement of radioactivity in solution was carried out by liquid scintillation counting (LSC). A qualitative analysis of the test solutions were conducted by thin layer chromatography (TLC).</p>
Statistics:	The mortality data was statistically analysed using the method of Weil for 24, 48, 72 and 96 hour LC ₅₀ values and 95% confidence intervals.
<u>Findings:</u>	
Analytical data:	The mean [¹⁴ C]-ethofumesate concentration over the study period was between 83.1 and 88.1% of the nominal test concentration. Hence, the results of the study are based on nominal test concentrations.

Table B. 9.2.1-1: Mortality and sub-lethal effects

Test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)						
	0 h	3 h	14 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 (1/10)	10 (1/10)
Solvent control	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
2.36	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.72	0 (0/10)	0 (0/10)	0 (0/10) ^a	0 (0/10) ^s	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a
9.44	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^{ade}	0 (0/10) ^a	0 (0/10) ^{ae}
18.88	0 (0/10) ^a	0 (0/10) ^{ab}	0 (0/10) ^{cd}	0 (0/10) ^{cd}	0 (0/10) ^{acd}	40 (4/10) ^{ae}	40 (4/10) ^{ace}
37.76	0 (0/10) ^{ab}	0 (0/10) ^{cd}	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 20.2 mg ai/L (95% C.I.: 16.1 – 25.4 mg ai/L)							
96 h NOEC = 2.36 mg ai/L (based on effects on the colour of the fish)							
96 h LOEC = 4.72 mg ai/L							

^a Dark in color, ^b Loss of equilibrium, ^c Fish immobile / convulsive on bottom of tank, ^d Fish gasping / surfacing, ^e Fish less active than in control

Conclusion:

The lowest concentration that resulted in 100% mortality within the period of the test was 37.76 mg ai/L.

No mortalities occurred at the three lowest test concentrations (2.36, 4.72 and 9.44 mg ai/L) over the 96 hour exposure period although 70% of the fish at 4.72 mg ai/L exhibited sub-lethal effects. Hence the NOEC was 2.36 mg ai/L and the LOEC was 4.72 mg ai/L.

Based on these results a LC₅₀ of 20.2 mg ai/L (nominal concentrations) was determined.

Comment RMS:

The study was conducted according to the OECD test guidelines 203 (1984) and the US EPA test guideline OPPTS 850.1075 (1985). The validity criteria regarding the acute toxicity test with fish have not changed significantly within the versions of the test guidelines according OECD and US EPA.

Taking into account the current valid test guidelines according to OECD (1992) and US EPA (1996) the acute fish study with the freshwater species rainbow trout is considered not acceptable. Even though the mortality in the control was 10 % (one fish out of 10), the dissolved oxygen concentration was not maintained throughout the test duration.

The dissolved oxygen throughout the test was less than 60% of the air saturation. After 72 hours of exposure the dissolved oxygen was observed to be between 45 and 69% in all treatment and control groups. At the end of the study the dissolved oxygen was measured to be between 46 and 64%. The reason for the low dissolved oxygen might be the high loading rate of fish in the test vessels. The loading rate was 1.3078 g fish/L. According to the test guidelines (OECD and US EPA) the

loading rate should be below 1.0 g fish/L (OECD) and below 0.8 g fish/LL (US EPA) for static tests.

Based on the evaluation of the study the acute fish toxicity test is not considered acceptable based on the low dissolved oxygen throughout the test.

Reference:	The acute toxicity of [¹⁴C]-ethofumesate to bluegill sunfish (<i>Lepomis macrochirus</i>) under semi-static conditions
Author(s), year:	██████████ 1991b
Report/Doc. number:	Study no. A83373, Reference no. M-155641-01-1
Guideline(s):	OECD test guideline 203 (1984), US EPA guideline (1985)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, Batch no.: CFQ 6469 (radiolabelled sample), purity: 97. 8% Batch no.: R000047 (technical), purity: 99.9%
Test species:	Bluegill sunfish (<i>Lepomis macrochirus</i>)
Holding of fish :	Test medium: Dilution water All fish were acclimatised to laboratory conditions for at least 14 days prior to commencement of the study. Environmental conditions: temperature 12°C ± 2°C, photoperiod 16 h light and 8 h dark, light intensity approximately 1500 lux Feeding of fish: Twice daily ad libitum, weekends only once per day The fish were not fed for 24 hours prior to use.
Number of organisms:	10 fish per controls and test concentrations
Age, length, weight:	Juvenile fish, ~ 12 weeks, 36.9 mm (average length), 0.7507 g (average weight)
Loading	0.375 g/L fish loading per test vessel
Type of test:	Semi-static
<u>Applied concentrations:</u>	
Nominal:	0 (control and solvent control), 0.94, 1.88, 3.75, 7.50, 15, 30 and 60 mg ai/L
Measured (mean):	- (control and solvent control), 1.09, 2.06, 4.04, 7.88, 15.57, 30.5, 56.23 mg ai/L
Solvent:	Dimethyl formamide (DMF), 0.5 mL/L
<u>Test conditions:</u>	
Water quality:	Dilution water, hardness: 73.5 – 79.33 mg /L as CaCO ₃ , alkalinity: 69.17 – 79.0 mg/L as CaCO ₃

Conductivity:	142.9 – 165.2 $\mu\text{s}/\text{cm}$
Temperature:	21.8 – 22.8 °C (mean)
pH:	7.02 – 7.20 (test start), 7.16 – 7.58 (test end), 7.18 – 7.37 (mean)
O ₂ content:	74.0 – 98.0 %
Light regime:	Light/dark cycle of 16/8, light intensity approximately 1500 lux
Feeding	The fish were not fed during the 96 hours study period.
Methods:	<p>The test was carried out in glass aquaria of ca. 28 litre capacity with internal dimensions of 450 mm x 250 mm x 250 mm (length x width x depth).</p> <p>At the initiation of the study ten fish were allocated at random to each test vessel.</p> <p>The test solutions were renewed after 48 hours to ensure oxygen concentrations were not significantly depleted, and to ensure maintenance of test solution concentrations.</p>
Test parameters:	<p>All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 24, 48, 72 and 96 hours.</p> <p>Measurements of temperature, pH, conductivity and dissolved oxygen were made in all treatment solutions at the start and end of the test and after 24, 48, and 72 hours. In addition, temperature was continuously measured in the control vessel.</p>
Analytical measurements:	<p>At the start and the end of the test samples of the stock and test solutions were taken for quantitative and qualitative analysis.</p> <p>Quantitative measurement of radioactivity in solution was carried out by liquid scintillation counting (LSC). A qualitative analysis of the test solutions were conducted by thin layer chromatography (TLC).</p>
Statistics:	The mortality data was statistically analysed using the method of Weil for 24, 48, 72 and 96 hour LC ₅₀ values and 95% confidence intervals.
<u>Findings:</u>	
Analytical data:	<p>The mean over the 96 hour study period ranged from 93.7 to 115.4% of nominal.</p> <p>Therefore for the purpose of the LC₅₀ calculations nominal values were used.</p>

Table B. 9.2.1-2: Mortality and sub-lethal effects

Test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)						
	0 h	3 h	6 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)	0 (0/10)	0 (0/10)
Solvent control	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
0.94	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
1.88	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
3.75	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
7.50	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
15.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
30.0	0 (0/10) ^a	0 (0/10) ^{ac}	0 (0/10) ^{ac}	0 (0/10) ^{abc}	30 (3/10) ^{ab}	90 (9/10) ^{ab}	100 (10/10)
60.0	0 (0/10) ^a	90 (9/10) ^{ab}	90 (9/10) ^{ab}	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 21.2 mg ai/L							
96 NOEC = 15 mg ai/L, 96 h LOEC = 30 mg ai/L							

^a Dark in colour, ^b Fish immobile, ^c Fish gasping / surfacing

Conclusion:

The lowest concentration that resulted in 100% mortality within the period of the test was 30.00 mg ai/L. No mortalities or sublethal effects were recorded at the five lowest concentrations (15.0, 7.5, 3.75, 1.88 and 0.94 mg ai/L) over the 96 hour exposure period. Hence the NOEC was 15.0 mg ai/L and the LOEC 30.0 mg ai/L.

Comment RMS:

The study was conducted according to the OECD test guidelines 203 (1984) and the US EPA test guideline OPPTS 850.1075 (1985). The validity criteria regarding the acute toxicity test with fish have not changed significantly within the versions of the test guidelines according OECD and US EPA.

Taking into account the current valid test guidelines according to OECD (1992) and US EPA (1996) the acute fish study with the freshwater species bluegill sunfish 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.

Based on the evaluation of the study the acute fish toxicity test is considered valid and acceptable to be used in the risk assessment.

Reference:	The acute toxicity of ethofumesate technical to the sheephead minnow (<i>Cyprinodon variegatus</i>) in a static system
Author(s), year:	1992
Report/Doc. number:	Study no. A83384, Reference no. M-155652-01-1
Guideline(s):	OECD test guideline 203 (1984), US EPA guideline (1985)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, Batch no.: CR 19291\2, purity: 97%
Test species:	Sheephead minnow (<i>Cyprinodon variegatus</i>)
Holding of fish :	Test medium: Synthetic sea water (salinity of 17 ‰) All fish were acclimatised to laboratory conditions for at least 48 hours prior to initiation of the study. Environmental conditions: temperature 22°C ± 1°C, photoperiod 16 h light and 8 h dark, light intensity approximately 150 foot candles Feeding of fish: Twice daily ad libitum, weekends only once per day The fish were not fed for 24 hours prior to use.
Number of organisms:	10 fish per controls and test concentrations
Age, length, weight:	Juvenile fish, ~ 3 months Mean weight and length of control fish taken at the end of the study: 2.3 cm (SD = 0.21 cm) and 0.314 g (SD = 0.071 g)
Loading	0.165 g/L fish loading per test vessel
Type of test:	Static

Applied concentrations:

Nominal:	0 (control and solvent control), 4, 7, 12, 19 and 32 mg ai/L
Measured (mean):	- (control and solvent control), 4.2, 7.1, 12, 17 and 28 mg a.s/L
Solvent:	Triethylene glycol (TEG), 0.5 mL/L

Test conditions:

Water quality:	Dilution water, hardness: 73.5 – 79.33 mg /L as CaCO ₃ , alkalinity: 69.17 – 79.0 mg/L as CaCO ₃
Salinity:	17‰ (throughout the test)
Temperature:	Range: 21.2 – 22.7 °C, mean: 22.1 °C (SD = 0.45 °C)
pH:	8.4 – 8.5 (test start), 7.9 – 8.1 (test end)
O ₂ content:	4.7 – 7.4 ppm (= mg O ₂ /L) The dissolved oxygen was > 60% of air saturation throughout the test.
Light regime:	Light/dark cycle of 16/8, light intensity approximately 125 foot candle
Feeding	The fish were not fed during the 96 hours study period.

Methods:	Test chambers were 19 L glass fish tanks containing ~ 15 L of test solution. Tank dimensions were ~ 40.1 cm x ~ 24.5 cm x ~ 20.4 cm (length x width x depth), with a test solution depth of ~ 18.4 cm. All test chambers were covered with glass sheets to prevent evaporation and entry of foreign materials. Test solutions were not aerated during the study.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 24, 48, 72 and 96 hours. Measurements of temperature, pH, salinity and dissolved oxygen were made in all treatment solutions at the start and end of the test and after 48 hours. In addition, temperature was continuously measured in the control vessel.
Analytical measurements:	Samples of all treatments were taken at test initiation (Day 0), prior to addition of the fish, and at test termination (96 hours). Samples were analysed for ethofumesate by High Performance Liquid Chromatography.
Statistics:	Mortality data was analysed using Toxdat. Due to the nature of the data from this test (i.e. only one partial kill), the Binomial method was reported. The slope of the dose - effect line was determined with least squares linear regression of mortality (as a proportion) versus log ₁₀ dose. Slope was determined using SAS/STAT software for personal computers.
Findings:	
Analytical data:	The mean over the 96 hour study period ranged between the 80 and 120% of the nominal test concentration.

Table B. 9.2.1-3: Mortality and sub-lethal effects

Test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)				
	0 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)
Solvent control	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
7.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
12.0	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
19.0	0 (0/10)	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a	0 (0/10) ^a
32.0	0 (0/10)	0 (0/10) ^{ab}	20 (2/10) ^{ab}	40 (4/10) ^{ab}	70 (7/10) ^{ab}
96 h LC ₅₀ = 25 mg ai/L					
96 h NOEC = 12 mg ai/L (based on behavioural effects)					

^a Loss of equilibrium, ^b Lethargic

Conclusion:	No mortalities or sublethal effects were recorded at the test concentrations 4, 7 and 12 mg ai/L over the 96 hour exposure period. Hence, the NOEC was 12.0 mg ai/L and the LOEC 19.0 mg ai/L. The LC ₅₀ was determined to be 25 mg ai/L based on mean measured concentrations.
--------------------	--

<u>Comment RMS:</u>	<p>The study was conducted according to no given test guideline. However, the study was conducted in general agreement with accepted guidelines. The validity criteria regarding the acute toxicity test with fish have not changed significantly within the versions of the test guidelines according OECD and US EPA.</p> <p>Taking into account the current valid test guidelines according to OECD (1992) and US EPA (1996) the acute fish study with the saltwater fish species 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.</p> <p>Based on the evaluation of the study the acute fish toxicity test is considered valid and acceptable to be used in the risk assessment.</p>
----------------------------	---

Reference:	Ethofumesate: Determination of acute toxicity (LC₅₀) to rainbow trout (96 h semi-static)
Author(s), year:	████████████████████ 1989
Report/Doc. number:	Study no. A87614, Reference no. M-161551-01-1
Guideline(s):	US EPA (Guidelines E, Subdivision 72-1)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, batch no.: not given, purity : > 97%
Test species:	Rainbow trout (<i>Oncorhynchus mykiss</i> , formerly known as <i>Salmo gairdneri</i>)
Holding of fish :	Test medium: Dechlorinated tap water Environmental conditions: temperature 13°C ± 2°C, photoperiod 16 h light and 8 h dark, artificial daylight.
Number of organisms:	5 fish per replicate, two replicates per test concentration and control
Age, length, weight:	44 – 57 mm length, 1.222 – 2.809 g weight (control group)
Loading	0.4 – 0.5 g/L fish loading per test vessel
Type of test:	Semi-static
<u>Applied concentrations:</u>	
Nominal:	0 (control), 2.0625, 4.125, 8.25, 16.5 and 33 mg ai/L
Measured (mean):	- (control), 1.76, 3.70, 7.34, 14.5 and 28.1 mg ai/L (mean measured concentrations of ethofumesate at 0 hours)
Solvent:	None

Test conditions:

Water quality:	Dechlorinated tap water, total hardness: 76 – 104 mg/L as CaCO ₃ , Alkalinity: 64 – 92 mg/L as CaCO ₃
Conductivity:	0.20 – 0.31 mS
Temperature:	12.9 – 13.7 °C (test start), 11.8 – 12.1 °C (test end)
pH:	8.2 – 8.6 (test start), 8.4 – 8.6 (test end)
O ₂ content:	69 – 80% (test start), 64 – 76 % (test end) Throughout the study the dissolved oxygen was > 60% (60 – 89%).
Light regime:	Light/dark cycle of 16/8, artificial daylight
Feeding	The fish were not fed throughout the duration of the tests or for the period 24 h before the initiation of the test.
Methods:	Tanks of 25 L capacity, of moulded glass construction, and covered with polypropylene lids to prevent dust contamination, were used for the tests. Test and control tanks were set up using 20 L final volumes of charcoal-filtered dechlorinated tap water. The charcoalfiltered dechlorinated tap water was aerated prior to tank preparation. Tanks were not aerated during the test but were prepared using preaerated charcoal-filtered dechlorinated tap water and fish were transferred to freshly prepared tanks at 12 h intervals.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 12, 24, 36, 48, 60, 72, 84 and 96 hours. At the termination of the definitive test, the length and weight of each fish in the control tank was recorded. The pH, temperature, conductivity and dissolved oxygen concentration were measured at the beginning and at 12 h intervals in all tanks throughout the test. Dissolved oxygen concentrations were also measured at 12 h after tank preparation in all tanks throughout the test. Water hardness and alkalinity were also measured.
Statistics	The intercept and dose response curve and hence LC ₅₀ (with 95% confidence limits) were estimated by applying the standard technique of maximum likelihood estimation to the probit model.

Findings:

Analytical data:	The mean ethofumesate concentration over the study period was between 78 and 99% of the nominal test concentration.
Biological effects	Swimming behaviour of the fish was observed throughout the test period. Loss of balance was noted in fish at a nominal concentration of 33 mg ai/L shortly after their addition to the test solutions, 100% mortalities were observed within 1 h exposure to Ethofumesate. Fish at a nominal concentration of 16.5 mg ai/L were noted to be darkened in appearance at 3 h and at further time points throughout the test.

Unusual swimming behaviour observed at 3 h and at further time points throughout the test included loss of equilibrium and lethargic swimming.

Fish at a nominal concentration of 8.25 mg ai/L were noted to be darkened in appearance at 48, 72 and 96 h. no other unusual characteristics were observed.

No unusual appearance or swimming behaviour was observed at nominal concentrations of 4.125, 2.0625 and 0 mg ai/L throughout the test period.

Table B. 9.2.1-4: Mortality after 96 h of exposure to ethofumesate

Test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)						
	0 h	3 h	12 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)	0 (0/10)	0 (0/10)
2.0625	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.125	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
8.25	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
16.5	0 (0/10)	10 (1/10)	40 (4/10)	50 (5/10)	70 (7/10)	80 (8/10)	80 (8/10)
33.0	0 (0/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 11.91 mg ai/L (based on mean measured concentrations)							
96 h NOEC = 4.125 mg ai/L							

Conclusion:

The highest measured concentration tested causing no mortalities within the test period was 7.31 mg ai/L. The lowest measured concentration tested causing any mortality within the test period was 14.2 mg ai/L.

Based on these results a LC₅₀ of 11.91 mg ai/L (mean measured concentrations) was determined.

Comment RMS:

The study was conducted according to the US EPA test guideline. The study was conducted in general agreement with accepted guidelines. The validity criteria given in the current valid test guidelines according OECD (203, 1992) and US EPA (OPPTS 850.1075) are met.

The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being: 0%). The constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test.

Based on the evaluation of the study the acute fish toxicity test is considered valid and acceptable to be used in the risk assessment.

However, the documentation of the methods and results of the study is poor. No information regarding the batch used is given. Additionally, no information on the age of the fish is given in the study.

Nevertheless, the RMS is of the opinion that the results of the study are acceptable

and should be used for the risk assessment.

Reference:	Ethofumesate: Determination of acute toxicity (LC₅₀) to bluegill sunfish (96 h, semi-static)
Author(s), year:	1990
Report/Doc. number:	Study no. A87615, Reference no. M-161552-01-1
Guideline(s):	US EPA (Guidelines E, Subdivision 72-1)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, purity : > 97%
Test species:	Bluegill sunfish (<i>Lepomis macrochirus</i>)
Holding of fish :	Test medium: Reconstituted water All fish were acclimatised to laboratory conditions for at least 12 days prior to commencement of the study. Environmental conditions: temperature 22°C ± 1°C, photoperiod 16 h light and 8 h dark, artificial daylight.
Number of organisms:	10 fish per test vessel
Age, length, weight:	30 - 32 mm length, 0.508 – 0.655 g weight (control group)
Loading	0.5 g/L fish loading per test vessel
Type of test:	Semi-static

Applied concentrations:

Nominal:	0 (control), 2.0625, 4.125, 8.25, 16.5 and 33 mg ai/L
Measured (mean):	- (control), 1.75, 3.55, 7.29, 14.2 and 21.1 mg ai/L
Solvent:	None

Test conditions:

Water quality:	Reconstituted water, total hardness: 30 - 38 mg/L as CaCO ₃ , Alkalinity: 16 - 20 mg/L as CaCO ₃
Conductivity:	0.18 – 0.23 mS
Temperature:	22.0 – 22.7 °C (test start), 22.0 – 22.5 °C (test end)
pH:	7.9 – 8.0 (test start), 7.9 (test end)
O ₂ content:	80 - 83% (test start), 56 - 67 % (test end) In some test vessels the dissolved oxygen was determined to be below 60% of air saturation.
Light regime:	Light/dark cycle of 16/8, artificial daylight
Feeding	The fish were not fed throughout the duration of the tests or for the period 24 h

	before the initiation of the test.
Methods:	<p>Tanks of 25 L capacity, of moulded glass construction, and covered with polypropylene lids to prevent dust contamination, were used for the tests. Test and control tanks were set up using 20 L final volumes of charcoal-filtered water. The charcoal-filtered water was aerated prior to tank preparation.</p> <p>Tanks were not aerated during the test but were prepared using preaerated charcoal-filtered water and fish were transferred to freshly prepared tanks at 12 h intervals.</p>
Test parameters:	<p>All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 12, 24, 48, 72 and 96 hours. At the termination of the test, the length and weight of each fish in the control tank was recorded.</p> <p>The pH, temperature, conductivity and dissolved oxygen concentration were measured at the beginning and at 12 h intervals in all tanks throughout the test. Dissolved oxygen concentrations were also measured at 12 h after tank preparation in all tanks throughout the test. Water hardness and alkalinity were also measured.</p>
Statistics	<p>The intercept and dose response curve and hence LC_{50} (with 95% confidence limits) were estimated by applying the standard technique of maximum likelihood estimation to the probit model.</p>
<u>Findings:</u>	
Analytical data:	<p>The mean ethofumesate concentration over the study period was between 78 and 99% of the nominal test concentration.</p>
Biological effects	<p>Swimming behaviour of the fish was observed throughout the test period. Unusual characteristics in fish at a nominal concentration of 33 mg ai/L included swimming close to the water surface, slow respiratory movements and very lethargic swimming. 100% mortalities were observed within 6 h exposure to Ethofumesate at this concentration.</p> <p>Fish at a nominal concentration of 16.5 mg ai/L were noted to show lethargic swimming at 36 h and at all further time points. A single fish at 96 h showed rapid respiratory movements and was incapable of other movements, including swimming, unless touched.</p> <p>Fish at a nominal concentration of 8.25 mg ai/L were noted to show lethargic swimming by comparison to the control fish at 96 h. no other unusual characteristics were noted at 8.25 mg ai/L during the test. No unusual appearance or swimming behaviour was observed at nominal concentrations of 4.125, 2.0625 and 0 mg ai/L throughout the test period.</p>

Table B. 9.2.1-5: Mortality and sub-lethal effects

Nominal test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)						
	0 h	3 h	12 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)	0 (0/10)	0 (0/10)
2.0625	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.125	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
8.25	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	20 (2/10)	20 (2/10)
16.5	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	10 (1/10)	40 (4/10)
33.0	0 (0/10)	90 (9/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 12.37 mg ai/L (95% C.I. : 9.31 – 16.34 mg ai/L)							
96 h NOEC = 3.55 mg ai/L							
based on mean measured concentrations							

Conclusion:

The highest mean measured concentration tested causing no mortalities within the test period was 3.56 mg ai/L. The lowest mean measured concentration tested causing any mortality within the test period was 7.32 mg ai/L.

Based on these results a LC₅₀ of 12.37 mg ai/L (mean measured concentrations) was determined.

Comment RMS:

The study was conducted according to no specified test guideline. The study was conducted in general agreement with accepted guidelines. The validity criteria given in the current valid test guidelines according OECD (203, 1992) and US EPA (OPPTS 850.1075, 1006) are not all met.

The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being; 0%). The constant conditions were maintained as far as possible throughout the test. However, the validity criteria regarding the dissolved oxygen were not met. In some of the treatment groups the dissolved oxygen was measured to be below 60%, i.e. about 56% of air saturation. Only slight deviation of the dissolved oxygen was observed. In addition, no adverse effects on fish due to low oxygen levels were observed throughout the test. Hence, the study is considered valid and might be used in the risk assessment for fish.

However, the documentation of the methods and results of the study is poor. No information regarding the batch used is given. Additionally, no information on the age of the fish is given in the study.

Nevertheless, the RMS is of the opinion that the study is acceptable for the use in the risk assessment. The identified deficiencies are not considered to have an effect on the validity of the study.

Reference:	Technical ethofumesate: 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. A83349, Reference no. M-155618-01-1
Guideline(s):	US EPA (Guidelines E, Subdivision 72-1)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, Batch no.: R000047, purity : 99.9%
Test species:	Mirror carp (<i>Cyprinus carpio</i>)
Holding of fish :	Test medium: Dechlorinated tap water All fish were acclimatised to laboratory conditions for at least 12 days prior to commencement of the study. Environmental conditions: temperature 22°C ± 2°C, photoperiod 16 h light and 8 h dark, artificial daylight.
Number of organisms:	5 fish per replicate, two replicates per test concentration and control
Age, length, weight:	41 - 48 mm length, 1.224 – 2.514 g weight (control group)
Loading	< 1.0 g/L fish loading per test vessel
Type of test:	Semi-static

Applied concentrations:

Nominal:	0 (control), 6.25, 12.5, 25, 50 and 100 mg ai/L
Measured (mean):	- (control), 2.79, 4.15, 6.51, 10.98 and 26.3 mg ai/L
Solvent:	Acetone, 0.1 g/L

Test conditions:

Water quality:	Dechlorinated tap water, total hardness: 80 - 84 mg/L as CaCO ₃
Conductivity:	0.20 – 0.27 mS
Temperature:	21.0 – 23.8 °C (test start), 21.6 – 22.5 °C (test end)
pH:	8.2 – 8.3 (test start), 8.2 – 8.3 (test end)
O ₂ content:	78 - 92% (test start), 82 - 91 % (test end) Throughout the study the dissolved oxygen was > 60% (71 - 95%).
Light regime:	Light/dark cycle of 16/8, artificial daylight
Feeding	The fish were not fed throughout the duration of the tests or for the period 24 h before the initiation of the test.
Methods:	Tanks of 25 L capacity, of moulded glass construction, and covered with polypropylene lids to prevent dust contamination, were used for the tests. Test and control tanks were set up using 20 L final volumes of charcoal-filtered dechlorinated tap water. The charcoalfiltered dechlorinated tap water was aerated

	<p>prior to tank preparation.</p> <p>Tanks were not aerated during the test but were prepared using preaerated charcoal-filtered dechlorinated tap water and fish were transferred to freshly prepared tanks at 24 h intervals.</p>
Test parameters:	<p>All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 24, 48, 72 and 96 hours. At the termination of the definitive test, the length and weight of each fish in the control tank was recorded.</p> <p>The pH, temperature, conductivity and dissolved oxygen concentration were measured at the beginning and at 12 h intervals in all tanks throughout the test. Dissolved oxygen concentrations were also measured at 12 h after tank preparation in all tanks throughout the test.</p> <p>Water hardness and alkalinity were also measured.</p>
Statistics	<p>The intercept and dose response curve and hence LC_{50} (with 95% confidence limits) were estimated by applying the standard technique of maximum likelihood estimation to the probit model.</p>
<u>Findings:</u>	
Analytical data:	<p>The mean ethofumesate concentration over the study period was between 12.0 and 73.2% of the nominal test concentration.</p> <p>The test material was sparingly soluble at all test concentrations. A fine white powder was visible on the floor of the tanks, covering about 10% of the tank floor area, together with a light surface film of powder. No material was evident in control tanks. Fish did not appear to consume undissolved material.</p>
Biological effects	<p>Swimming behaviour of the fish was observed throughout the test period. Abnormal swimming behaviour at 100 mg ai/L included erratic swimming and loss of equilibrium. Fish at 50 mg ai/L exhibited lethargic swimming throughout, particularly 72-96 hours after initiation of the test, and the eyes of fish at 96 hours were noted to protrude dramatically. No abnormal swimming behaviour was found at 25-6.25 mg ai/L technical ethofumesate or in the control tank.</p>

Table B. 9.2.1-6: Mortality after 96 h of exposure to ethofumesate

Nominal test concentration [mg ai/L]	Mortality [%] (no. of dead fish / no. of treated fish)						
	0 h	3 h	6 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)	0 (0/10)	0 (0/10)
6.25	0 (0/10)	0 (0/10)	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)	0 (0/10)	0 (0/10)
25	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
50	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	70 (7/10)	70 (7/10)
100	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
96 h LC ₅₀ = 10.92 mg ai/L (95% C.I. : 8.11 – 14.84 mg ai/L)							
96 h NOEC = 6.51 mg ai/L							
based on mean measured concentrations							

Conclusion:

The highest mean measured concentration tested causing no mortality within the period of the test was 6.70 mg ai/L.

The lowest mean measured concentration tested causing 100% mortality within the period of the test was 26.mg ai/L. Based on these results a LC₅₀ of 10.92 mg ai/L (mean measured concentrations) was determined.

Comment RMS:

The study was conducted according to no given test guideline. However, the study was conducted in general agreement with accepted guidelines. The validity criteria given in the current valid test guidelines according OECD (203, 1992) and US EPA (OPPTS 850.1075, 1006) are met.

The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being: 0%). The constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test.

Hence, the study is considered valid and might be used in the risk assessment for fish.

Reference:	Acute toxicity in rainbow trout (<i>Salmo gairdneri</i>)
Author(s), year:	██████ 1991a
Report/Doc. number:	Study no. A87614, Reference no. M-352116-01-1
Guideline(s):	OECD 203, EEC-Directive 79/831, Annex V.
GLP:	Yes
Deviations:	None
Validity:	Additional information

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, Batch no.: 09/06/91, purity : 98%
Test species:	Rainbow trout (<i>Oncorhynchus mykiss</i> , formerly known as <i>Salmo gairdneri</i>)
Holding of fish :	Test medium: Dechlorinated tap water Prior to the initiation of the test, the fish were acclimatized for a minimum of 14 days. Environmental conditions: temperature 15°C ± 1.5°C, photoperiod 16 h light and 8 h dark, 600-800 lux
Number of organisms:	5 fish per replicate, two replicates per test concentration and control
Length, weight:	5.9 cm length (mean), 2.2 g weight (mean)
Loading	Not given
Type of test:	Semi-static

Applied concentrations:

Nominal:	0 (control), 6.9, 9.7, 13.5, 19.0, 26.5, 37.0, 51.8 and 73.0 mg ai/L
Measured (mean):	Not given
Solvent:	None

Test conditions:

Water quality:	Dechlorinated tap water, hardness: 14 °dH
Conductivity:	Not given
Temperature:	16.4 – 17.1 °C (test start), 15.1 – 16.1 °C (test end)
pH:	7.26 – 7.98 (test start), 7.86 – 7.96 (test end)
O ₂ content:	7.8 – 9.7 mg O ₂ /L (test start), 7.4 – 10.0 mg O ₂ /L (test end) Throughout the study the dissolved oxygen was > 60%.
Light regime:	Light/dark cycle of 16/8, artificial daylight
Feeding	The fish were not fed throughout the duration of the tests or for the period 24 h before the initiation of the test.
Methods:	For each concentration, two 12L glass container were used. Each test vessel contained 5 fish (10 L water). Tanks were aerated continuously using a membrane pump system. The analytical controls concerning the concentration and stability of the test article were performed by means of GC analysis.

Test parameters:	<p>All test vessels were monitored for mortality and sub-lethal effects after 2-4, 24, 48, 72 and 96 hours. At the termination of the definitive test, the length and weight of 20 fish were recorded.</p> <p>The pH, temperature, conductivity and dissolved oxygen concentration were measured at the beginning and at 12 h intervals in all tanks throughout the test.</p> <p>Water hardness was also measured.</p>
Statistics	<p>Due to the nature of the data, a statistical calculation of the LC_{50} was not possible.</p> <p>Therefore, the LC_{50} was calculated as a geometric mean from the LC_0 and LC_{100}.</p>
Findings:	
Analytical data:	<p>The results of the analytical control measurements show that all concentrations levels were maintained at a constant level throughout the test. However, a deviation of ca. 20% as opposed to the nominal initial concentration is observed.</p>
Biological effects	<p>At a concentration level of 26.5 mg ai/L, 5 fish survived. However, two of these fish were found lying on their sides on the bottom of the aquarium.</p> <p>The fish appeared quiet and displayed a tendency to stay at the bottom of the aquaria at concentration levels of 13.5 mg ai/L and 19 mg ai/L. At or below concentrations of 9.7 mg ai/L, no abnormal effects were observed.</p>

Table B. 9.2.1-7: Mortality after 96 h of exposure to ethofumesate

Nominal test concentration [mg ai/L]	Mortality [%]				
	2-4 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
6.9	0	0	0	0	0
9.7	0	0	0	0	0
13.5	0	0	0	0	0
19.0	0	0	0	0	0
26.5	0	40	50	50	50
37.0	0	100	100	100	100
51.8	0	100	100	100	100
73.0	0	100	100	100	100
96 h LC_{50} = 26.5 mg ai/L (mean from LC_0 and LC_{100})					
96 h NOEC = 9.7 mg ai/L (based on behavioural effects)					

Conclusion:	Based on these results a LC_{50} of 26.5 mg ai/L (nominal concentrations) was determined. Due to the effects observed in the test, the NOEC was determined at a concentration of 9.7 mg ai/L.
--------------------	---

Comment RMS:	The study was conducted according to the OECD test guideline (1984). The validity criteria given in the current valid test guidelines according OECD (203, 1992) and US EPA (OPPTS 850.1075) are met.
---------------------	---

The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being: 0%). The constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test.

The information on the mean measured concentrations given in the study report is poor. Hence, no calculation of mean measured concentrations could be conducted. However, based on the available information the mean measured concentrations are considered to be below 80% of the nominal test concentrations. As the toxicity endpoints of the study are based on nominal concentrations the results should be used with caution.

Even under consideration of the deficiencies the RMS is of the opinion that the study could be used as additional information.

Reference: **Acute toxicity in golden orfe (*Leuciscus idus*)**

Author(s), year:	██████ 1993
Report/Doc. number:	Study no. 80-91-2312-01-93, Reference no. M-352126-01-1
Guideline(s):	OECD 203, EEC-Directive 79/831, Annex V.
GLP:	Yes
Deviations:	None
Validity:	Additional information

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, Batch no.: 20/03/93
Test species:	Golden orfe (<i>Leuciscus idus</i>)
Holding of fish :	Test medium: Dechlorinated tap water Prior to the initiation of the test, the fish were acclimatized for a minimum of 14 days. Environmental conditions: temperature $18 \pm 2^{\circ}\text{C}$, photoperiod 16 h light and 8 h dark, 600-800 lux
Number of organisms:	5 fish per replicate, two replicates per test concentration and control
Length, weight:	5.45 cm length (mean), 1.55 g weight (mean)
Loading	Not given
Type of test:	Static
<u>Applied concentrations:</u>	
Nominal:	0 (control), 0.92, 1.6, 2.9, 5.2, 9.3, 16.5, 29.3 and 52.2 mg ai/L
Measured (mean):	Not given

Solvent:	None
<u>Test conditions:</u>	
Water quality:	Dechlorinated tap water, hardness: 14 °dH,
Conductivity:	Not given
Temperature:	18.1 – 18.2 °C (test start), 19.5 – 19.8 °C (test end)
pH:	7.25 – 7.47 (test start), 7.99 – 8.18 (test end)
O ₂ content:	9.7 – 10.0 mg O ₂ /L (test start), 7.9 – 9.9 mg O ₂ /L (test end) Throughout the study the dissolved oxygen was > 60%.
Light regime:	Light/dark cycle of 16/8, artificial daylight
Feeding	The fish were not fed throughout the duration of the tests or for the period 24 h before the initiation of the test.
Methods:	For each concentration, two 12L glass container were used. Each test vessel contained 5 fish (10 L water). Tanks were aerated continuously using a membrane pump system. Analytical control measurements of the actual concentrations of the test article during the preliminary and the main test were performed by means of HPLC analysis.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 2-4, 24, 48, 72 and 96 hours. At the termination of the definitive test, the length and weight of 20 fish were recorded. The pH, temperature, conductivity and dissolved oxygen concentration were measured at the beginning and at 12 h intervals in all tanks throughout the test. Water hardness was also measured.
Statistics	Due to the nature of the data, a statistical calculation of the LC ₅₀ was not possible. Therefore, the LC ₅₀ was calculated as a geometric mean from the LC ₀ and LC ₁₀₀ .
<u>Findings:</u>	
Analytical data:	The saturated stock solution was analysed only prior to the initiation of the main test. During the main test, three representative concentration levels were analysed upon initiation of the test and thereafter every 24 h. In principle, the analytical values support the assumption that the test article concentration is stable over the entire duration of the test. However, the final (96 h) values show a sudden decline (in contrast to the findings of the preliminary test). This can be explained by the fact that these samples had been frozen (for operational reasons) and were filtered after thawing. Thus did not yield total recovery of the test article upon thawing.
Biological effects	During the main test, at or below nominal concentration levels of 9.3 mg ai/L, no abnormal effects in comparison to the control group were noted. At a concentration level of 16.5 mg ai/L, most of the fish showed a reduced activity and displayed the tendency to stay at the bottom of the test aquaria.

On the basis of these observations, the NOEC was determined at a concentration level of 9.3 mg ai/L.

Table B. 9.2.1-8: Mortality after 96 h of exposure to ethofumesate

Nominal test concentration [mg ai/L]	Mortality [%]				
	2-4 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
0.92	0	0	0	0	0
1.6	0	0	0	0	0
2.9	0	0	0	0	0
5.2	0	0	0	0	0
9.3	0	0	0	0	0
16.5	0	0	0	0	0
29.3	0	100	100	100	100
52.2	100	100	100	100	100
96 h LC ₅₀ = 22.0 mg ai/L (mean from LC ₀ and LC ₁₀₀)					
96 h NOEC = 9.3 mg ai/L (based on behavioural effects)					

Conclusion:

Based on these results a LC₅₀ of 22.0 mg ai/L (nominal concentrations) was determined. Due to the effects observed in the test, the NOEC was determined at a concentration of 9.3 mg ai/L.

Comment RMS:

The study was conducted according to the OECD test guideline (1984). The study was conducted in general agreement with accepted guidelines. The validity criteria given in the current valid test guidelines according OECD (203, 1992) and US EPA (OPPTS 850.1075) are met.

The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being 0%). The constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test.

The information on the mean measured concentrations given in the study report is poor. Hence, no calculation of mean measured concentrations could be conducted. However, based on the available information the mean measured concentrations are considered to be below 80% of the nominal test concentrations. As the toxicity endpoints of the study are based on nominal concentrations the results should be used with caution.

Even under consideration of the deficiencies the RMS is of the opinion that the study could be used as additional information.

B.9.2.2. Long-term and chronic toxicity to fish

For the first EU peer-review of the active substance ethofumesate prolonged toxicity studies with fish were submitted addressing the chronic risk to fish. However, these studies do not fulfil the new data requirements under Regulation 1107/2009. Thus, a new fish full life cycle study has been performed with the active substance ethofumesate.

A fish life cycle study with the zebrafish (*Danio rerio*) was submitted addressing the long-term risk to fish. The life cycle study including a P- and F₁-generation was conducted according to the OECD guidelines 210 (fish early life stage toxicity test) and 215 (fish juvenile growth test) and the draft OECD guideline “fish two – generation test”.

Reference:	Zebrafish (<i>Danio rerio</i>), Life Cycle test - Flow through conditions
Author(s), year:	██████████ 2013
Report/Doc. number:	Study no. EBADL027, Reference no. M-464613-01-1
Guideline(s):	OECD 210 (1992), OECD 215 (2000), OECD “Draft proposal for a new guideline: Fish Two-generation Test” (2002)
GLP:	Yes
Deviations:	<ul style="list-style-type: none"> - In the course of the study a further concentration step was set at nominal 0.156 mg ai/L. Additionally, a second control treatment was prepared. - The survival rates of larvae/juvenile fish were estimated by digital photography. A first photo data on day 14 post fertilisation was skipped. <p>The changes of the study protocol had no impact on the study integrity.</p>
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, batch no. : AE B049913-01-08, purity : 98.3%
Test species:	Zebrafish (<i>Danio rerio</i>)
Holding of fish :	<p>Parental fish (maximum age : 2 years) were held in aquaria with a total volume of 150 L. Holding water is of the same quality as used in the test (purified tap water). Environmental conditions: temperature 25°C ± 2°C, photoperiod 12 h light and 12 h dark, light intensity approximately 1000 lux</p> <p>Feeding of adult fish: Daily ad libitum with TetraMin® Hauptfutter and brine shrimp nauplii (<i>Artemia salina</i>)</p> <p>Feeding of fish larvae: Breeding food</p> <p>Only healthy fish without diseases and abnormalities were used as parental fish for the production of fertilised eggs. Fertilised eggs (microscopic determination of > 4 cell stage) were transferred by means of a widened and de-burred pipette tip into</p>

	the test chambers.
Number of organisms:	4 replicates per test concentration and controls 25 eggs per fry chambers, 2 fry chambers per aquarium, in total 200 eggs in four replicates. After 28 days, the fish from the two fry chambers of each replicate were pooled and randomly reduced to 30 individuals and released into the test vessels. After 56 days, the fish number was reduced to 20 individuals.
Age:	Freshly fertilized eggs
Type of test:	Flow-through test (water flow rate of 5.2 L/h, resulting in a daily turnover of approximately 5 volumes)
<u>Applied concentrations:</u>	
Nominal:	0 (control), 0.156 ^a , 0.313, 0.625, 1.25, 2.5 and 5.0 ^b mg ai/L ^a In the course of the study a further concentration step was set at nominal 0.156 mg ai/L. Additionally, a second control treatment (control II) was prepared. ^b Due to limited size of the flow through device, the highest treatment level at 5.0 mg ai/L was terminated after 60 days.
Measured (mean):	- (control), 0.156, 0.306, 0.620, 1.26, 2.47 and 4.99 mg ai/L
Solvent:	None
<u>Test conditions:</u>	
Water quality:	Purified drinking water (according OECD 215), total hardness: 1.0 – 1.2 mmol/L
Temperature:	25 ± 2 °C (measured: 24.3 – 26.5 °C)
pH:	7.7 – 8.6
O ₂ content:	79 - 105 % saturation
Light regime:	Light/dark cycle of 12/12, light intensity approximately 1000 lux
Feeding	Larvae were fed daily ad libitum with breeding food. From day 9 on, brine shrimp nauplii (<i>Artemia salina</i>) were added ad libitum. From day 16 on ground flake food was added ad libitum to the daily food.
Methods:	The in life phase was started with the introduction of fertilised eggs. After 28 days, the fish number was randomly reduced per replicate for the investigation of juvenile growth. After 56 days, the fish numbers were randomly reduced to 30 fish per replicate for the investigation of reproduction to 20 fish per replicate. Starting with day 70 of exposure, glass spawning trays were introduced and monitored daily for spawned eggs. The time until first spawns was recorded. The P-generation was terminated after the F ₁ generation passed the early life stage phase of 28 days. All fish were measured for length and weight. To start the F ₁ generation, 50 fertilised eggs per test vessel were placed in stainless free fry chambers. After 28 days, the F ₁ fish were sacrificed and measured for length and weight.
Test parameters:	Mortalities of different life stages, hatching rates (P- and F ₁ generation,

respectively), juvenile and adult growth, spawning performance, fertilisation rate, and sex ration were recorded.

All fish were observed daily for mortality and any other abnormalities in appearance and behaviour.

Between hatch and 28 days of the P- and F₁-generation, larvae/juvenile fish were photographed on day 21 and day 28 and the survival rates were estimated. Lengths of the P-fish were measured by digital photography after 28 and 56 days.

Fish weight was determined by weighing out wet and dried fish with both tissue and glass beaker and finally calculating the weight difference between. The single dry weight per fish was calculated by dividing the total group weight by the number of surviving fish at termination of the ELS phase.

For the time interval between day 28 and 56 the specific growth rate based on length was calculated.

The time of first spawning, identified as first day at which eggs were found in the spawning tray, was recorded.

Analytical measurements: The test item concentrations were measured in the test vessels three times per week during the initial two weeks of the study and once weekly thereafter.

The samples were analysed for the content of the test item using LC-MS/MS.

Statistics: All statistical tests and probit analysis were conducted using the software ToxRat Professional 2.10.

Findings:

Analytical data: The overall arithmetic mean measured concentrations per replicate were calculated to be between 92.4 and 107% of the nominal values. The overall mean measured concentrations, determined for each test level were between 97.7 and 101% of the nominal concentrations.

Thus, the effect values were evaluated based on nominal test item concentrations.

Effects on fish (P-generation): Early life stage: The hatching success was not affected and was > 90% in all treatments at the end of the hatching period (day 8 post fertilization (pf)). On day 6

pf, a slight delay in hatch was observed at the highest treatment level, which was detected to be statistical significantly different compared to the control.

The post hatch survival after 28 days pf, was found to be significantly reduced at 5.0 mg ai/L and ≥ 2.5 mg ai/L. respectively.

Fish growth (based on length) was found to be significantly reduced at ≥ 0.313 mg ai/L.

Table B. 9.2.2-1: P-generation – Hatch, survival and growth, 28 days pf (SD)

Test concentration [mg ai/L]	Hatch [%]				Post-hatch survival [%]		Length, day 28 pf [cm]
	Day 5 pf	Day 6 pf	Day 7 pf	Day8 pf	Day 21 pf	Day 28 pf	
Control I	66.0 ± 5.9	83.5 ± 7.5	93.5 ± 7.5	100 ± 0.0	79.5 ± 9.1	77.0 ± 6.6	0.98 ± 0.03
Control II	86.0 ± 11.2	98.0 ± 2.8	100 ± 0.0	nd	79.5 ± 5.5	79.5 ± 5.5	0.90 ± 0.03
0.156	73.5 ± 12.0	96.0 ± 4.9	97.5 ± 3.0	nd	79.6 ± 4.4	79.6 ± 4.4	0.95 ± 0.05
0.313	56.7 ± 11.6	71.1 ± 12.4	92.5 ± 6.4	99.0 ± 1.1	86.9 ± 1.1	86.9 ± 1.1	0.89 ± 0.05*
0.625	55.3 ± 11.3	67.2 ± 9.0	93.5 ± 7.9	93.5 ± 7.9	88.3 ± 6.6	85.5 ± 5.3	0.83 ± 0.05*
1.25	64.5 ± 9.3	80.5 ± 8.9	98.0 ± 1.6	99.5 ± 1.0	75.9 ± 10.6	74.9 ± 9.9	0.85 ± 0.07*
2.5	62.0 ± 16.1	75.5 ± 13.6	98.5 ± 1.9	99.5 ± 1.0	72.4 ± 8.7	65.4 ± 5.5 ^a	0.80 ± 0.05*
5.0	40.9 ± 1.7	64.5 ± 6.9*	97.5 ± 1.0	98.0 ± 1.6	61.8 ± 15.6*	57.2 ± 16.5 ^a	0.72 ± 0.05*
NOEC _{time of hatch} = 2.5 mg ai/L NOEC _{post-hatch survival} = 1.25 mg ai/L NOEC _{growth} = 0.156 mg ai/L							

nd...not determined, SD...Standard deviation

* Significantly different compared to the control (p < 0.05), Williams test, one-sided smaller

^a The check for variance homogeneity was not passed for this data set. However, Williams test was performed since it represents the most powerful multiple test. The respective test for non-homogenous variances, the Welch t-test, showed no significant difference at any treatment level.

Effects on fish (P-generation): Juvenile growth: no effects on survival of the juvenile stage could be observed. Growth in terms of length was statistical significantly reduced at ≥ 1.25 mg ai/L. The “pseudo” specific growth rate, based on length measurements performed on Day 28 and 56 pf, was not negatively affected. A significant increase was detected at ≥ 0.625 mg ai/L.

Table B. 9.2.2-2: P-generation – Survival and growth, day 56 pf (SD)

Test concentration [mg ai/L]	Survival between Day 28 and 56 pf [%]	Length, Day 56 pf [cm]	“Pseudo” specific growth rate (based on length)
Control I	98.3 ± 3.3	1.95 ± 0.04	2.53 ± 0.14
Control II	98.3 ± 1.9	2.12 ± 0.01	2.86 ± 0.14
0.156	100 ± 0.0	2.18 ± 0.07	2.96 ± 0.20
0.313	100 ± 0.0	1.89 ± 0.05	2.77 ± 0.23
0.625	96.6 ± 3.9	1.88 ± 0.07	2.97 ± 0.13 **
1.25	95.8 ± 5.0	1.88 ± 0.05 *	2.87 ± 0.21 **
2.5	98.3 ± 1.9	1.78 ± 0.03 *	2.87 ± 0.14 **
5.0	97.3 ± 3.3	1.74 ± 0.08 *	2.81 ± 0.29 **
NOEC _{growth} = 0.625 mg ai/L			

SD...Standard deviation

* Significantly different compared to the control (p < 0.05), Williams test, one-sided smaller

** Significantly different compared to the control (p < 0.05), Williams test, one-sided greater

Effects on fish (P-generation):

Reproduction: Compared to the controls, there was no significant difference with regard to the time to first spawning.

In one replicate of the additional control (control II), only irregular spawning was observed. This group was excluded from the overall evaluation of the reproductive parameter as well as the other parameters of the adult life stage.

The egg number per day and female as well as the fertilisation rate were not affected.

Table B. 9.2.2-3: P-generation – Reproduction (SD)

Test concentration [mg ai/L]	Time to first spawning [d]	Egg number/day/female	Fertilisation rate [%]
Control I	107.3 ± 5.6	23.2 ± 2.1	93.2 ± 2.0
Control II	96.8 ± 7.0	19.3 ± 10.3	94.4 ± 0.5
0.156	91.5 ± 5.2	13.3 ± 5.0	90.8 ± 3.1
0.313	107.3 ± 5.9	20.5 ± 2.9	93.8 ± 2.0
0.625	101.5 ± 3.3	22.4 ± 4.5	93.9 ± 1.7
1.25	110.3 ± 8.2	19.8 ± 4.6 ^a	94.0 ± 2.2
2.5	108.3 ± 6.4	19.6 ± 6.1 ^a	91.9 ± 3.2
NOEC _{reproduction} = 2.5 mg ai/L			

SD...Standard deviation

^a In one replicate of treatment 1.25 mg ai/L (A) and of treatment 2.5 mg ai/L (B) regular spawning of fish and consequently assessment of reproduction by counting of eggs were delayed. Regular spawning was fulfilled when the fish groups showed fertilisation rates ≥ 80% and total egg numbers of ≥ 15 eggs on three successive days. To prevent a delayed start of F₁ early life stage phase, egg counting was stopped as soon as collected data were sufficient to allow calculation of mean values for these replicates (i.e. on day 143). The advance termination had no impact on the quality of results.

Effects on fish (P-generation):

Termination: no test item related effect on the survival of the adult fish was observed. With regard to growth, a decrease of both length and weight was detected for males and females.

No visible effects on the sex ratio of fish were observed. The percentage of females was quite high through all treatments and in the controls. However, since the reproductive output was satisfying, there were no hints for a negative impact on the study outcome. Historical data of the test facility showed that even up to a percentage of around 75% females, a sufficient reproductive success (fecundity and fertility) can be derived.

Table B. 9.2.2-4: P-generation – Survival, growth and sex ratio, test termination (SD)

Test conc. [mg ai/L]	Survival [%]	Males		Females		Sex ratio [% males]	Sex ratio [% females]
		Length [cm]	Weight [g]	Length [cm]	Weight [g]		
Control I	93.6 ± 4.8	4.0 ± 0.1	0.54 ± 0.02	3.9 ± 0.1	0.594 ± 0.07	35.5 ± 14.6	64.5 ± 14.6
Control II	97.5 ± 2.9	3.9 ± 0.2	0.522 ± 0.1	3.9 ± 0.1	0.625 ± 0.1	27.5 ± 10.2	69.0 ± 4.4
0.156	95.0 ± 4.1	3.8 ± 0.1 ¹	0.460 ± 0.03	3.7 ± 0.02*	0.527 ± 0.04	29.0 ± 5.4	71.0 ± 5.4
0.313	93.8 ± 4.8	3.7 ± 0.1*	0.410 ± 0.05*	3.7 ± 0.04*	0.517 ± 0.01*	22.5 ± 7.2	77.5 ± 7.2
0.625	90.0 ± 7.1	3.8 ± 0.1*	0.439 ± 0.03*	3.7 ± 0.04*	0.501 ± 0.04*	42.5 ± 17.3	57.5 ± 17.3
1.25	98.8 ± 2.5	3.8 ± 0.2*	0.441 ± 0.04*	3.8 ± 0.2*	0.518 ± 0.06*	28.7 ± 4.4	70.0 ± 3.7
2.5	91.3 ± 8.5	3.7 ± 0.2*	0.385 ± 0.05*	3.6 ± 0.1*	0.472 ± 0.07*	23.9 ± 12.2	76.1 ± 12.2
NOEC _{survival} = 2.5 mg ai/L NOEC _{length} = 2.5 mg ai/L ^a NOEC _{weight} = 0.156 mg ai/L NOEC _{sex ratio} = 2.5 mg ai/L							

SD...Standard deviation

* Significantly different compared to the control (p < 0.05), Williams test, one-sided smaller

^a The statistical evaluation revealed significant difference at ≥ 0.313 mg ai/L for male and ≥ 0.156 mg ai/L for female. However, the calculated differences of all treatment levels compared to the control were found to be < 10% in all test concentrations. The differences of the treatment level ≤ 0.125 mg ai/L were found to be even < 5% compared to control. Furthermore, no dose response relationship could be observed within this concentration range with exception of the highest treatment level, in which the effect on growth reduction was slightly higher than in the other groups. Thus, this observation was considered to be not biologically relevant.

Effects on fish (F₁-
generation):

Early life stage: The F₁ generation was prepared by sampling eggs from the parental fish and keeping them for hatching.

Hatch of the F₁ larvae was > 90% in the controls and at ≤ 1.25 mg ai/L. At the highest test concentration, 2.5 mg ai/L the hatching success was found to be statistical significantly reduced.

The post hatch survival was not negatively affected. Furthermore, no effect on growth in terms of lengths and weight was observed.

Table 9.2.2-5: F₁-generation – Hatch, survival and growth (SD), Day 28 pf

Test conc. [mg ai/L]	Hatch [%]			Post hatch survival [%]		Length [cm]	Group dry weight [mg]	Single dry weight [mg]
	Day 5 pf	Day 6 pf	Day 7 pf	Day 21 pf	Day 28 pf			
Control I	75.0 ± 14.1	91.5 ± 3.0	92.5 ± 4.4	98.3 ± 2.2	95.1 ± 3.7	0.87 ± 0.01	15.5 ± 1.3	0.36 ± 0.05
Control II	95.4 ± 1.2	95.4 ± 1.2	95.4 ± 1.2	88.3 ± 8.2	88.3 ± 8.2	0.84 ± 0.01	14.6 ± 1.1	0.35 ± 0.02
0.156	95.5 ± 1.0	97.0 ± 2.0	97.0 ± 2.0	89.6 ± 8.9	86.0 ± 12.3	0.84 ± 0.05	15.9 ± 5.6	0.37 ± 0.10
0.313	75.6 ± 6.7	89.5 ± 6.4	92.5 ± 7.2	98.9 ± 2.1	98.9 ± 2.1	0.88 ± 0.02	20.0 ± 3.5	0.43 ± 0.04
0.625	72.7 ± 16.9	92.0 ± 1.6	94.0 ± 1.6	89.4 ± 14.1	88.8 ± 13.6	0.84 ± 0.04	13.5 ± 5.8	0.31 ± 0.09
1.25	64.5 ± 5.0	88.0 ± 5.4	90.5 ± 8.2	89.4 ± 9.9	89.4 ± 9.9	0.91 ± 0.02	17.3 ± 6.0	0.42 ± 0.12
2.5	62.5 ± 12.6	69.0 ± 17.2*	72.5 ± 18.3*	97.4 ± 5.1	96.8 ± 6.4	0.89 ± 0.05	15.4 ± 3.1	0.45 ± 0.05
NOEC _{hatch} = 1.25 mg ai/L NOEC _{post hatch survival} = 2.5 mg ai/L NOEC _{growth} = 2.5 mg ai/L								

* Significantly different compared to the control ($p < 0.05$), Williams test, one-sided smaller

Conclusion:

Based on the data derived from the study, the growth in terms of length of parental fish larvae (P-generation), and furthermore of length and weight of the parental adult fish (P-generation) was found to be the most sensitive endpoint. No effect on growth (based on length and weight) were observed for the F₁ (filial) generation. The hatching success and post hatch survival of the fish was affected in the P-generation (early life stage) and for hatching success in the F₁-generation, but these parameters were less sensitive. The parameters reproduction and sex ratio were not affected by the exposure to the test item ethofumesate.

Based on the most sensitive parameter growth of parental early life stages and adults, the overall NOEC was 0.156 mg ai/L, based on nominal concentrations.

Comment RMS:

The study was conducted according to three different test guidelines, the OECD test guidelines 210 and 215 and the OECD draft test guideline “fish two-generation test”. For the evaluation of the study the validity criteria of all used test guidelines were considered.

The early life stage of the study was conducted according to the OECD test guideline (1992 and 2013). For the test to be valid the following conditions apply:

- the dissolved oxygen concentration must be between 60 and 100% of the air saturation value throughout the test;
- the water temperature must not differ by more than $\pm 1.5^{\circ}\text{C}$ between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species ($20 \pm 2^{\circ}\text{C}$ according OECD 210, 1992 and $26 \pm 1.5^{\circ}\text{C}$ according OECD 2010, 2013);

- evidence must be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within $\pm 20\%$ of the mean measured values;
- overall survival of fertilised eggs in the controls must be greater than or equal to the given limits (hatching success at least 70% and post hatch success at least 70-75%).

According to the OECD test guideline 210 (version of the years 1992 and 2013) the fish early life stage test is considered valid. All validity criteria according to the OECD test guideline 210 (1992) and the current valid OECD test guideline 210 (2013) are met.

The juvenile growth was conducted according to the OECD test guideline 215 (2000). For the test to be valid the followings conditions apply:

- the mortality in the controls must not exceed 10% at the end of the test;
- the mean weight of fish in the controls must have increased enough to permit the detection of the minimum variation of growth rate considered as significant (recommended range for initial fish weight: 0.05-0.1 g);
- the dissolved oxygen concentration in each test vessel was greater than 60% of the air saturation value throughout the exposure period.
- the water temperature must not differ by more than $\pm 1^\circ\text{C}$ between test chambers at any one time during the test and should be maintained within a range of 2°C within the temperature ranges specified for the test species ($21\text{-}25^\circ\text{C}$).

According to the OECD test guideline 215 (2000) the fish juvenile growth test is considered valid. All validity criteria according to the OECD test guideline 215 (2000) are met.

In addition to the validity criteria given in the OECD test guideline 210 and 215 performance criteria are listed in the draft OECD guideline for the two-generation fish test. The following criteria should be considered for judging the acceptability of the data:

- Water quality characteristics should remain within the limits of tolerance depicted in Tables 1 and 2;
- There should be documentation of purity of the test material, all as delivery of chemical to the fish(e.g. concentrations of the chemical in test water);
- There should be more than 90% survival in the control animals in all test phases over the duration of the chemical exposure, and the control fish in each replicates in the two spawning phases should be spawn regularly;
- There could be greater than 80% fertility and hatchability of eggs and embryos, respectively, from the control animals.

The temperature was in a range between 24.3 – 26.5 °C throughout the test which is in line with the validity criteria according to the mentioned OECD test guidelines.

The hatch and post hatch success in the controls (control I and II) was greater than 80% (P- and F₁ generation). The survival of larvae/fish was greater than 90% in the controls.

No effects on the spawning were observed in the control groups. The spawning was regular and in time.

Based on the evaluation of the study the chronic fish toxicity test is considered acceptable.

Reference:	Ethofumesate – Fathead minnow (<i>Pimephales promelas</i>) early life stage toxicity test
Author(s), year:	██████████ 1991
Report/Doc. number:	Study no. A83372, Reference no. M-155640-01-1
Guideline(s):	US EPA Guideline 72-4
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Reference:	Ethofumesate technical: Statistical re-evaluation of the fish early life stage toxicity study with Fathead minnow (<i>Pimephales promelas</i>) by ██████████ 1991
Author(s), year:	██████████ 2013
Report/Doc. number:	Reference no. M-470756-01-1
Guideline(s):	None
GLP:	No

Material and methods:

Test substance:	Ethofumesate techn., CAS no. : 26225-79-6, batch no. : CR19291/2, purity : 97%
Test species:	Fathead minnow (<i>Pimephales promelas</i>)
Holding of fish :	Brood fish (3 females and 1 male) were held in aquaria with a total volume of 19 L. Holding water is of the same quality as used in the test (reconstituted, dechlorinated tap water). Environmental conditions: mean temperature 25.5°C (range: 24.5 – 26.0 °C), mean pH 7.5 (range: 7.5 – 7.6) Feeding of adult fish: Once daily with Tetra Conditioning Food and twice daily with brine shrimp nauplii (<i>Artemia salina</i>)

	Only healthy fish without diseases and abnormalities were used as parental fish for the production of fertilised eggs. Embryos were ≤ 48 hours old at test initiation.
Number of organisms:	2 replicates per test concentration and control, 35 embryos per replicate
Age:	Embryos were ≤ 48 hours old at test initiation.
Type of test:	Flow-through test
<u>Applied concentrations:</u>	
Nominal:	0 (control), 3.25, 5.25, 8.5, 16 and 25 mg ai/L
Measured (mean):	- (control), 2.56, 4.17, 7.04, 13.3 and 23.2 mg ai/L
Solvent:	None
<u>Test conditions:</u>	
Water quality:	Reconstituted, dechlorinated tap water, total hardness: 40 to 48 mg/L
Temperature:	24.9 – 25.3 °C
pH:	7.34 – 7.43
O ₂ content:	7.5 – 8.4 mg/L (dissolved oxygen > 60% of air saturation)
Conductivity:	217 – 259 μ S
Light regime:	Light/dark cycle of 16/8, light intensity approximately 430 lux
Methods:	Seventy fathead minnow embryos were randomly distributed among two replicates (35 embryos per replicate) of each treatment five days after initiation of the flow-through system. The maximum loading rate at the conclusion of the test was 0.25 g/L. The test consisted of five concentrations of test substance and a dilution water control as previously described.
Test parameters:	Dissolved oxygen concentration, pH, temperature, and conductivity were measured at 24 h intervals. Alkalinity and hardness of the control and the highest treatment with living organisms were analysed weekly.
Analytical measurements:	The concentration of ethofumesate was analysed in all chambers on day one, and one replicate from each treatment group (alternated with each sampling time) was analysed weekly until the termination of the study.
Statistics:	In the original study report the most sensitive endpoints fry growth expressed as wet weight and standard length were statistically analysed based on individual fish. The means based on individual fish of each of the two replicates at each treatment level were compared separately with the control using a one-tailed Dunnett's tests. For this analysis the fish of the two control replicates were pooled. As a result the analysis of fry growth revealed at the lowest (standard length) and at the second lowest concentration level (standard length and wet weight) statistical differences to the control in one replicate of each treatment, whereas the second replicate of the treatment levels did not show any statistical differences. However, the statistical procedure to compare each replicate separately with a control based on individual organisms does not reflect the state of the art in statistical analyses. For example it is stated by the most recent version of OECD

guideline 210 (2013): “In all analyses, the test chamber, not the individual fish, is the unit of analysis and the experimental unit and both hypothesis tests and regression should reflect that”. Thus, the statistical analysis as reported in the original study report is considered as not reliable.

Therefore, this statement presents a statistical re-evaluation of the original study data based on state-of-the-art approaches in statistical analysis.

NOEC Determination:

Biological data (hatching success/embryo survival, fry survival and growth data (standard length and wet weight)) for the replicate chambers of each concentration were grouped together for analysis. Replicate means were used for statistical analysis since each test chamber (aquarium) was an experimental unit based on the design of the test system. Data in percent were arcsine transformed before analysis. For each parameter analysed the following statistical tests were conducted:

- Shapiro Wilk-test procedure in order to test the correspondence with normal distribution
- Levene's-test to check homogeneity of variances
- One-sided William's test on multiple pair-wise comparisons was used subsequently to determine a significant difference between the treatment groups and the control with conclusions of statistical significance based on a 95 % confidence level ($\alpha = 0.05$).

Regression Estimates:

ECx-values were estimated by Probit analysis using linear max. likelihood regression. The observations used were replicate means (length and weight) or replicate proportions (hatching success/embryo survival, fry survival).

Findings:

Analytical data:

The mean measured concentrations of ethofumesate averaged 83% (77-94%) of nominal and remained stable throughout the 28-day exposure period.

Table B. 9.2.2-6: Survival and growth of larvae/fry, day 28

Test concentration [mg ai/L]	Survival after 28 d [%]	Length [mm]	Wet weight [mg]
Control	94.0	15.3	49.7
2.56	95.5	15.1	49.8
4.17	95.5	14.7	45.1
7.04	89.0	14.0*	39.6*
13.3	90.0	11.7*	21.5*
23.2	0.0 *	nd	
28 d NOEC _{larval/fry survival} = 13.3 mg ai/L, EC ₁₀ = 12.2 mg ai/L (95% C.I. = 7.34 – 15.28 mg ai/L) 28 d NOEC _{growth} = 4.17 mg ai/L 28 d EC _{10 length} = 7.31 mg ai/L (95% C.I. = 6.35 – 8.08 mg ai/L) 28 d EC _{10 weight} = 4.93 mg ai/L (95% C.I. = 2.96 – 6.27 mg ai/L)			

nd...not determined

* Significantly different compared to the control ($p < 0.05$), Williams test, one-sidedConclusion:

The overall chronic 28-day-NOEC observed in this study is 4.17 mg ai/L and the respective overall chronic 28-day-LOEC is 7.04 mg ai/L based on fry growth (standard length and wet weight). Based on the most sensitive endpoint fry growth expressed as wet weight the overall 28-day-EC₁₀ is 4.93 mg ai/L. All endpoints are based on mean measured concentrations.

Comment RMS:

The study was conducted according to the US EPA test guideline 72-4. The study is in line with the current test guideline (OECD 210, 2013) regarding the early life stage test with fish. The environmental conditions (dissolved oxygen > 60% of the air saturation, water temperature between test chambers or between successive days should not differ more than $\pm 1.5^\circ\text{C}$) were acceptable throughout the test. Biological criteria for acceptability of the test were met in this study. Spawns used to supply embryos for the study had > 90% fertility/survival. Survival of embryos/fry was 94% in the controls over the study period.

Based on the evaluation of the study the ELS fish toxicity test is considered acceptable.

In addition to the fish early life stage test (■■■■■ 1991) and the fish full life cycle test (■■■■■ 2013) three prolonged toxicity test with the rainbow trout were submitted. According to the new data requirements prolonged toxicity tests (21 day) are no longer a data requirement. Hence, the studies were not evaluated in detail for the renewal of the EU approval. The study summaries given in the DAR for the first EU approval are given below as additional information.

██████, 1993*Methods*

The prolonged toxicity (21-days) of ethofumesate (purity 97%) to rainbow trout, *Oncorhynchus mykiss*, was determined under flow-through conditions. Fish with an average length of 56 mm and an average wet weight of 2.7 g were exposed to five nominal concentrations of ethofumesate, 0.047, 0.19, 0.75, 3 and 12 mg/L. Stock solutions were prepared with ethofumesate in Tween 80 and tap water. The stock solutions were delivered via Hamilton dispenser units into mixing flasks receiving filtered (5 µm) active carbon treated tap water. The flow rate was 100 l test medium per test tank and 24 hour. In addition, fish were exposed to tap water and Tween 80, and tap water alone as controls. The test was conducted in aquaria, each containing 50 l of test medium, at a temperature of 13.5 - 15.5°C. Groups of ten rainbow trouts were distributed to each test aquarium resulting in a loading of 0.17 - 0.20 g/L test solution. The fish were fed commercial fish food (Kliba Forellenfutter 33-364) daily throughout the test. Test water characteristics were; total hardness as CaCO₃ 26.5 - 41.2 mg/L, alkalinity as CaCO₃ 23.3 - 27.8 mg/L, pH 7.8 - 8.4 and conductivity 483 - 802 µS/cm.

Results

At the highest test concentration (12 mg/L) mortality started on day 10 and increased up to 30% on day 19. No mortality was observed in the lower concentrations or in the controls. Hence the threshold level of lethal effect was between 3 and 12 mg/L. No statistically significant effects on fish growth was observed up to a nominal concentration of 3 mg/L, except at 0.75 mg/L where the fish length were significantly different from the tap water control but not the Tween 80 control. In the Tween 80 control, and in the 0.75 and 12 mg/L treatments, sublethal effects such as loss of equilibrium, dark pigmentation and abnormal swimming behaviour were observed. No sublethal responses were observed in the tap water control or in the 0.19 and 3 mg/L treatments. The sublethal effects in the 0.75 mg/L treatment was not pronounced and was judged as being of no significance. NOEC and the threshold level for sublethal effects were reported to 3 mg/L and between 3 and 12 mg/L, respectively. The 21-days LC₅₀ was >12 mg/L. All values were based on nominal concentrations. The mean measured concentrations ranged from 82 to 111% of nominal concentrations.

Comments

The study was conducted in compliance with GLP and with OECD guideline 204.

██████ et al., 1990*Methods*

The prolonged toxicity (21-days) of ethofumesate (purity 99.9%) to rainbow trout, *Oncorhynchus mykiss*, was determined under semi-static conditions. Fish with an average length of 72 mm and an average wet weight of 3.6 g (n = 10) were exposed to five nominal concentrations of ethofumesate, 1.0, 3.0, 9.0, 27 and 80 mg/L. Test solutions were prepared by adding appropriate amounts of ethofumesate to 15 l reconstituted water. The test was conducted in glass aquaria, each containing 15 l of test medium, at a temperature of 15°C ± 1°C. Two replicate aquaria were used for each test concentration including two control aquaria. Groups of five rainbow trouts were distributed to each aquarium resulting in a loading of 1.2 g/L test solution. The test solutions were renewed 3 times per week. The fish were fed commercial fish food (Silvercup) daily throughout the test and the

test solutions were aerated. Test water characteristics were; total hardness as CaCO_3 196 - 250 mg/L , alkalinity not given, pH 7.4 - 7.9 and conductivity not given.

Results

At the highest nominal test concentration (80 mg/L) 100% mortality occurred on day two, and at the second highest test concentration (27 mg/L) 10% mortality occurred on day 21. No mortalities were observed in the lower test concentrations or in the controls. The threshold level of lethal effect based on measured concentrations was 19 mg/L . At the two highest test concentrations sublethal effects included reduced or severely reduced mobility. Slightly reduced mobility was observed in the 9.0 mg/L treatment on day 16, 19 and 20. No sublethal responses were observed at lower concentrations or in the controls. A statistically significant reduction in fish growth was observed at a nominal concentration of 27 mg/L . NOEC and the threshold level for sublethal effects based on measured concentrations were reported to 2.1 mg/L and 6.2 mg/L , respectively. The 21-day LC_{50} was 22 mg/L (probit analysis) based on measured concentrations. The measured concentrations ranged from 45 to 74% of nominal concentrations.

Comments

The study was conducted in compliance with GLP and was conducted in agreement with OECD guideline 204. The fish loading was higher than recommended, however, this is not considered to have affected the results of the study.

■, 1991b

The prolonged toxicity (21-days) of ethofumesate to rainbow trout, *Oncorhynchus mykiss*, was determined under semi-static conditions. Findings in this study (21-d LC_{50} 18.8 mg/L and NOEC 0.8 mg/L) were in agreement with the results from the prolonged toxicity studies on rainbow trout submitted by AgrEvo (Knacker, 1990 and ■, 1993). The study has not been evaluated.

B.9.2.3. Potential for endocrine disruption

Population relevant effects of ethofumesate on fish were studied in an early life-stage test (ELS) in Fathead minnow (*Pimephales promelas*) and in a fish full life-cycle test (FFLC) in Zebra fish (*Danio rerio*). Growth of the fish larvae was affected in the ELS test at concentrations > 4.17 mg/L. All other endpoints were affected only at higher concentrations. In the FFLC with Zebra fish growth of parental adult fish was the most sensitive parameter with slight effects at concentrations above 0.156 mg/L. However, no clear dose response relationship could be observed with exception of the highest treatment level (2.5 mg/L), in which the effect on growth reduction was slightly higher than in the other groups. Furthermore, there was no effect on growth of the filial generation at ≥ 2.5 mg/L. Significant mortality was observed in larvae of parental and filial generation at > 1.25 mg/L. Neither sex ratio nor reproduction of parental fish was affected at concentrations up to 2.5 mg/L. Hatch of larvae of parental and filial generation was not affected up to concentration levels of 2.5 and 1.25 mg/L, respectively.

While some slight growth effects were seen, a fish population is not likely to be adversely affected by these effects. Furthermore, since there is no indication from toxicology of a potential effect on the thyroid or other endocrine organs, it can be ruled out that these effects on growth are endocrine mediated. No further testing is indicated to evaluate the endocrine disrupter potential of ethofumesate to fish.

B.9.2.4. Bioconcentration in fish

As the log P_{ow} of the active substance and its metabolites is below the trigger ($\log P_{ow} < 3$), no evaluation of the bioconcentration potential in fish is needed. Hence, the bioaccumulation studies submitted for the first EU approval of the active substance were not evaluated and summarised by the RMS of the RAR.

However, the study summaries given in the DAR are included as additional information.

██████ *et al.*, 1992

Methods

The bioaccumulation of ^{14}C -ethofumesate (purity >97%) in bluegill sunfish, *Lepomis macrochirus*, was estimated in a flow-through system at a temperature of 20.4 - 23.0°C. Fish with a mean wet weight of 0.87 g were exposed to ethofumesate at a nominal concentration of 0.124 mg/L for a period of 28 days followed by a 14 days depuration period. The test was conducted in two 50 l glass tanks with 68 fish receiving ethofumesate stock solution and charcoal-filtered dechlorinated tap water (265 l per day) and 58 fish receiving dilution water alone. The fish were fed daily during the study. Test water characteristics were: total hardness as CaCO_3 52 - 70 mg/L, alkalinity as CaCO_3 50 - 66 mg/L, pH 7.7 - 8.5 and conductivity 160 - 240 $\mu\text{S}/\text{cm}$.

Results

Most of the radioactivity (<71%) was accumulated in the viscera. An apparent steady-state for viscera was obtained after 24 hours with a BCF of 1280 mL/g fresh weight. The time to apparent steady-state for muscle and carcass were 3 days with a BCF of 36 and 43 mL/g, respectively. The mean BCF for whole fish was 144 mL/g. The elimination was rapid with over 99% of the radioactivity eliminated within 3 days. In all tissues radioactive components which showed co-chromatography with ethofumesate and metabolite M1, M2 and M5 (identical with xx, see fig 7.1) were found. This indicates transformation of ethofumesate to metabolite M1, M2 and M5 as probable metabolic pathway. The measured exposure concentration was 0.121 mg/L.

Comments

The study was conducted in compliance with GLP and US EPA guideline 165-4.

██████, 1991

Methods

The bioaccumulation of ^{14}C -ethofumesate (purity >97%) in bluegill sunfish, *Lepomis macrochirus*, was estimated in a flow-through system at a temperature of $22 \pm 1^\circ\text{C}$. Bluegill with a mean wet weight of 1.53 g and an average length of 43.6 mm were exposed to ethofumesate at a mean measured ethofumesate concentration of 0.56 mg/L for a period of 28 days. The test was conducted in two glass aquaria containing 142.5 l test medium,

each with 105 bluegills. One aquarium received ethofumesate stock solution in acetone and dechlorinated reverse osmosis treated water (1440 l per 24 hours) and the other aquarium received water and acetone (100µg/L) only. The exposure was followed by a 14 day depuration period with dilution water only. The fish were fed daily during the study. Test water characteristics were:, total hardness as CaCO₃ 60 - 95 mg/L , alkalinity as CaCO₃ 62 - 81 mg/L , pH 6.6 - 7.2 and conductivity 140 - 149 µS/cm.

Results

Most of the radioactivity was accumulated in the non-edible portion of the fish. The time to apparent steady-state for viscera was 1 day with a BCF of 595 mL/g fresh weight. The time to apparent steady-state for edible flesh and carcass was 6 hours with a BCF of 17 and 25 mL/g, respectively. The BCF for whole fish was 67 mL/g. The elimination was rapid with approximately 99% of the radioactivity eliminated by day 3. Based on one compartment kinetics for whole fish an uptake rate coefficient of 251 mL/g/day and a depuration rate coefficient of 3.49/day, were calculated. This corresponds to a depuration half-life of 0.199 days, a time to 90% of steady-state of 0.66 days and a BCF of 72 mL/g. After 28 days of exposure approximately 30% of the radioactivity in the fish was characterised as parent compound, and identified major metabolites were NC 20645 (41%) and NC 9607 (3.5%).

Comments

The study was conducted in compliance with GLP and generally in accordance with recognised test guidelines.

B.9.2.5. Acute toxicity to aquatic invertebrates**Active substance:**

Reference:	Determination of the acute toxicity of [¹⁴C]-ethofumesate to <i>Daphnia magna</i>
Author(s), year:	Barber, I., 1991
Report/Doc. number:	Study no. A83370, Reference no. M-155638-01-1
Guideline(s):	OECD guideline 202 (1984), US EPA guideline 540/9-85-005 (1985)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate, technical grade (radio-labelled) Technical ethofumesate, purity: 97.5% w/w, batch no.: CR 19291/3 [¹⁴ C]-labelled ethofumesate, purity: 97.78%, batch no.: CFQ 6191
Test species:	Water flea (<i>Daphnia magna</i>)
Number of organisms:	3 replicates each with 10 daphnids per treatment, control and solvent control
Age:	First instar, > 6 hours and < 24 hours old
Type of test, duration:	Static test, 48 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 5.13, 8.55, 14.25, 23.75, 39.58 and 65.95 mg ai/L
Measured (mean):	Not given
Solvent:	Acetone (0.5 mL) and Tween 80 (0.5 mL)

Test conditions:

Water quality:	Dilution water, total hardness: 67 – 71 mg/L as CaCO ₃ , alkalinity: 67.3 – 70.3 mg/K as CaCO ₃ , conductivity: 154 – 162.5 µS/cm
Temperature:	19.5 – 20.0 °C
pH:	7.84 – 8.25 (0 - 48 h)
O ₂ content:	78 - 98 % saturation
Light regime:	16 hours light / 8 hours darkness
Test parameters:	<p>Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed.</p> <p>Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was also recorded continuously.</p> <p>For chemical analysis (liquid scintillation counter, thin layer chromatography) of ethofumesate in the test media samples were taken at test initiation (0 h) and termination (48 h).</p>
Statistics:	The EC ₅₀ and 95 % confidence limits were calculated using the moving average

method of Weil.

Findings:

Analytical data: The analytical data indicated that the [^{14}C]-ethofumesate concentrations were maintained within 20% of nominal throughout the duration of the study.
The mean measured concentrations are in a range of 93.9 and 118.0% of nominal test concentrations. Hence, the endpoint is based on nominal concentrations.

Effects: The [^{14}C]-ethofumesate was found to cause immobilisation of first instar daphnids at concentrations > 8.55 mg/L, such that the NOEC (immobilisation) was 8.55 mg/L and the LOEC (immobilisation) was 14.25 mg/L.

Table B. 9.2.5-1: Effects on daphnids (*D. magna*) exposed to technical ethofumesate

Ethofumesate [mg ai/L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	3.33	6.67
Solvent control	3.33	13.33
5.13	0	3.33
8.55	0	6.67
14.25	23.33	73.33
23.75	20.0	86.67
39.58	16.67	90.0
65.95	96.67	100
48 h EC ₅₀ = 13.52 mg ai/L (95 % C.I. 11.76 – 15.53 mg ai/L)		
48 h NOEC = 8.55 mg ai/L		
Based on nominal concentrations		

Conclusion:

The acute toxicity of [^{14}C]-ethofumesate to *Daphnia magna* has been investigated. The lowest concentration resulting in significant immobilisation of first instar daphnid neonates over a 48 hr exposure period (i.e. LOEC) was 14.25 mg/L, and the highest concentration resulting in significant immobilisation (i.e. NOEC) was 8.55 mg/L.

The 48-hour EC₅₀ was calculated as 13.52 mg/L based on nominal concentrations.

Comment RMS:

The study was conducted according to the OECD (1984) and US EPA (1985) test guideline. However, the validity criteria given in the former (OECD 202, 1984) and current test guidelines (OECD 202, 2004 and US EPA, OPPTS 850.1075) are not met regarding the immobility of daphnids in the control groups.

In the solvent control the immobility of daphnids was 13.3% and hence more than 10% as stated in the test guideline (OECD and US EPA). The immobility in the water control was below 10% (being: 3.3%).

The dissolved oxygen concentration at the end of the test was greater than 3 mg/L in all test vessels (control and treatment groups). The measured dissolved oxygen

was greater than 6 mg/L at test termination (78 – 94 % of air saturation).

In the solvent control and, at each increasing [¹⁴C]-ethofumesate concentration the test solutions were observed to be increasingly opaque after 48 hours. The study director argued that this was due to increased bacterial growth caused by the presence of Tween 80 and that it is considered that this did not affect the results obtained in this study. However, under consideration of the high solvent control mortality (> 10%) the RMS is of the opinion that the bacterial growth might cause adverse effects on the survival of daphnids.

The RMS is of the opinion that the results of the study are not acceptable and hence should not be used for the risk assessment.

Reference:	The acute toxicity of ethofumesate to <i>Daphnia magna</i>
Author(s), year:	Douglas, M.T. & James, C.M., 1990
Report/Doc. number:	Study no. A87618, Reference no. M-161557-01-1
Guideline(s):	OECD guideline 202 (1984), EEC Directive 67/548 Annex VI C.2
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance: Ethofumesate, technical grade, purity: > 97%, batch no.: 04402/2

Test species: Water flea (*Daphnia magna*)

Number of organisms: 2 replicates each with 10 daphnids per treatment and control

Age: First instar

Type of test, duration: Static test, 48 hours

Applied concentrations:

Nominal: 0 (control and solvent control), 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg ai/L

Mean measured: Not given

Solvent: None

Test conditions:

Water quality: Dechlorinated and aged laboratory tap water, total hardness: ~ 350 mg/L as CaCO₃

Temperature: 21 °C

pH: 8.2 – 8.3 (0 - 48 h)

O₂ content: 7.9 – 8.3 mg/L (> 60 % saturation)

Light regime: 16 hours light / 8 hours darkness

Test parameters: Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During

the exposure the daphnids were not fed.

Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was also recorded 24 h after test start.

Findings:

Table B.9.2.5-2: Effects on daphnids (*D. magna*) exposed to technical ethofumesate

Ethofumesate [mg ai/L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
1.0	0	5
1.8	5	15
3.2	0	10
5.6	5	5
10	10	15
18	5	15
32	60	75
56	75	100
100	95	100
48 h EC ₅₀ = 22 mg ai/L (95 % C.I. 18 - 27 mg ai/L) 48 h NOEC = 5.6 mg ai/L		

Conclusion:

The acute toxicity of ethofumesate to *Daphnia magna* has been investigated.

The highest concentration resulting in significant immobilisation (i.e. NOEC) was 5.6 mg/L.

The 48-hour EC₅₀ was calculated as 22 mg/L based on nominal concentrations.

Comment RMS:

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

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

However, the reporting of the test methods and test results is poor and important information (e.g. analytical measurements, statistical analyses) is not given.

The RMS is of the opinion that the reliability of the results is not given considering that no analytical measurements were conducted. Hence, the results of the study should not be used in the risk assessment.

Reference:	Acute toxicity in <i>Daphnia magna</i> – test article: ethofumesate techn.
Author(s), year:	Thun, S., 1993
Report/Doc. number:	Study no. 80-91-2312-02-93, Reference no. M-352128-01-1
Guideline(s):	OECD guideline 202 (1984), EEC Directive 79/831, Annex V
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate, technical grade, batch no.: 20/03/93
Test species:	Water flea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control
Age:	First instar, 6 – 24 hours old
Type of test, duration:	Static test, 48 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 1.3, 2.3, 4.1, 7.3, 13, 23.1, 41.1 and 73.2 mg ai/L
Mean measured:	Not given
Toxic reference:	K ₂ Cr ₂ O ₇ (0.4 and 1.4 mg/L)
Solvent:	None

Test conditions:

Water quality:	Synthetic test water (Elendt medium), total hardness: 14.5 °dH, pH: 7.5 – 8.5, conductivity: 0.049 µs/cm
Temperature:	18.0 – 19.1 °C
pH:	7.15 – 7.51 (0 - 48 h)
O ₂ content:	8.6 – 9.6 mg/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness, 600 – 700 lux
Test parameters:	<p>Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed.</p> <p>Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test.</p>
Analytical measurements:	Upon initiation of the preliminary and the main test, analytical control measurements were performed by means of HPLC analysis. The stock solution and two representative concentration levels were analysed for both tests.
Statistics:	The statistical calculation of the EC ₅₀ values was performed by means of the Probit analysis according to Finney.

Findings:

Analytical measurements:	The analytical data indicated that the [¹⁴ C]-ethofumesate concentrations were maintained within 20% of nominal throughout the duration of the study.
--------------------------	---

Table 9.2.5-3: Effects on daphnids (*D. magna*) exposed to technical ethofumesate

Ethofumesate [mg ai/L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
1.3	0	0
2.3	0	0
4.1	0	0
7.3	0	0
13.0	0	0
23.1	20	20
41.1	95	95
73.2	100	100
48 h EC ₅₀ = 28.1 mg ai/L (95 % C.I. 23.8 – 31.7 mg ai/L) 48 h NOEC = 13 mg ai/L		

Conclusion:

The acute toxicity of ethofumesate to *Daphnia magna* has been investigated. Due to the observations made during the main test, the NOEC was determined at a concentration of 13.0 mg/L. The 48-hour EC₅₀ was calculated as 28.1 mg/L based on nominal concentrations.

Comment RMS:

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

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

However, analytical measurements of the test concentrations were conducted at the test start. At test start the mean measured concentrations were within 80 and 120% of the nominal concentrations. However, no analytical measurements were conducted at the end of the test.

Hence, the toxicity endpoints should be considered with caution.

The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Reference:	The acute toxicity of ethofumesate technical to the mysid shrimp, <i>Mysidopsis bahia</i> in a static system
Author(s), year:	Schupner, J.K. & Stachura, B.J., 1992
Report/Doc. number:	Study no. A83389, Reference no. M-155657-01-1
Guideline(s):	FIFRA Guideline 72-3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate, technical grade, batch no.: CR 19291/2, purity: 97%
Test species:	Mysid shrimp (<i>Americamysis bahia</i> , formerly known as <i>Mysidopsis bahia</i>)
Number of organisms:	2 replicates each with 10 mysid shrimp per treatment, control and solvent control
Age:	Juveniles, < 24 hours old
Type of test, duration:	Static test, 96 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 6, 11, 18, 30 and 50 mg ai/L
Mean measured:	- (control and solvent control), 2.5, 5.2, 8.0, 14.4 and 25.1 mg ai/L
Solvent:	Triethylene glycol (TEG), 0.5 mL/L

Test conditions:

Water quality:	Synthetic sea water , salinity 20 - 21 ‰
Temperature:	20 - 22 °C
pH:	8.2 – 8.4 (0 - 96 h)
O ₂ content:	6.0 – 7.3 mg/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness, 128 foot candles
Test parameters:	Mortality and sublethal effects were assessed after 0, 24, 48, 72 and 96 hours. During the exposure the mysid shrimps were fed with <i>artemia nauplii</i> ad libitum. Measurements of pH, temperature and dissolved oxygen concentrations were made at the test start, 48 hours after the start and at the end of the test. Temperature was monitored continuously.
Analytical measurements:	Samples of all treatments were taken at test initiation (Day 0), prior to addition of the mysids, and at test termination (96 hours). Samples were analysed for ethofumesate by High Performance Liquid Chromatography.
Statistics:	Mortality data was analysed using Toxdat, a multi-method program which determines the LC ₅₀ and 95% confidence interval using the Binomial, Moving Average, and Probit methods. The LC ₅₀ values are reported based on the method that gave the narrowest confidence interval. The Probit result was reported for the 72 and 96 hour time periods. The moving average result was reported for the 48 hour time period. All values are based on study mean concentrations as

analytically determined.

Findings:

Analytical measurements: The analytical data indicated that the mean measured ethofumesate concentrations were between 42 and 50 % of the nominal test concentrations. Hence, the results are based on mean measured concentrations.

Table B.9.2.5-4: Effects on mysid shrimp (*Americamysis bahia*) exposed to technical ethofumesate

Ethofumesate [mg ai/L] (mean measured)	Mean cumulative mortality [%]				
	0 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Solvent control	0	0	0	5	5
2.5	0	0	10	10	10
5.2	0	0	15	20	30
8.0	0	0	60 ^b	75 ^c	85 ^a
14.4	0	0	60 ^{ac}	90 ^{ac}	100
25.1	0	40 ^{ab}	100	100	100
96 h LC ₅₀ = 5.4 mg ai/L (95% C.I. 4.5 – 6.4 mg ai/L)					
96 h NOEC < 2.5 mg ai/L					

^a Erratic swimming, ^b surfacing, ^c Lethargic

Conclusion:

The 96 hour LC₅₀ of ethofumesate technical to mysid shrimp, *Americamysis bahia* was determined under the static test conditions of this study, is 5.4 mg/L (95% C.I. 4.5 - 6.4 mg/L) based on mean measured concentrations. The NOEC is less than 2.5 mg/L.

Comment RMS:

The study was conducted to the US EPA test guideline. The mortality in the control groups was below 10% (being: 5% in the solvent control and 0% in the control) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.

The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Metabolites:

Reference:	Acute toxicity of BCS-BB94377 (tech.) to the waterflea <i>Daphnia magna</i> in a static laboratory test system – limit test
Author(s), year:	Riebschläger, T., 2012a
Report/Doc. number:	Study no. E 320 4318-1, Reference no. M-434284-02-1
Guideline(s):	OECD guideline 202 (2004), EEC Regulation no 440/2008, Method C.2 (2008)
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:

Test substance:	BCS-BB94377 (further names: NC 8493), batch no.: SES 10116-5-2, purity: 99.8% w/w
Test species:	Waterflea (<i>Daphnia magna</i>)
Number of organisms:	12 replicates each with 5 daphnids per treatment and control group
Age:	First instar, < 24 hours old
Type of test, duration:	Static test (renewal of the test solution after 24 hours), 48 hours, limit test

Applied concentrations:

Nominal:	0 (control) and 10 mg/L
Mean measured:	- (control) and 10.4 mg/L
Solvent:	None
Toxic reference	K ₂ CR ₂ O ₇ , tested at least twice a year, 24 h EC ₅₀ = 0.87 mg/L

Test conditions:

Water quality:	Artificial water (Elendt M7), total hardness: 231 mg/L as CaCO ₃ (= 13 °dH), alkalinity: 53 mg/L as CaCO ₃ (= 3 °dH), conductivity: 579 – 581 µS/cm
Temperature:	20.2 – 23.2 °C
pH:	7.9 (0 – 48 h)
O ₂ content:	8.7 – 9.0 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, max. 1200 lux
Test parameters:	<p>Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed.</p> <p>Measurements of pH, temperature and dissolved oxygen concentrations were made at the test start, 24 hours after the start and at the end of the test. Temperature was monitored continuously.</p>
Analytical measurements:	The water samples were analysed with HPLC-UV. Water samples were taken at test start, after 24 hours and at test end.
Statistics:	Based on the given results, statistical EC ₅₀ evaluations were not applicable.

Findings:

Analytical measurements: The analytical data indicated that the mean measured ethofumesate concentrations were between 104 and 108% of the nominal test concentrations. Hence, the results are based on nominal concentrations.

Biological effects: No immobility or other effects on behaviour occurred in the treatment or control group within 48 hours of exposure.

Conclusion: Due to the absence of treatment-related effects during 48 hours of static-renewal exposure to a limit concentration of 10 mg BCS-BB94377/L, the corresponding EC₅₀ is higher than 10 mg/L.

Comment RMS: The study was conducted according to the OECD 202 (2004) and EC test guideline (C.2). The validity criteria given in the test guidelines are met. The mortality in the control groups was below 10% (being: 0%) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.

In the study report the study exposure system is described as a static system. However, the test medium was renewed after 24 hours and hence the exposure is considered to be semi-static.

The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Reference:	Acute toxicity of NC 8493 to <i>Daphnia magna</i> in a 48-hour static test
Author(s), year:	Juckeland, D., 2013a
Report/Doc. number:	Study no. 13 10 48 015 W, Reference no. IDD00072
Guideline(s):	OECD guideline 202 (2004), Directive 92/69/EEC, Method C.2 (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	NC 8493, batch no.: EPP/VMV 541.A, purity: 99.9%
Test species:	Waterflea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control group, 3 additional replicates for measuring, analysis and retain specimen
Age:	First instar, < 24 hours old
Type of test, duration:	Static test, 48 hours, limit test
<u>Applied concentrations:</u>	
Nominal:	0 (control) and 100 mg/L
Mean measured:	- (control) and 105.2 mg/L
Solvent:	None
Toxic reference	K ₂ Cr ₂ O ₇ (potassium dichromate) tested in a separate study to verify the sensitivity of the test system, 48 h EC ₅₀ = 1.37 mg/L

Test conditions:

Water quality:	Reconstituted water according to ISO 6341, total hardness: 200 mg/L as CaCO ₃ , conductivity: ≤ 10µS/cm
Temperature:	19.5 – 21.2 °C
pH:	7.84 – 7.99 (0 – 48 h)
O ₂ content:	8.6 – 9.1 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, approx. 1000 lux
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the test start and at the end of the test. Temperature was monitored continuously.
Analytical measurements:	The water samples were analysed with HPLC-UV. Water samples were taken at test start and at test end.
Statistics:	A calculation and statistical evaluation was not applicable (limit test).

Findings:

Analytical measurements:	The analytical data indicated that the mean measured ethofumesate concentrations were between 101 and 110% of the nominal test concentrations. Hence, the results are based on nominal concentrations.
--------------------------	--

Biological effects: No immobility or other effects on behaviour occurred in the treatment or control group within 48 hours of exposure.

Conclusion: Due to the absence of treatment-related effects during 48 hours of static exposure to a limit concentration of 100 mg NC 8493/L, the corresponding EC₅₀ is higher than 100 mg/L.

Comment RMS: The study was conducted according to the OECD 202 (2004) and EC test guideline (C.2). The validity criteria given in the test guidelines are met.
The mortality in the control groups was below 10% (being: 0%) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.
The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Reference: Acute toxicity of BCS-AV65501 (tech.) to the waterflea *Daphnia magna* in a static-renewal laboratory test system – limit test

Author(s), year: Riebschläger, T., 2012b

Report/Doc. number: Study no. E 320 4317-1, Reference no. M-434289-02-1

Guideline(s): OECD guideline 202 (2004), EEC Regulation no 440/2008, Method C.2 (2008)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: BCS-CU88901 (tech.), sodium-salt of BCS-AV65501 (further names: NC20645), batch no.: SES 11754-3-8, purity: 69.2% BCS-CU88901 w/w

Test species: Waterflea (*Daphnia magna*)

Number of organisms: 12 replicates each with 5 daphnids per treatment and control group

Age: First instar, < 24 hours old

Type of test, duration: Semi-static test (renewal of the test solution after 24 hours), 48 hours, limit test

Applied concentrations:

Nominal: 0 (control) and 10 mg/L

Mean measured: - (control) and 10.2 mg/L

Solvent: None

Toxic reference K₂Cr₂O₇, tested at least twice a year, 24 h EC₅₀ = 0.87 mg/L

Test conditions:

Water quality: Artificial water (Elendt M7), total hardness: 231 mg/L as CaCO₃ (= 13 °dH),

	alkalinity: 53 mg/L as CaCO ₃ (= 3 °dH), conductivity: 590 - 593 µS/cm
Temperature:	20.0 – 21.0 °C
pH:	7.8 - 7.9 (0 – 48 h)
O ₂ content:	8.4 – 8.8 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, max. 1200 lux
Test parameters:	<p>Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed.</p> <p>Measurements of pH, temperature and dissolved oxygen concentrations were made at the test start, 24 hours after the start and at the end of the test. Temperature was monitored continuously.</p>
Analytical measurements:	The water samples were analysed with HPLC-UV. Water samples were taken at test start, after 24 hours and at test end.
Statistics:	Based on the given results, statistical EC ₅₀ evaluations were not applicable.
<u>Findings:</u>	
Analytical measurements:	The analytical data indicated that the mean measured ethofumesate concentrations were between 102 and 104% of the nominal test concentrations. Hence, the results are based on nominal concentrations.
Biological effects:	No immobility or other effects on behaviour occurred in the treatment or control group within 48 hours of exposure.
<u>Conclusion:</u>	Due to the absence of treatment-related effects during 48 hours of static-renewal exposure to a limit concentration of 10 mg BCS-AV65501/L, the corresponding EC ₅₀ is higher than 10 mg /L.

<u>Comment RMS:</u>	<p>The study was conducted according to the OECD 202 (2004) and EC test guideline (C.2). The validity criteria given in the test guidelines are met.</p> <p>The mortality in the control groups was below 10% (being: 0%) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.</p> <p>The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.</p>
---------------------	---

Reference:	Acute toxicity of NC 20645 to <i>Daphnia magna</i> in a 48-hour static test
Author(s), year:	Juckeland, D., 2013b
Report/Doc. number:	Study no. 13 10 48 030 W, Reference no. IDD00071
Guideline(s):	OECD guideline 202 (2004), Directive 92/69/EEC, Method C.2 (1992)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

<u>Material and methods:</u>	
Test substance:	NC20645, batch no.: EEP / RH1079.1, purity: 95.6%
Test species:	Waterflea (<i>Daphnia magna</i>)
Number of organisms:	4 replicates each with 5 daphnids per treatment and control group, 3 additional replicates for measuring, analysis and retain specimen
Age:	First instar, < 24 hours old
Type of test, duration:	Static test, 48 hours, limit test
<u>Applied concentrations:</u>	
Nominal:	0 (control) and 100 mg/L
Mean measured:	- (control) and 104.1 mg/L
Solvent:	None
Toxic reference	K ₂ Cr ₂ O ₇ (potassium dichromate) tested in a separate study to verify the sensitivity of the test system, 48 h EC ₅₀ = 1.37 mg/L
<u>Test conditions:</u>	
Water quality:	Reconstituted water according to ISO 6341, total hardness: 200 mg/L as CaCO ₃ , conductivity: ≤ 10µS/cm
Temperature:	19.5 – 21.1 °C
pH:	7.96 – 8.17 (0 – 48 h)
O ₂ content:	8.62 – 9.24 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, approx. 1000 lux
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the test start and at the end of the test. Temperature was monitored continuously.
Analytical measurements:	The water samples were analysed with HPLC-UV. Water samples were taken at test start and at test end.
Statistics:	A calculation and statistical evaluation is not applicable (limit test).
<u>Findings:</u>	
Analytical measurements:	The analytical data indicated that the mean measured ethofumesate concentrations were between 105 and 113% of the nominal test concentrations. Hence, the results are based on nominal concentrations.

Biological effects: No immobility or other effects on behaviour occurred in the treatment or control group within 48 hours of exposure.

Conclusion: Due to the absence of treatment-related effects during 48 hours of static exposure to a limit concentration of 100 mg NC 20645/L, the corresponding EC₅₀ is higher than 100 mg /L.

Comment RMS: The study was conducted according to the OECD 202 (2004) and EC test guideline (C.2). The validity criteria given in the test guidelines are met.

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

The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Reference: Acute toxicity of ethofumesate acetic acid to the waterflea *Daphnia magna* in a static laboratory test system – limit test

Author(s), year: König, N., 2013

Report/Doc. number: Study no. E 320 4449-6, Reference no. M-444843-01-1

Guideline(s): OECD guideline 202 (2004), EEC Regulation no 440/2008, Method C.2 (2008)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: Ethofumesate acetic acid (further names: AE B049913), batch no.: SES 12013-7-3, purity: 91% w/w

Test species: Waterflea (*Daphnia magna*)

Number of organisms: 12 replicates each with 5 daphnids per treatment and control group

Age: First instar, < 24 hours old

Type of test, duration: Static test, 48 hours, limit test

Applied concentrations:

Nominal: 0 (control) and 10 mg/L

Mean measured: - (control) and 10.3 mg/L

Solvent: None

Toxic reference K₂CR₂O₇, tested at least twice a year, 24 h EC₅₀ = 0.83 mg/L

Test conditions:

Water quality:	Artificial water (Elendt M7), total hardness: 213 mg/L as CaCO ₃ (= 12 °dH), alkalinity: 53 mg/L as CaCO ₃ (= 3 °dH), conductivity: 617 µS/cm
Temperature:	20.2 – 20.3 °C
pH:	7.8 - 7.9 (0 – 48 h)
O ₂ content:	9.1 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, max. 1200 lux
Test parameters:	<p>Immobility and sublethal effects were assessed after 0, 24 and 48 hours. During the exposure the daphnids were not fed.</p> <p>Conductivity, total hardness and alkalinity were measured in the dilution media at test start. Measurements of pH, dissolved oxygen and temperature were conducted at the start and the end of the test. Additionally, temperature was monitored continuously.</p>
Analytical measurements:	The water samples were analysed with HPLC-UV. Water samples were taken at test start and at test end.
Statistics:	Based on the given results, statistical EC ₅₀ evaluations were not applicable.
<u>Findings:</u>	
Analytical measurements:	The analytical data indicated that the mean measured ethofumesate concentrations were between 103 and 106% of the nominal test concentrations. Hence, the results are based on nominal concentrations.
Biological effects:	No immobility or other effects on behaviour occurred in the treatment group within 48 hours of exposure. Only one immobilised individual (being 1.7%) was observed in the untreated control within 48 hours of exposure.
<u>Conclusion:</u>	Due to the absence of treatment-related effects during 48 hours of static exposure to a limit concentration of 10 mg ethofumesate acetic acid/L, the corresponding EC ₅₀ is higher than 10 mg /L.

<u>Comment RMS:</u>	<p>The study was conducted according to the OECD 202 (2004) and EEC test guideline (C.2). The validity criteria given in the test guidelines are met.</p> <p>The mortality in the control groups was below 10% (being: 1.7%) and the dissolved oxygen was greater than 3 mg/L throughout the test duration. Based on the validity criteria the study is considered valid.</p> <p>The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.</p>
---------------------	--

B.9.2.6. Long-term and chronic toxicity to aquatic invertebrates**B.9.2.6.1. Reproductive and development toxicity to *Daphnia magna***

Reference:	An assessment of the effects of ethofumesate on the reproduction of <i>Daphnia magna</i>
Author(s), year:	Douglas, M.T., James, C.M. and Macdonald, I.A., 1990
Report/Doc. number:	Study no. A87619, Reference no. M-161558-01-1
Guideline(s):	OECD 202 (Part 2, 1984)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., batch no.: P-04402/2, purity: 97% w/w
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 hours old
Type of test, duration:	Semi-static test, Medium renewal 3 times per week, 21 days

Applied concentrations:

Nominal:	0 (control), 0.32, 1.0, 3.2, 10 and 32 mg/L
Solvent:	None

Test conditions:

Water quality:	Dechlorinated and aged laboratory tap water, total hardness: 350 mg/L as CaCO ₃
Temperature:	21 ± 1 °C
pH:	8.2 – 8.3
O ₂ content:	7.8 – 8.5 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	<p>The live and dead <i>Daphnia</i> of the "parental" (P₁) generation were counted daily and recorded together with observations on the general condition and size of the <i>Daphnia</i> as compared with the controls. At each test media renewal the numbers of live and dead "filial" (F₁) <i>Daphnia</i> were recorded. The number of <i>Daphnia</i> with eggs or young in the brood pouch plus the number of discarded unhatched eggs was also determined at this time.</p> <p>Each vessel received approximately 5 ml of a mixed unicellular algal culture supplemented with fry fish food (Liquifry®), daily.</p> <p>Temperature was recorded daily for each flask. Dissolved oxygen, pH and temperature were measured before and after- each test media renewal.</p>
Analytical measurements:	Verification of test concentration (HPLC) was carried out on Days 0 (fresh media), 2, 5, 7, 9, 12, 14, 16, 19 and 21 (expired media).

Statistics: EC₅₀ values for immobilisation (mortality) of the parental *Daphnia* were calculated according to the method of Thompson and Weil. EC₅₀ values for the effects on reproduction were determined by fitting logistic response curves to the data.

Findings:

Analytical measurements: The analytical data indicated that the mean measured ethofumesate concentrations were between 79 and 136% of the nominal test concentrations. Hence, the results are based on nominal concentrations.

Lethal effects on P₁: Mortality (immobilisation) occurred predominantly within 48 hours of exposure to the highest test concentration (32 mg/L), but appreciable further mortality also occurred throughout the study in three of the remaining test concentrations 1.0, 3.2 and 10 mg/L, until Day 14 of exposure. Thereafter only occasional mortalities occurred resulting in almost identical EC₅₀ values at Day 14 and 21.

Sub-lethal effects on P₁: A high number of unhatched eggs were noted in the two highest test concentrations at which survivors reached reproductive age (3.2 and 10 mg/L). no other sub-lethal adverse effects were noted with parental *Daphnia* at any exposure level.

Table B. 9.2.6.1-1: Effects on daphnids (*Daphnia magna*) exposed to technical ethofumesate

Ethofumesate [mg ai/L] (nominal)	% survival of P ₁	no. live young	no. dead young	no. unhatched eggs
Control	95	1663	0	16
0.32	93	1638	0	14
1.0	60	716	5	30*
3.2	48	233	8	130*
10	48	2	2	380*
32	0	-	-	0
21 d EC ₅₀ (P ₁ survival) = 4 mg ai/L (95% C.I. 3 – 5 mg ai/L) 21 d EC ₅₀ (P ₁ reproduction) = 1.35 mg ai/L (95% C.I. 0.97 – 1.84 mg ai/L) 21 d EC ₅₀ (P ₁ egg production) = 0.77 mg ai/L (95% C.I. 0.54 – 1.11 mg ai/L) 21 d NOEC = 0.32 mg ai/L (survival, reproduction) based on nominal concentrations				

* Statistically significant compared to the control, according to Williams' test, α 0.05

Effects on F₁: The number of dead young daphnids was insignificant in all treatment and control groups (< 1 dead young/female).

Conclusion:

Prolonged exposure of *Daphnia magna* to Ethofumesate resulted in progressive mortality of parental P₁ generation *Daphnia* up to Day 14.

Impairment of reproduction occurred with all survivors at exposure levels of 1.0 mg/L and above, with large numbers of non-viable eggs being produced in the test concentrations, 3.2 and 10 mg/L. Despite this feature, the impairment of

reproduction was primarily due to adverse effects on total egg production rather than subsequent inhibition of embryo development and hatching. Progressive deterioration in reproduction was not apparent.

The 21-day NOEC has been determined to be 0.32 mg ai/L.

Comment RMS:

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

The mortality in the control groups was below 20% (being: 5%) and the dissolved oxygen was greater than 60% of the air saturation throughout the test duration.

The pH in the controls and of at least the most concentrated solutions was given in the study. The deviation from the initial values was ≤ 0.3 units.

The first young were born in the controls after 7 days (maximum 9 days). The average cumulative number of young per female in the controls after three broods was ≥ 20 at a temperature of 20 ± 1 °C (being: 44 young per female at 21 °C)

The RMS is of the opinion that the study is acceptable and should be used in the risk assessment.

Reference: **21 d Daphnia-Reproduction Test**

Author(s), year: Bellmann, W., 1992

Report/Doc. number: Study no. 40730.315-202-II, Reference no. M-352134-01-1

Guideline(s): OECD 202 (Part 2), 1984

GLP: Yes

Deviations: None

Validity: Additional information

Material and methods:

Test substance: Ethofumesate techn., batch no.: 08/05/92, purity: not specified

Test species: Waterflea (*Daphnia magna*)

Number of organisms: 4 replicates each with 10 daphnids per treatment and control group

Age: Not given

Type of test, duration: Semi-static test, Medium renewal every 2 to 3 days

Feeding: Unicellular green algae (*Desmodesmus subspicatus*, formerly known as *Scenedesmus subspicatus*), daily

Applied concentrations:

Nominal: 0 (control), 1.0, 3.2, 10, 31.6 and 100 mg/L

Mean measured: Not given

Solvent: None

Test conditions:

Water quality:	Synthetic test water (M4-medium), conductivity: 0.05 µS/cm
Temperature:	21 - 23 °C
pH:	7.8 – 8.6
O ₂ content:	92.1 – 100% air saturation
Light regime:	16 hours light / 8 hours darkness, approx. 1000 lux
Test parameters:	<p>At test medium renewal the adult Daphnia were observed and the young counted and removed from the vessels. The adult Daphnia were transferred with specially prepared Pasteur pipettes. Subsequently, the young were counted and the number of living and dead animals was noted.</p> <p>The pH, temperature, and O₂ concentrations were measured at the beginning and at the end of each renewal period.</p>
Analytical measurements:	The analytical control measurements were performed by means of GC analysis.
Statistics:	<p>For the determination of the EC₅₀ values the method by Spearman-Kärber was used.</p> <p>The calculation of the quotients (number of offspring/number of adults) was performed for each parallel concentration level and time. The comparison of the concentration levels was done by means of a U-test (2-tailed, corrected for ties) according to Mann/Whitney.</p>

Findings:

Analytical measurements:	Up to a test concentration of 10 mg ai/L the recovery of the active substance was greater than 100%. At higher test concentrations, between 31.6 mg ai/L and 100 mg ai/L a slight sedimentation of the test article at the bottom of the vessels was observed.
Biological effects:	<p>At the concentration level of 100.0 mg/L and from the day 13 until the end, the adult animals appeared smaller and paler than the animals in all other concentration levels. Also at the same concentration level the developing eggs and embryos in the brood pouch showed a greenish colour.</p> <p>A mortality of 100 % was determined at the highest concentration of 100.0 mg/L after 3 days and at 31.6 mg/L after 6 days respectively. A mortality of 62.5 % and 52.5 % was observed at concentrations of 10.0 mg/L and 3.2 mg/L. At or below 1.0 mg/L, the mortality rate was fairly parallel to the control group.</p>

Table B. 9.2.6.1-2: Effects on daphnids (*Daphnia magna*) exposed to technical ethofumesate at day 21

Ethofumesate [mg ai/L] (nominal)	Immobilisation of adults [%]	No. live young	No. dead young	No. of offspring per adult	No. of dead young per adult
Control	12.5	420	13	12.37	0.37
1.0	20.0	344	15	11.22	0.47
3.2	52.5	39	26	3.42 *	1.37
10.0	62.5	0	33	2.20 *	2.20 *
31.6	100	-	-	0	0
100	100	-	-	0	0
21 d EC ₅₀ = 3.8 mg ai/L (95% C.I. 2.8 – 5.0 mg ai/L) based on immobilisation					
21 d EC ₅₀ = 2.7 mg ai/L (95% C.I. 2.1 – 3.4 mg ai/L) based on reproduction					
21 d NOEC = 1.0 mg ai/L (based on reproduction)					

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

Conclusion:

Due to the distribution of the data, the statistically derived EC₅₀ value for the reproduction was 2.7 mg ai/L and the EC₅₀ value for the immobilisation was 3.8 mg ai/L. The NOEC based on reproduction and immobilisation was determined at 1.0 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. In the study report it is stated that the number of offspring per female was around 20 on day 12 of the test (> 40 on day 21).

The first young should have been born in the controls after a maximum of nine days (being: 6-8 days).

Based on the validity criteria the study is considered valid.

The measured concentrations ranged between 11 and 150% of the nominal values, and the results based on nominal concentrations cannot be considered to be reliable. The endpoint has to be recalculated based on the measured concentrations.

The RMS is of the opinion that the NOEC should be used as additional information.

Reference:	The chronic toxicity of ethofumesate to <i>Daphnia magna</i>
Author(s), year:	Adema, D.M.M. and de Rulter, A., 1989
Report/Doc. number:	Study no. A83345, Reference no. M-155614-01-1
Guideline(s):	OECD 202 (Part 2), 1984
GLP:	Yes
Deviations:	None
Validity:	Additional information

Material and methods:

Test substance:	Ethofumesate techn., batch no.: not given, purity: not specified
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 every 2 to 3 days
Feeding:	Unicellular green algae (<i>Chlorella pyrenoidosa</i>) and some “sludge extract”, daily

Applied concentrations:

Nominal:	0 (control), 0.1, 0.32, 1.0, 3.2, 10 and 32 mg/L
Mean measured:	Not given
Solvent:	None

Test conditions:

Water quality:	Groundwater (including several salts), hardness: 215 mg/L as CaCO ₃
Temperature:	20 ± 1 °C
pH:	7.6 – 8.6
O ₂ content:	7.4 - 11.1 mg/L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	At test medium renewal the adult <i>Daphnia</i> were observed and the young counted and removed from the vessels; the condition and the size of the original test animals were qualitatively compared with those of the control animals. The pH, temperature, and O ₂ concentrations were measured at the beginning and at the end of each renewal period.

Analytical measurements:	At the start of the test (just after dosing) about 100 mL samples were taken from the control and the test solutions containing 0.32, 1.0, 3.2, 10 and 32 mg of test substance per L (nominal) and at t = 9 d, just after dosing, from the control and the test solutions containing 0.10, 0.32, 1.0, 3.2 and 10 mg/L. At t = 2 days (samples after 48 hours) and at t = 12 days (samples after 72 hours) about 100 ml samples were taken from the spent test solutions containing 1.0 and 10 mg of test substance (nominal) per L. The analytical control measurements were performed by means of GC (gas-liquid chromatographic method) analysis.
--------------------------	---

Statistics: The LC₅₀ values and their confidence interval were calculated by means of a parametric model developed by Kooijman.

The EC₅₀ values and their confidence interval were calculated by means of a maximum likelihood fitting procedure on a logistic model.

Statistical significance for mortality was determined with a binomial test with a 95% significance level combining the results of the quadruplicates. Statistical significance for reproduction was determined with the one tailed Student t-test with a 95% significance level using the mean number of young per female in each of the four replicates as observed values.

In both cases the observations at each concentration were compared with those of the control.

Findings:

Analytical measurements: The average measured test concentrations just after dosing were determined to be 77% of the nominal test concentrations. The average measured concentration in “spent” solutions was 75% of the nominal. The overall average concentration during the whole exposure period was 76% of the nominal test concentrations.

Biological effects: No significant (binomial test, $p = 0.95$) effects on mortality were found at 10 mg/L and the lower concentrations tested. At 32 mg/L all animals died within 7 days of exposure.

At 1.0 mg/L, at $t = 19$ d and $t = 21$ d many eggs were released instead of living young. At 3.2 mg/L almost all eggs were released as such and almost no young were born. Therefore the NOEC was stated to be 0.32 mg/L.

Table B. 9.2.6.1-3: Effects on daphnids (*Daphnia magna*) exposed to technical ethofumesate at day 21

Ethofumesate [mg ai/L] (nominal)	Immobilisation of adults [%]	Cumulative number of young born alive per female	
		Number	% of control
Control	0.0	118 ^c	-
0.1	0.0	126 ^c	107
0.32	5.0	117 ^c	99
1.0	0.0	91 ^a	77 *
3.2	10.0 ^b	0.4 ^a	0.3 *
10	2.5 ^b	0 ^a	0 *
32	100 **	-	-
21 d EC ₅₀ = 13.5 mg ai/L (95% C.I. 10.7 – 17.0 mg ai/L) based on immobilisation 21 d EC ₅₀ = 1.2 mg ai/L (95% C.I. 0.9 – 1.6 mg ai/L) based on reproduction 21 d NOEC = 0.32 mg ai/L (based on reproduction)			

* Statistically significant compared to the control, one tailed Student t-test, $p = 0.95$

** Statistically significant compared to the control, binominal test, $p = 0.95$

^a Many eggs were released instead of living young

^b Colour of adults “greenish” instead of the red-brown of the control animals.

^c No undeveloped eggs were found

Conclusion:

Due to the distribution of the data, the statistically derived EC₅₀ value for the reproduction was 1.2 mg ai/L and the EC₅₀ 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.

No mean measured concentrations were given in the study report even though the recovery of the test concentration was below 80%. Hence, the results based on nominal concentrations cannot be considered to be reliable.

Based on the available analytical measurements mean measured concentrations of 0.1, 0.25, 0.75, 2.35, 7.42 and 26.0 mg ai/L was determined by the RMS. The mean measured concentrations are in a range of 73 and 100% of nominal test concentrations. Under consideration of mean measured concentrations a NOEC of 0.25 mg ai/L was determined. However, taking into account the poor information on analytical measurements given in the study report the endpoint should be considered with caution.

Hence, the RMS is of the opinion that the NOEC should be used as additional information.

B.9.2.6.2. Reproductive and development toxicity to an additional aquatic invertebrate species

No chronic studies on an additional aquatic invertebrate species are required since ethofumesate is not an insecticide and does not show an insecticidal mode of action.

B.9.2.6.3. Development and emergence in *Chironomus* species

No chronic study on the development and emergence in *Chironomus* species was provided during the first EU approval of the active substance, since ethofumesate is not an insect growth regulator.

B.9.2.6.4. Sediment dwelling organisms

For the first EU approval of the active substance a chronic toxicity study with the sediment dwelling organisms *Chironomus riparius* was submitted (Addendum to the DAR, 2000). In addition, two studies with sediment dwelling organisms were submitted for the renewal of the EU approval.

The summary and evaluation of the studies is given below.

Reference:	Ethofumesate: Chronic toxicity to the sediment dwelling organisms <i>Chironomus riparius</i> (BBA method)
Author(s), year:	Mattock, S.D., 1998
Report/Doc. number:	Study no. A91783, Reference no. M-168438-01-1
Guideline(s):	BBA guideline
GLP:	Yes
Deviations:	<ul style="list-style-type: none"> - The hardness of the water used in this study was slightly above the range specified in the protocol, i.e. 62.1 to 69.7 mg/L and not 40 to 60 mg/L. - The test guidelines state that the pH should be within 6.0 to 9.0. However on occasions during the study the pH fell below 6.0. <p>Neither of the deviations are considered likely to have had any impact on the outcome of this study.</p>
Validity:	Acceptable

Material and methods:

Test substance:	<p>Unlabelled test material: Ethofumesate techn., batch no.: CR 19291/02/940701, purity: 97.7%</p> <p>Radio-labelled test material: [Benzene ring-U-¹⁴C] ethofumesate, batch no.: 901B-1, purity: > 98%</p>
Test species:	Midge (<i>Chironomus riparius</i>)
Number of organisms:	6 replicates each with 25 larvae per treatment and control groups, 2 replicates for analytical measurements
Age:	First instar larvae, approx. 1 day old
Type of test, duration:	Static test, 28 days, limit test
Feeding:	Ground TetraMin TM , every second day, 0.058 g per replicate
<u>Applied concentrations:</u>	
Nominal:	0 (control and solvent control) and 5.0 mg/L

Mean measured:	- (control and solvent control) and 3.2 mg/L (overlying water)
Solvent:	Acetone
<u>Test conditions:</u>	
Water quality:	Water, hardness: 62.1 – 64.7 mg/L as CaCO ₃ , conductivity 223 – 295 µS
Temperature:	18.8 °C (mean), 18.4 – 19.4 °C (range)
pH:	5.4 – 7.6
O ₂ content:	86 – 104 % air saturation
Light regime:	16 hours light / 8 hours darkness
Test sediment:	Artificial soil according OECD guideline 207. Mixture of moss peat, silver sand and clay in a dry weight ratio of 1:7:2, respectively. pH: 6.0 ± 0.5
Test system:	The test vessels were 3000 mL volume glass beakers, containing, in the 310 ± 4 g of test sediment (equivalent to 260 ± 3 g of dry sediment) and 2500 mL of overlying water. Test vessels were filled with sediment and water, aerated using long form glass pasteur pipettes and conditioned for seven days before addition of larvae. After addition of the larvae all test vessels were covered with cling film and aeration was re-started following application of the test material.
Test parameter:	The dissolved oxygen concentration, pH, temperature and conductivity of the overlying water were determined at the start of the test and at weekly intervals thereafter. The ambient, minimum and maximum temperature of the overlying water was determined daily, in one of the control replicate test vessels. The test vessels were observed daily for emergence. The number of emergent adults were recorded and removed daily. The sex of the emergent midges was recorded.
Analytical measurements:	Samples of overlying water, in triplicate, were taken for liquid scintillation counting (LSC) on days 0 (approximately one hour), 3, 7, 14, 21 and 28. Samples of pore water were taken for LSC counting on days 0 (approximately one hour), 7 (from additional analytical test vessels) and 28. Samples for sediment analysis were taken from additional analytical test vessels on days 0 (approximately one hour) and 7, samples taken on day 28 were taken from one of the replicate test vessels. Radioactivity was determined by LSC. Samples for water analysis were taken from an additional test vessel on day 0 (approximately one hour). The procedural recovery for the analysis, determined by LSC counting was 103%.
Statistics:	Pooled male and female emergence data were used for the interpretation of the results. The emergence rate (ER) and development rate (X) were calculated according to the guidelines. The calculated variables ER and X were analysed

using one-way analysis of variance (ANOVA).

Findings:

Analytical measurements: The initial concentration in the overlying water was 5.22 mg/L [^{14}C]-ethofumesate after correction for the percentage of radioactivity present as ethofumesate (97.9%). By the end of the study the concentration of [^{14}C]-ethofumesate equivalent had reduced to 3.2 mg/L. Since measured [^{14}C]-ethofumesate concentration was close to nominal at the start of the study the toxicity of [^{14}C]-ethofumesate to *C. riparius* was based on the nominal initial concentration. The initial pore water concentrations were determined to be 0.29 mg/L [^{14}C]-ethofumesate equivalent, and these reached 2.30 mg/L [^{14}C]-ethofumesate equivalents by the end of the study. The initial sediment concentrations were determined to be 2.120 mg/kg [^{14}C]-ethofumesate equivalents and these reached 18.334 mg/kg [^{14}C]-ethofumesate equivalents by the end of the study.

Biological effects: Emergence was first observed on day 14 in one replicate in each of the control groups and the 5.0 mg/L test treatment. By day 20, emergence of *C. riparius* was complete, with the exception of one replicate in the 5.0 mg/L test treatment where there was one emergent adult on day 28. There were no apparent effects on the development of male and female midges. The development rate [%/day] for both the solvent control and 5.0 mg/L treatment was 6.3. There were no significant differences ($p > 0.05$) in emergence, time to first emergence, or development rate between the solvent control and the 5.0 mg/L treatment.

Table B. 9.2.6.4-1: Emergence summary data, day 28

Ethofumesate [mg ai/L] (nominal)	Number emerged (sum of all replicates)			Emergence [%]
	Male	Female	Total	
Control	50	79	129	86
Solvent control	48	81	129	86
5.0	67	71	138	92
28 d NOEC = 3.2 mg ai/L (based on emergence) based on mean measured concentrations				

Conclusion:

Ethofumesate, applied at a concentration of 5 mg/L to a sediment-water system had no significant effect on total emergence, development rate or time to first emergence of *Chironomus riparius*. Hence, a NOEC of 5 mg ai/L based on nominal concentrations was determined.

Comment RMS:

The study was conducted according to the BBA test guideline (1994). The study protocol is in line with the current valid test guideline according OECD 219

(2004). The validity criteria given in the BBA test guideline are covered by the current valid test guidelines according OECD.

The emergence in the controls (solvent and negative control) was at least 70% at the end of the test (being: 86%). The emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels (being: 14-20). The water temperature should not differ by more than $\pm 1.^\circ\text{C}$. The water temperature in the test vessels was in line with the validity criterion. Only in three test vessels there was a slightly higher difference in temperature over the test period.

At the end of the test, pH and the solved oxygen concentration should be measured in each vessel.

The oxygen concentration should be at least 60% of the air saturation at the temperature used, and the pH of overlying water should be in the 6 – 9 range in all test vessels. In the study the oxygen concentration was between 86 and 104% and the pH was between 5.4 and 7.6.

According to the BBA guideline (1994) no validity criteria considering temperature are given. However, it is stated that the pH should be between 6 and 9 in all test vessels.

Even though the validity criteria were not met regarding the environmental conditions (pH and temperature) the study is considered acceptable, considering that the pH and temperature are only slightly below the recommended values. In addition, no adverse effects on the emergence and the emergence rate were observed.

In the study report a NOEC of 5.0 mg ai/L based on nominal concentrations is stated. However, at the end of the study a mean measured concentration of 3.2 mg ai/L in the overlying water was determined. This is 64% of the nominal test concentration. Hence, the NOEC should be 3.2 mg ai/L based on mean measured concentrations.

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

Reference:	Sediment-water chironomid toxicity test using water spiked with ethofumesate
Author(s), year:	Desmares-Koopmans, M.J.E., 2002
Report/Doc. number:	Study no. 324089, Reference no.: IDD00073
Guideline(s):	OECD 219 (draft, 2001)
GLP:	Yes
Deviations:	<p>- The pH in two control vessels on day 28 was 5.8 and 5.9, respectively. Thus deviation of 0.2 and 0.1 unit, respectively from the protocolled range (6 – 9) were noted.</p> <p>- One test vessel of the solvent control was broken on day 27. Thereafter no more observations were made.</p> <p>- Four to five days before the application of the test substance, egg packets were taken from the culture and deposited into small vessels in culture medium. Thus, egg packets were taken from the culture on nominal days -5 and -4, instead of on nominal days -6 and -5 as stated in the guideline.</p> <p>The deviations were considered to have no effect on the outcome of the study.</p>
Validity:	Acceptable

Material and methods:

Test substance:	Unlabelled test material: Ethofumesate techn., batch no.: EFS-106, purity: 98.93% Radio-labelled test material: [Benzene ring-U- ¹⁴ C] ethofumesate, batch no.: CFQ12729, purity: 98.5 – 99.4%
Test species:	Midge (<i>Chironomus riparius</i>)
Number of organisms:	6 replicates each with 20 larvae per treatment and control groups
Age:	First instar larvae, approx. 2-3 day old
Type of test, duration:	Static test, 28 days, limit test
Feeding:	Trouvit, daily, from day -1 to 27

Applied concentrations:

Nominal:	0 (control and solvent control) and 4.4 mg ai/L
Mean measured:	- (control and solvent control) and 2.42 mg ai/L (overlying water)
Solvent:	Acetone

Test conditions:

Water quality:	ISO-medium, hardness: 200 mg/L as CaCO ₃
Temperature:	19.3 - 20.1 °C
pH:	5.8 – 8.2
Hardness:	179 – 232 mg/L as CaCO ₃
O ₂ content:	5.8 – 9.4 mg O ₂ /L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness, light intensity 688 – 728 lux
Test sediment:	Artificial soil according OECD guideline 207.

	Mixture of 5% sphagnum peat, 20% kaolin clay and 75% industrial sand pH: 6.7, organic carbon: 1.6% of dry weight
Test system:	A layer of ca. 1,5 cm of formulated sediment (mean weight 85.03 ± 0.23 g) was added to each test vessel (600 mL volume glass beakers). Thereafter 6 cm of ISO medium (mean weight 270 ± 0.04 g) was added to the sediment. Thus the height ratio sediment : overlying water was 1 : 4. Twenty larvae of the first larval stage were allocated randomly to each test vessel with a pipette. One day after adding the test substance was added to the water column using a pipette. The water was mixed gently without disturbing the sediment.
Test parameter:	The dissolved oxygen concentration, pH, temperature and conductivity of the overlying water were determined at the start of the test and at weekly intervals thereafter. The ambient, minimum and maximum temperature of the overlying water was determined daily, in one of the control replicate test vessels. The test vessels were observed daily for emergence. The number of emergent adults were recorded and removed daily.
Analytical measurements:	Samples of overlying water, in triplicate, were taken for liquid scintillation counting (LSC) on days 0 (approximately 5 minutes), 7, and 28. Samples of pore water were taken for LSC counting on days 0 (approximately 5 minutes), 7 (from additional analytical test vessels) and 28. Samples for sediment analysis were taken from additional analytical test vessels on days 0 (approximately 5 minutes) and 7, samples taken on day 28 were taken from one of the replicate test vessels. Radioactivity was determined by LSC.
Statistics:	Statistical analyses were conducted using the software TOXSTAT.
Findings:	
Analytical measurements:	The mean measured concentration of the active substance in the overlying water was between 84.9% (5 min after spiking) and 53.8% (28 days after spiking), corresponding to a mean measured concentration of 2.42 mg ai/L. The recovered activity in the pore water was between 0.04% (5 minutes after spiking) and 0.19% (after 28 days).

Table B. 9.2.6.4-2: Emergence summary data, day 28

Ethofumesate [mg ai/L] (nominal)	Number emerged (sum of all replicates)			Emergence rate
	Male	Female	Total	
Control	54	32	86	0.72
Solvent control	52	37	89	0.74
4.4	47	32	79	0.66

Table B. 9.2.6.4-3: Mean development time and rate after 28 d of exposure

Ethofumesate [mg ai/L] (nominal)	Mean development time [d]	Mean development rate [1/d]
Control	20.7	0.049
Solvent control	21.1	0.048
4.4	20.2	0.050

Conclusion:

Ethofumesate, applied at a concentration of 4.4 mg/L to a sediment-water system had no significant effect on total emergence, development rate or time to first emergence of *Chironomus riparius*. Hence, a NOEC of 4.4 mg ai/L based on nominal concentrations was determined.

Comment RMS:

The study was conducted according to the OECD draft test guideline 219 (2001). The study protocol is in line with the current valid test guideline according OECD 219 (2004). The validity criteria given in the OECD test guideline (2001 and 2004) were met.

The mortality in the controls should not exceed 30% at the end of the test (being: 0.0%).

The emergence in the controls (solvent and negative control) was at least 70% at the end of the test (being: 72-74%). The emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.

In the present test eff packets were taken from the cultures on nominal days -5 and -4, instead of on nominal days -6 and -5 as stated in the guideline. Thus, the larvae exposed in the test are one day younger. Since exposure of younger larvae is a worst-case scenario, this deviation is considered to have no effect on the final test results. On day 24, 58% of the midges emerged in the blank control and 54% in the solvent control. This pattern of emergence of midges, before and after day 24, was comparable in the controls and the test concentration.

The water temperature should not differ by more than $\pm 1^{\circ}\text{C}$. The water temperature in the test vessels was in line with the validity criterion.

At the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel.

The oxygen concentration should be at least 60% of the air saturation at the temperature used, and the pH of overlying water should be in the 6 – 9 range in all test vessels. In the study the oxygen concentration was >60% and the pH was between 5.8 and 8.2.

Even though the validity criteria were not met regarding the environmental conditions (pH) the study is considered acceptable, considering that the pH are

only slightly below the recommended values.

In addition, no adverse effects on the emergence and the emergence rate were observed.

In the study report a NOEC of 4.4 mg ai/L based on nominal concentrations is stated. However, at the end of the study a mean measured concentration of 2.42 mg ai/L in the overlying water was determined. This is 55% of the nominal test concentration. Hence, the NOEC should be 2.42 mg ai/L based on mean measured concentrations.

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

Reference:	Assessment of side effects of ethofumesate technical on the larvae of the midge, <i>Chironomus riparius</i> with laboratory test method
Author(s), year:	Stäbler, D., 2003
Report/Doc. number:	Study no. 20021050/01-ASCr, Reference no. M-IDD00074
Guideline(s):	BBA guideline (1995), OECD draft guideline 219 (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., batch no.: 1997/1, purity: 98.59%
Test species:	Midge (<i>Chironomus riparius</i>)
Number of organisms:	6 replicates each with 25 larvae per treatment and control groups, additional 18 vessels for the analytical control.
Age:	First instar larvae, approx. 1-3 day old
Type of test, duration:	Static test, 28 days
Feeding:	Tetra Min®, daily, 1 mg food per larvae

Applied concentrations:

Nominal:	0 (control and solvent control), 50 and 100 mg ai/L
Mean measured:	- (control and solvent control) 12.9 and 33 mg ai/L (overlying water)
Solvent:	Acetone

Test conditions:

Water quality:	Dechlorinated drinking water and deionised water, pH = 6.5 – 8.5
Temperature:	19.2 – 20.8 °C
pH:	7.99 – 8.87

Hardness:	179 – 232 mg/L as CaCO ₃
O ₂ content:	7.5 – 10 mg O ₂ /L (> 60% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test sediment:	Artificial soil according OECD guideline 207. Mixture of 10% sphagnum peat, 20% kaolin clay, 69% industrial sand and approx. 1% calcium carbonate
Test system:	A layer of ca. 2-3 cm of sediment (310 g wet weight) was added to each test vessel (2 L volume glass beakers). Thereafter 15 - 16 cm of water (1600 mL medium) was added to the sediment. Larvae of the first larval stage were allocated randomly to each test vessel with a pipette. Immediately after application the test vessels were closed with a plastic cover which offered an opening for gas exchange and the aeration was started.
Test parameter:	The test vessels were observed three times per week to make a visual assessment of any behavioural effects. During the period of expected emergence (normally starting at day 10 and lasting until day 24) a daily check of emerged midges was performed. The sex and number of emerging adults were recorded daily. The oxygen concentration, water temperature and pH were recorded in all test vessels at the start and the end of the test.
Analytical measurements:	Samples of the overlying water, pore water and the sediment were taken 1 hour, 7 days and 29 days after application. The analytical samples were taken from addition parallel test vessels. The overlying water was analysed using HPLC method.
Statistics:	The calculation of the NOEC multiple t-tests such as Dunnett or pairwise U-test (0.05, one-sided) were performed.

Findings:

Analytical measurements:	The analytical data showed a precipitation of ethofumesate immediately after start of the test. In the overlying water mean measured concentrations of ethofumesate of 24 – 40% were measured after test start. After 28 d of exposure the measured concentrations in the overlying water were 23 – 30% of nominal concentrations. In the pore water the mean measured concentrations were in range of 0.4 – 1.2% (0 d) and 0.7 – 1.4% (28 d) of nominal concentrations. In the sediment the mean measured concentrations were in a range of 68 – 93% (0 d) and 58 – 79% (28 d) of nominal concentrations.
--------------------------	--

Table B. 9.2.6.4-4: Emergence summary data, day 28

Ethofumesate [mg ai/L] (nominal)	Number emerged (sum of all replicates)			Emergence [%]	Emergence rate	Development rate
	Male	Female	Total			
Control	57	86	143	95.3	1.375	0.0611
Solvent control	55	80	135	90.0	1.282	0.0624
50	85	51	136	90.7	1.321	0.0689
100	37	46	83	55.3 *	0.770 *	0.0622

* Statistically significant compared to the solvent control, Dunnett's test, $p \leq 0.05$, one-sided

The low emergence rate at the highest test concentration is based on the missing emergence of midges in two vessels. In the other vessels the emergence of midges was similar to the controls and the 50 mg/L treatment group.

In the 50 mg/L treatment group the sex ratio was different to the sex ratio observed in the other groups.

Conclusion:

Ethofumesate, applied at a concentration of 50 mg/L to a sediment-water system had no significant effect on total emergence, development rate or time to first emergence of *Chironomus riparius*. At the highest test concentration 100 mg/L an inhibition of emergence of 44.7% was observed. Hence, a NOEC of 50 mg ai/L (corresponding to 12.9 mg ai/L mean measured) was determined. The EC₅₀ (emergence, development) was determined to be greater than 100 mg/L (corresponding to 33 mg ai/L mean measured).

Comment RMS:

The study was conducted according to the BBA test guideline (1991) and the OECD draft test guideline 219 (2001). The study protocol is in line with the current valid test guideline according OECD 219 (2004). The validity criteria given in the OECD test guideline (2001 and 2004) were met.

The mortality in the controls should not exceed 30% at the end of the test (being: 0.0%).

The emergence in the controls (solvent and negative control) was at least 70% at the end of the test (being: 90-95%). The emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.

Main emergence of midges was observed between day 14 and 22 in the control and between day 14 and 24 in the solvent control.

The water temperature should not differ by more than $\pm 1^\circ\text{C}$. The water temperature in the test vessels was in line with the validity criterion.

At the end of the test, pH and the solved oxygen concentration should be measured in each vessel.

The oxygen concentration should be at least 60% of the air saturation at the

temperature used, and the pH of overlying water should be in the 6 – 9 range in all test vessels. In the study the oxygen concentration was > 60% and the pH was between 7.99 and 8.87.

Even though the validity criteria were not met regarding the duration of emergence (1 midge emerged on day 24 in the solvent control) the study is considered acceptable.

In addition, no adverse effects on the emergence and the emergence rate were observed.

In the study report a NOEC of 50 mg ai/L based on nominal concentrations is stated. However, the mean measured concentration was 12.9 mg ai/L in the overlying water. This is 25.8% of the nominal test concentration. Hence, the NOEC should be 12.9 mg ai/L based on mean measured concentrations.

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

B.9.2.7. Effects on algal growth

Laboratory studies on the toxicity to algae with the active substance ethofumesate were submitted for the first EU approval of the active substance. For the first EU approval only one algae species was tested, i.e. *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*). As ethofumesate is a herbicide a study with additional algae species has to be provided according to the EU data requirements. Hence, laboratory studies with the green algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), the diatom *Nitzschia palea*, the blue green algae *Anabaena flos-aquae* and the saltwater diatom *Skeletonema costatum* were submitted.

In addition, studies with the metabolites NC 8493, NC 20645 and BCS-CW35117 were conducted.

The study summaries are given below.

Active substance:

Reference:	The algistatic activity of ethofumesate
Author(s), year:	Douglas, M.T., Halls, R.W.S. and Macdonald, I.A., 1990
Report/Doc. number:	Study no. A87620, Reference no. M-161559-01-1
Guideline(s):	OECD test guideline 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate tech., batch no. : P-04402/2, purity : 97%
Test species:	Green alga (<i>Desmodesmus subspicatus</i> formerly known as <i>Scenedesmus subspicatus</i>)
Number of organisms:	2.44×10^5 cells/mL (control); 3 replicates per treatment and control group
Type of test, duration:	Static test, 72 hours

Applied concentrations:

Nominal:	0 (control), 1.25, 2.5, 5, 10 and 20 mg ai/L
Solvent:	None

Test conditions:

Water quality:	Algal nutrient medium
Temperature:	24 ± 1 °C
pH:	7.2 (0 h), 7.2 – 7.3 (72 h)
Incubation:	Continuous illumination at ~ 7000 lux
Analytical measurements:	For chemical analysis (High Performance Liquid Chromatography) of test the substance, samples of test solution were taken at test initiation and at test termination.
Test parameters:	Samples were taken at 0, 24, 48, and 72 hours and the absorbance measured at 665

nm. The cell densities of the control cultures at initiation and at termination were determined by direct counting with the aid of a haemocytometer.

Measurements of pH and temperature were made at initiation and at termination.

Findings:

Analytical data: Mean measured concentrations were in the range of 85 - 95% of nominal concentrations over the whole test duration. Hence, the results are based on nominal concentrations.

Morphological effects: After 72 h of exposure no abnormalities were observed in any of the control or treatment groups.

Table B. 9.2.7-1: Effects of technical ethofumesate on the green algae *Desmodesmus subspicatus*

Ethofumesate [mg/L] (nominal)	Biomass		Growth rate	
	Area under the curve (72 h)	% inhibition relative to the control	0 – 24 h	% inhibition relative to the control
Control	5.064	-	0.019	-
1.25	5.056	0	0.018	1
2.5	3.272	35	0.010	47
5	2.156	57	0.010	45
10	1.652	67	0.009	53
20	1.144	77	0.003	86

Conclusion:

72 h E_0C_{50} = 3.9 mg ai/L
 24 h E_rC_{50} = 9.0 mg ai/L
 72 h NOEC = 1.25 mg ai/L (biomass and growth rate)
 based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (1984). The study was conducted in general agreement with the current valid test guideline. The test medium used in the study was identical to the OECD TG 201 test medium. In addition, the environmental conditions were in line with the OECD test guideline.

According to the validity criteria given in the OECD test guideline (1984) the study is considered acceptable.

The cell concentration in the control groups increase by a factor of at least 16 within 3 days. The mean cell density of control at the start and at the end of the test (72 h) was 2.44×10^5 cells/mL and 3.90×10^6 cells/mL. This is a decrease of the cell concentration by a factor of 16.

The validity criteria given in the current valid test guidelines (OECD 201, 2006)

cannot be discussed as no statistical analyses were conducted. Based on the results of the study (no information on the calibration is available) no additional statistical analyses could be conducted by the RMS.

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

Reference:	A study of the toxicity of algae of ethofumesate technical
Author(s), year:	Knacker, T., 1989
Report/Doc. number:	Study no. A83343, Reference no. M-155612-01-1
Guideline(s):	OECD 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate tech., CAS no. 26225-79-6, batch no. : R000047, purity : 99.9%
Test species:	Green alga (<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), 1.0, 3.162, 10.0, 31.623, 100, 316.228 and 1000 mg ai/L
Toxic reference	Potassium dichromate, E _b C ₅₀ = 0.6 mg/L, E _r C ₅₀ = 0.9 mg/L
Solvent:	None

Test conditions:

Water quality:	Algal medium II
Temperature:	23 ± 1 °C
pH:	7.7 – 7.8 (0 h), 7.7 – 7.9 (96 h)
Incubation:	Continuous illumination, universal white
Test parameters:	The test substance was not soluble in the range of 10 to 1000 mg/L. Therefore, below the test substance concentration of 10 mg/L the cell number was determined spectrophotometrically, above the concentration of 10 mg/L a counting chamber was used in addition to the spectrophotometer. For the two highest test substance concentrations only the counting chamber was used. For chemical analysis (High Performance Thin Layer Chromatography) of the test

substance, samples of test solution were taken at test initiation and at test termination. Measurements of pH and temperature were made at initiation and at termination.

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 measured test concentrations were in the range of 35 and 132% of nominal concentrations.

The values measured for the nominal concentrations of 1000 and 316 mg/L were not taken into consideration since according to the solubility and the analytical data the test material was not homogeneously distributed in the test vessels.

Morphological effects: After 72 h of exposure no abnormalities were observed in any of the control or treatment groups.

Table B. 9.2.7-2: Effects of technical ethofumesate on the green algae *Desmodesmus subspicatus*

Ethofumesate [mg/L] (nominal)	Biomass		Growth rate	
	Area under the curve (0 - 96 h)	% inhibition relative to the control	Growth rate 24 – 96 h	% inhibition relative to the control
Control	2657.3	-	0.052	-
1.0	814.1	69.4	0.030	41.8
3.162	609.3	77.1	0.023	55.7
10.0	278.2	89.5	0.013	75.5
31.623	182.1	93.1	0.006	88.5
100.0	285.7	89.2	0.004	91.8

Conclusion: 96 h E_bC_{10} = 0.0001 mg ai/L

96 h E_bC_{50} = 0.06 mg ai/L

96 h E_rC_{10} = 0.06 mg ai/L

96 h E_rC_{50} = 1.8 mg ai/L

96 h NOEC < 1.0 mg ai/L (biomass and growth rate)

based on nominal concentrations

Comment RMS: The study was conducted according to the OECD test guideline 201 (1984). The study was conducted in general agreement with the current valid test guideline. The test medium used in the study was identical to the OECD TG 201 test

medium. In addition, the environmental conditions were in line with the OECD test guideline.

According to the validity criteria given in the OECD test guideline (1984) the study is not considered acceptable. At the test start the mean number of cells in the control was 1×10^4 cells/mL. Under consideration of a cell concentration of 1.25×10^5 cells/mL at the end of the study the increase of cells in the control is not acceptable. According to the OECD test guideline the cell concentration in the control groups should increase by a factor of at least 16 within 3 days.

The test results are based on nominal concentrations. However, based on the mean measured concentrations ($< 80\%$ and $> 120\%$) the results should be based on mean measured concentrations.

In addition, it should be considered that results of the range finding test and the definitive test are not in line. In the range finding test no significant inhibition was observed at a concentration of 1.0 mg ai/L. However, in the definitive test a significant growth inhibition was observed.

The validity criteria given in the current valid test guidelines (OECD 201, 2006) cannot be discussed as no statistical analyses were conducted.

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

Reference:	Alga growth inhibition test
Author(s), year:	Bellmann, W., 1992
Report/Doc. number:	Study no. 40730.315-201, Reference no. M-359250-01-1
Guideline(s):	OECD 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance:	Ethofumesate tech., CAS no. 26225-79-6, batch no.: 08/05/92, purity: not specified
Test species:	Green alga (<i>Desmodesmus subspicatus</i> formerly known as <i>Scenedesmus subspicatus</i>)
Number of organisms:	1.1×10^4 cells/mL; 3 replicates per treatment group 0.8 and 1.5 mg ai/L and 2 replicates per treatment group 3.1 – 98.4 mg ai/L

	4 replicates per control group
Type of test, duration:	Static test, 72 hours
<u>Applied concentrations:</u>	
Nominal:	0 (control), 0.8, 1.5, 3.1, 6.2, 12.3, 24.6, 49.2 and 98.4 mg ai/L
Toxic reference	None
Solvent:	None
<u>Test conditions:</u>	
Water quality:	Algal medium II
Temperature:	21 - 25 °C
pH:	8.0 – 8.1 (0 h), 8.1 – 8.7 (72 h)
Incubation:	Continuous illumination, universal white, > 8000 lux
Test parameters:	The cell concentration in each vessel was determined at the beginning of the test and after 24, 48, and 72 hours by measuring the fluorescence in a fluorimeter (wavelength $\lambda = 685$).
Statistics:	The E_bC_x and E_rC_x values were estimated from the graph of the regression analysis after log transformation of the concentration values.
<u>Findings:</u>	
Morphological effects:	At test concentrations of 49.2 and 98.4 mg/L a certain agglutination of algae cells at the bottom of the vessels were observed. This might be the reason for the questionable results (no cell counts at the beginning of the test) for these two concentrations.

Table B. 9.2.7-3: Effects of technical ethofumesate on the green algae *Desmodesmus subspicatus*

Ethofumesate [mg/L] (nominal)	Biomass	Growth rate
	% inhibition relative to the control	% inhibition relative to the control
Control	-	-
0.8	- 0.9	- 2.9
1.5	10.8	3.0
3.1	57.1	22.7
6.2	77.9	41.0
12.3	85.7	54.1
24.6	92.7	68.7
49.2	93.2	99.9
98.4	106	100

<u>Conclusion:</u>	72 h E_bC_{10} = 1.5 mg ai/L
	72 h E_bC_{50} = 2.7 mg ai/L
	72 h NOE_bC = 0.8 mg ai/L
	72 h E_rC_{10} = 2.0 mg ai/L
	72 h E_rC_{50} = 10. mg ai/L

72 h NOE_rC = 1.1 mg ai/L
based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline 201 (1984). The study was conducted in general agreement with the former and current valid test guideline. The test medium used in the study was identical to the OECD TG 201 test medium. In addition, the environmental conditions were in line with the OECD test guideline.

According to the validity criteria given in the OECD test guideline (1984) the study is considered acceptable. At the test start the mean number of cells in the control was 1.1×10^4 cells/mL. Under consideration of a cell concentration of 4.1×10^5 cells/mL at the end of the study the increase of cells in the control is greater than a factor of 16 (being: 37).

The validity criteria given in the current valid test guidelines (OECD 201, 2006) cannot be discussed as no statistical analyses were conducted. Based on the results of the study (no cell counts at the two highest test concentrations at test start) no additional statistical analyses could be conducted by the RMS.

In addition, no information on analytical measurements was given in the study report.

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

Reference:	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with ethofumesate (techn.)
Author(s), year:	Bruns, E. (2. Amendment) and Dorgerloh, M., 2008
Report/Doc. number:	Study no. E 323 3418-4, Reference no. M-302092-03-1
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: Ethofumesate tech., CAS no. 26225-79-6, batch no. : EWFA002886, purity : 97% w/w (analysed)

Test species:	Green alga, <i>Pseudokirchneriella subcapitata</i> (formerly known as <i>Selenastrum capricornutum</i>)
Number of organisms:	1 x 10 ⁴ cells/mL; 3 replicates per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 72 hours
<u>Applied concentrations:</u>	
Nominal:	0 (control and solvent control), 0.179, 0.572, 1.83, 5.86, 18.8 and 60 mg ai/L
Mean measured:	- (control and solvent control), 0.144, 0.495, 1.74, 5.91, 16.0 and 14.6 mg ai/L
Solvent:	Acetone (100 µL/L)
<u>Test conditions:</u>	
Water quality:	Nutrient medium
Temperature:	22.0 – 22.2 °C
pH:	8.2 (0 h), 8.1 – 8.7 (72 h)
Incubation:	Continuous illumination, 7770 - 8390 lux (mean: 8128 lux)
Test parameters:	<p>Morphological examination of cells using a microscope was made over the exposure period on each study day. Cell numbers per volume (as a surrogate for biomass per volume) and possible alterations in algae cells such as unusual cell size were estimated by direct algae cell counting under a microscope.</p> <p>The pH and the temperature was measured at each observation time in all test levels and the controls.</p> <p>Samples were analysed (HPLC-UV) for the actual concentration of ethofumesate present in the test medium of all treatment levels and the controls on day 0 and 3.</p>
Statistics:	Shown are rounded values, but all calculations were carried out using Microsoft Excel® spreadsheets. All further statistical evaluations were done using the commercial program ToxRat Professional.
<u>Findings:</u>	
Analytical data:	<p>The analytical findings of ethofumesate in all test concentrations except the highest found on day 0 were 70 % to 101 % of nominal and of 81 % to 104 % of nominal on day 3. In the highest test concentration only 24% (day 0) and 25 % (day 3) of nominal were found. This can be explained by the limited solubility of ethofumesate in the nutrient medium used in the study, because the maximum water solubility of ethofumesate is reported at about 40 mg/L. Results are based on nominal and geometric mean measured test concentrations.</p>
Morphological effects:	After 72 h of exposure no abnormalities were observed in any of the control or treatment groups.

Table B. 9.2.7-4: Effects of technical ethofumesate on the green alga *Pseudokirchneriella subcapitata*

Ethofumesate [mg/L] (mean measured)	Mean cell numbers after 72h per mL	Doubling time of algae cells [d]	Average specific growth rates	
			Growth rate [d] (0 – 72 h)	% inhibition relative to the controls
Control	358000	0.583	1.189	-
Solvent control	343000	0.592	1.170	-
Pooled control	350000	0.588	1.179	-
0.144	402000	0.563	1.231	- 4.4
0.495	453000	0.545	1.271	- 7.8
1.74	442000	0.550	1.261	- 7.0
5.91	295000	0.615	1.127	4.4
16.0	77000	1.10	0.633 *	46.3
14.6	70000	1.08	0.642 *	45.5

* Significantly different compare to the pooled control, based on Williams multiple sequential t-test, α 0.05, one-sided smaller

Conclusion:

72 h $E_{rC_{50}}$ = 16.3 mg ai/L (95% C.I. 15.4 – 17.7 mg ai/L)

96 h NOEC = 5.91 mg ai/L (growth rate)

based on mean measured concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006).

The study is in line with the test guideline and all validity criteria are met.

The biomass in the control cultures increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the biomass increased by a factor of 35, corresponding to a growth rate of 1.189 per day.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 28.3%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 5.9%

In the study report statistical analyses considering the growth rate were conducted. However, no information of the biomass/yield was given in the report. Hence, the RMS conducted additional statistical analyses using the software ToxRat®.

Based on the re-evaluation of the results the following endpoints (based on mean measured concentrations) were determined.

72 h $E_{yC_{10}}$ = 5.054 mg ai/L (95% C.I. = 4.188 - 5.803 mg ai/L)

72 h E_yC_{50} = 9.683 mg ai/L (95% C.I. = 8.883 – 10.451 mg ai/L)

NOE_yC = 5.91 mg ai/L

72 h E_rC_{10} = 7.295 mg ai/L (95% C.I. = 5.296 – 8.675 mg ai/L)

72 h E_rC_{50} = 16.347 mg ai/L (95% C.I. = 15.431 – 17.685 mg ai/L)

NOE_rC = 5.91 mg ai/L

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

Reference:	Alga, Growth inhibition test (<i>Nitzschia palea</i> 96 [h])
Author(s), year:	Scheerbaum, D., 1998
Report/Doc. number:	Study no. A91866, Reference no. M-168517-01-1
Guideline(s):	OECD 201 (1984)
GLP:	Yes
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance: Ethofumesate tech., CAS no. 26225-79-6, batch no. : 28/02/98, purity : 98.8% w/w (analysed)

Test species: Diatoms, *Nitzschia palea*

Number of organisms: 1×10^4 cells/mL; 3 replicates per treatment group and control group

Type of test, duration: Static test, 96 hours

Applied concentrations:

Nominal: Not given

Mean measured: - (control), 0.86, 2.2, 5.2, 11.4, 25 and 51 mg ai/L

Solvent: None

Test conditions:

Water quality: Bacillariophycean medium according to SAG, pH approx. 8.1 ± 0.2

Temperature: 22 - 23 °C

pH: 7.48 – 8.03 (0 h), 7.80 – 8.42 (96 h)

Incubation: Continuous illumination, 4690 - 6030 lux

Test parameters: After 0 h fluorescence measurement the test substance was applied out of the prepared dilutions. Cell density was measured for each test flask via Chlorophyll-fluorescence (Impulsfluorometer), excitation at 435 nm, emission at 685 nm. Each replicate was measured 6-fold.

After 96 h algae were transferred from any treatment where growth was inhibited by more than 50 % to fresh medium and allowed to grow for a further 96 h to

determine whether the effect of the substance is reversible.

The pH-value at the beginning of the test was measured out of a mixture of two additional replicates of each dosage level and control. At the end it was measured from a mixture of all replicates. The room temperature was measured continuously.

Analytical measurements: The test substance concentrations have been analytically verified using HPLC. Sampling was carried out directly at test start (0 h) and at the test end (96 h).

Statistics: Calculations of the EC_x values and the confidence intervals were carried out via probit analysis using software SigmaPlot rel. 3.02 (1995). Cell densities, growth rates and biomass integrals were calculated using software Excel 5.0.

NOEC: One way analysis of variance (ANOVA) and Dunnett's test of biomass integrals and growth rate, respectively were carried out for the determination of statistically significant differences compared to control replicates. When running a one way analysis of variance a normality test and an equal variance test had been done first. P-values for both normality and equal variance test were 0.05.

For NOEC determination of growth rate after 24 and 48 h the normality test failed. Paired t-tests were carried out.

Findings:

Morphological effects: Microscopic evaluation of the cells at the start of the incubation revealed no morphological abnormalities.

Recovery: After 96 h a sample of cells from the highest treatment, 51 mg/L, were transferred to fresh untreated medium and incubated for a further 96 h. Algal growth was observed, indicating that the effect of the test substance was algistatic, and reversible, at the limit of solubility, 51 mg/L.

Table B. 9.2.7-5: Effects of technical ethofumesate on the diatom *Nitzschia palea*

Ethofumesate [mg/L] (mean measured)	Biomass		Average specific growth rates	
	Biomass integral	% inhibition relative to the controls	Growth rate [d] (0 – 96 h)	% inhibition relative to the controls
Control	31066	-	0.76	-
0.86	30225	-14	0.84	-10
2.2	28053	-16	0.81	-6
5.2	35664	-53 *	0.92	-21
11.4	15345	63 *	0.55 *	28
25.0	11458	81 *	0.41 *	46
51.0	3071	100 *	0.0 *	100

* Significantly different compare to the pooled control, based on Williams multiple sequential t-test, α 0.05, one-sided smaller

Negative values show increase of growth

Conclusion: 96 h $E_{rC_{50}}$ = 17.1 mg ai/L (95% C.I. = 16.2 – 18.1 mg ai/L)

96 h E_bC_{50} = 11.7 mg ai/L (95% C.I. = 10.9 – 12.6 mg ai/L) 96 h NOEC = 5.2 mg ai/L
(biomass and growth rate)
based on mean measured concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 1984). The study is in line with the OECD test guideline. The used test species, the diatom *Nitzschia palea* is not stated in the test guidelines (OECD 201, 1984 and 2006) as proposed test species. Hence, the validity criteria given in the test guidelines have to be considered with caution.

In general the study is in line with the stated test guidelines. However, the number of replicates used for the control groups is low (3 replicates). According to the OECD test guideline (OECD 201, 1984 and 2006) the test design should include preferably three replicates at each test concentration and ideally twice that number of controls.

The increase of cell density as a validity criterion has been changed to 10 fold within 96 h. According to the OECD test guideline (2006) the criterion (16-fold increase of cell density in the control) may not be met when species that grow slower than those mentioned in the test guideline are used. In this case, the test period should be extended to obtain at least a 16-fold growth in the control cultures, while the growth has to be exponential throughout the test.

In the control cultures the increase of the cell density was determined to be 11 (after 72 h) and 21 (after 96 h).

In the study report statistical analyses considering the growth rate and the biomass were conducted. However, no information on the coefficient of was given in the report. Hence, the RMS conducted additional statistical analyses using the software ToxRat®.

Based on the re-evaluation of the results the following endpoints (based on mean measured concentrations) were determined.

96 h E_yC_{10} = 5.341 mg ai/L (95% C.I. = 1.326 – 7.763 mg ai/L)

96 h E_yC_{50} = 11.796 mg ai/L (95% C.I. = 8.373 – 16.607 mg ai/L)

NOE_yC = 5.2 mg ai/L

96 h E_rC_{10} = 8.555 mg ai/L (95% C.I. = 0.646 – 13.982 mg ai/L)

96 h E_rC_{50} = 21.603 mg ai/L (95% C.I. = 12.454 – 36.132 mg ai/L)

NOE_rC = 5.2 mg ai/L

The results of the statistical analyses are well in line with the results given in the study report. However, the validity criteria according OECD test guideline 201 (2006) are not met.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 130%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 10.3%. According to the OECD test guideline (OECD 201, 2006) the value should not exceed 10% for less frequently tested species, like *Nitzschia palea*.

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

Reference:	Toxicity of ethofumesate technical to the blue green algae <i>Anabaena flos-aquae</i>
Author(s), year:	Banman, C.S., Daly, R.A. and Lam, C.V., 2009a
Report/Doc. number:	Study no. EBADL008, Reference no. M-349150-01-1
Guideline(s):	FIFRA guideline 123-2 (1982), OPPTS guideline 850.5400 (1996 draft) and OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: Ethofumesate tech., CAS no. 26225-79-6, batch no. : EWFA002886, purity : 97% w/w

Test species: Blue green algae, *Anabaena flos-aquae*

Number of organisms: 1×10^4 cells/mL; 3 replicates per treatment group and control group

Type of test, duration: Static test, 96 hours

Applied concentrations:

Nominal: 0 (control and solvent control), 0.0823, 0.25, 0.74, 2.22, 6.67 and 20.0 mg ai/L

Mean measured: - (control and solvent control), 0.0683, 0.22, 0.66, 1.92, 5.56 and 18.0 mg ai/L

Solvent: Acetone (0.1 mL/L)

Test conditions:

Water quality: AAP medium

Temperature: 23.4 – 23.9 °C (mean: 23.7 °C)

pH:	7.5 (0 h), 9.0 – 9.5 (96 h)
Conductivity:	88 – 92 µmhos/cm
Incubation:	Continuous illumination, 2200 lux
Test parameters:	Each day, density was determined in the three test replicates at each test concentration using a light microscope and an Improved Neubauer hemocytometer. Temperature was measured hourly. The pH was measured on Day 0, 3 and 4 and the conductivity was measured on Day 0 and Day 4.
Statistics:	The EC ₅₀ was determined using the Logistic Model or Bruce/Versteeg Cumulative Normal Model using nonlinear (weighted) regression analysis. Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively.
<u>Findings:</u>	
Analytical data:	Mean measured recoveries were within the range of 83 to 90% of the nominal concentrations. The toxicity values were calculated based on the nominal concentrations.
Morphological effects:	No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-6: Effects of technical ethofumesate on the blue green alga *Anabaena flos-aquae*

Ethofumesate [mg/L] (nominal)	Biomass		Average specific growth rates	
	Area under the growth curve (96 h)	% inhibition relative to the controls	Growth rate (0 – 96 h)	% inhibition relative to the controls
Control	4476.2	-	0.056173	-
Solvent control	3670.2	-	0.054433	-
Pooled control	4073.2	-	0.055303	-
0.0823	3479.2	14.6	0.054039	2.3
0.25	3947.5	3.1	0.055974	0.4
0.74	3455.3	15.2	0.054038	2.3
2.22	3746.1	8.0	0.055283	0.0
6.67	3439.9	15.5	0.054705	1.1
20.0	3197.9	21.5	0.054382	1.7

<u>Conclusion:</u>	96 h EC ₅₀ > 20 mg ai/L (biomass and growth rate) 96 h NOEC = 20 mg ai/L (biomass and growth rate) based on nominal concentrations
--------------------	---

<u>Comment RMS:</u>	The study was conducted according to the OECD test guideline (OECD 201, 2006). The study is in line with the OECD test guideline. The used test species, the
---------------------	--

blue green algae *Anabaena flos-aquae* is stated in the test guidelines (OECD 201, 2006) as proposed test species.

In general the study is in line with the stated test guidelines. However, the number of replicates used for the control groups is low (3 replicates). According to the OECD test guideline (OECD 201, 2006) the test design should include preferably three replicates at each test concentration and ideally twice that number of controls.

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

In the control cultures the increase of the cell density was determined to be 63 (after 72 h) and 220 (after 96 h).

Based on the statistical analyses the validity criteria are not met considering the coefficient of variation in the control groups.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 43%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 10%. According to the OECD test guideline (OECD 201, 2006) the value should not exceed 10% for less frequently tested species, like *Anabaena flos-aquae*.

The validity criteria contained in OECD Guideline 201, Inhibition of Algal Growth (2006), for section-by-section growth rates and average specific growth rates were derived using data from studies done with green algae species such as *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. These criteria can seldom be met with non-green algae and diatom species such as *Anabaena flos-aquae*, *Navicula pelliculosa*, and *Skeletonema costatum*.

As such, it is inappropriate to use these criteria in evaluating the regulatory acceptability of studies conducted with non-green species.

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 ethofumesate technical to the saltwater diatom <i>Skeletonema costatum</i>
Author(s), year:	Banman, C.S., Daly, R.A. and Lam, C.V., 2009b
Report/Doc. number:	Study no. EBADL009, Reference no. M-347965-01-1
Guideline(s):	FIFRA guideline 123-2 (1982), OPPTS guideline 850.5400 (1996 draft) and OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: Ethofumesate tech., CAS no. 26225-79-6, batch no. : EWFA002886, purity : 97% w/w

Test species: Saltwater diatom, *Skeletonema costatum*

Number of organisms: 1×10^4 cells/mL; 3 replicates per treatment group and control group

Type of test, duration: Static test, 96 hours

Applied concentrations:

Nominal: 0 (control and solvent control), 1.25, 2.5, 5.0, 10.0 and 20.0 mg ai/L

Mean measured: - (control and solvent control), 1.06, 2.36, 4.31, 9.21 and 18.2 mg ai/L

Solvent: Acetone (0.1 mL/L)

Test conditions:

Water quality: Enriched saltwater (ES) media

Temperature: 18.7 – 19.9 °C

pH: 7.9 – 8.8

Salinity: 26 ppt

Incubation: 16 h light, 8 h dark, 3850 - 4650 lux

Test parameters: Each day, density was determined in the three test replicates at each test concentration using a light microscope and an Improved Neubauer hemocytometer.

Temperature was measured hourly. The pH was measured on Day 0, 3 and 4 and the salinity was measured on Day 0 and Day 4.

Statistics: The EC₅₀ was determined using the Logistic Model or Bruce/Versteeg Cumulative Normal Model using nonlinear (weighted) regression analysis. Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively.

Findings:

Analytical data: Mean measured recoveries were within the range of 85 to 94% of the nominal concentrations. The toxicity values were calculated based on the nominal

concentrations.

Morphological effects: No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-7: Effects of technical ethofumesate on the saltwater diatom *Skeletonema costatum* – growth rate

Ethofumesate [mg/L] (nominal)	Average specific growth rates			
	Growth rate (72 h)	% inhibition relative to the controls	Growth rate (96 h)	% inhibition relative to the controls
Control	0.065279	-	0.051181	-
Solvent control	0.065659	-	0.050734	-
Pooled control	0.065469	-	0.050957	-
1.25	0.065653	0.0	0.050780	0.3
2.5	0.065803	-0.2	0.051873	-1.8
5.0	0.064503	1.8	0.050739	0.4
10.0	0.061605	6.2 *	0.050196	1.5
20.0	0.047419	27.6 *	0.046622	8.5 *

* Statistically significant compared to the pooled control, Dunnett's one-tailed test, $p \leq 0.05$

Negative values indicate an increase of growth.

Table B. 9.2.7-8: Effects of technical ethofumesate on the saltwater diatom *Skeletonema costatum* – biomass

Ethofumesate [mg/L] (nominal)	Biomass			
	Area under the growth curve (72 h)	% inhibition relative to the controls	Area under the growth curve (96 h)	% inhibition relative to the controls
Control	1906.2	-	4837.2	-
Solvent control	1977.4	-	4882.4	-
Pooled control	1941.8	-	4859.8	-
1.25	1957.0	-0.8	4861.0	0.0
2.5	1903.5	2.0	4999.5	-2.9
5.0	1693.2	12.8 *	4487.2	7.7
10.0	1516.1	21.9 *	4005.1	17.6 *
20.0	497.4	74.6 *	1896.5	61.0 *

* Statistically significant compared to the pooled control, Dunnett's one-tailed test, $p \leq 0.05$

Negative values indicate an increase of growth.

Conclusion:

72 h E_bC_{50} = 14.5 mg ai/L (95% C.I. = 13.8 – 15.3 mg ai/L)

96 h E_bC_{50} = 17.1 mg ai/L (95% C.I. = 16.4 – 17.8 mg ai/L)

72 h and 96 h E_rC_{50} > 20 mg ai/L

72 h NOEC = 5.0 mg ai/L (growth rate) and 2.5 mg ai/L (biomass)

based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006). The used test species, the saltwater diatom *Skeletonema costatum* is stated in the test guidelines (OECD 201, 2006) as proposed test species.

In general the study is in line with the stated test guidelines. However, the number of replicates used for the control groups is low (3 replicates). According to the OECD test guideline (OECD 201, 2006) the test design should include preferably three replicates at each test concentration and ideally twice that number of controls.

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

In the control cultures the increase of the cell density was determined to be 110 (after 72 h) and 136 (after 96 h).

Based on the statistical analyses the validity criteria are met considering the coefficient of variation in the control groups.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 9%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 1.6%.

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.

Metabolites:

Reference:	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with ethofumesate-NC8493 (AE C508493)
Author(s), year:	Bruns, E., 2012a
Report/Doc. number:	Study no. E 323 4324-1, Reference no. M-436372-01-1
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate-NC8493 (code: AE C508493), batch no. : SES 10116-5-2, purity : 99.8% (analysed)
Test species:	Green algae, <i>Pseudokirchneriella subcapitata</i>
Number of organisms:	1 x 10 ⁴ cells/mL; 3 replicates per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 72 hours

Applied concentrations:

Nominal:	0 (control and solvent control), 0.367, 1.17, 3.76, 12.0 and 38.5 mg/L
Mean measured:	0 (control and solvent control), 0.406, 1.285, 4.335, 13.7 and 42.55 mg/L
Solvent:	Dimethylformamid (DMF), 100 µL/1000 mL

Test conditions:

Water quality:	Nutrient medium according to the OECD guideline
Temperature:	19.8 – 22.1 °C
pH:	7.6 – 8.2
Conductivity:	Not given
Incubation:	Continuous lightning, 6860 – 7350 lux (mean: 7050 lux)
Test parameters:	Morphological examinations of cells using a microscope were made over the exposure period on each study day. Cell numbers per were estimated photometrically. For this purpose, small samples were taken on day 1, day 2, and day 3 of the exposure period. The extinctions were determined at a wave length of 578 nm. The pH was measured at each observation time in all test levels and the control. The temperature was measured continuously. Samples were analysed for the actual concentration of ethofumesate-NC8493 (AE C508493) present in the test medium of all treatment levels and the control on day 0 and 3.
Statistics:	Shown are rounded values, but all calculations were carried out using Microsoft

Excel®. All further statistical evaluations were done using the commercial program ToxRat Professional.

Findings:

Analytical data: The analytical findings of ethofumesate-NC8493 (AE C508493) in the treatment levels found on day 0 were 111 % to 118 % of nominal (average 115 %). On day 3 analytical findings of 98.1 % to 113 % of nominal (average 108 %) were found. All results are based on nominal test concentrations of the metabolite.

Morphological effects: No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-9: Effects of metabolite NC 8493 on the green algae *Pseudokirchneriella subcapitata*

NC 8493 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
Control	634740	1.383	-
Solvent control	646400	1.389	-
Pooled control	640570	1.386	-
0.376	623690	1.378	0.6
1.17	172950	0.950	31.5 *
3.76	91230	0.737	46.8 *
12.0	87290	0.722	47.9 *
38.5	92800	0.743	46.4 *

* Statistically significant compared to the pooled control, William multiple sequential t-Test, $\alpha \leq 0.05$, one-sided smaller

Conclusion:

72 h E_rC_{50} = 20.7 mg ai/L (95% C.I. = 9.23 – 111 mg/L)
 72 h E_yC_{50} = 0.865 mg/L (95% C.I. = 0.411 – 1.048 mg/L)
 72 h NOEC = 0.367 mg/L (yield and growth rate)
 based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006).

In general the study is in line with the stated test guidelines and all validity criteria are met.

The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the cell density increased by a factor of 64 (pooled controls) which is in line with the OECD test guideline.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 15.6%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 0.9%.

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:	Effects of NC 8493 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test under static conditions
Author(s), year:	Juckeland, D., 2013c
Report/Doc. number:	Study no. 13 10 48 014 W, Reference no. IDD00076
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	NC8493, batch no. : EPP/VMV 541.A, purity : 99.9%
Test species:	Green algae, <i>Desmodesmus subspicatus</i>
Number of organisms:	5 x 10 ³ cells/mL; 3 replicates per treatment group and 6 replicates per control group, 1 additional vessel for analysis and the retained specimen (control and treatment group)

Type of test, duration: Static test, 72 hours

Applied concentrations:

Nominal:	0 (control), 1.54, 2.92, 5.54, 10.5 and 20.0 mg/L
Mean measured:	0 (control), 1.33, 2.01, 3.85, 6.91 and 15.1 mg/L
Solvent:	None

Test conditions:

Water quality:	Nutrient medium according to the OECD guideline
Temperature:	23.4 – 24.3 °C
pH:	8.02 – 9.02
Conductivity:	Not given
Incubation:	Continuous lightning, average 109 µE/m ² /s
Test parameters:	At 24, 48 and 72 hours after the start of the test, the biomass (number of cells per mL) in all control and treatment vessels were determined by direct counting (actual microscopic cell count).
	The pH was measured at the beginning and at the end of the test. Measurement of temperature was recorded continuously.

Concentrations of the metabolite NC 8493 test item in the test solutions at the test start (0 h) and at the test end (72 h) were analysed using a HPLC method with UV-detection.

Statistics: Statistical analysis was performed using the software ToxRat Professional 2.10.06.

Findings:

Analytical data: The measured concentrations of NC 8493 in the test solution remained 100% of the nominal values at the start of the test and within a range of 43-76% of the nominal values at the end of the test. Therefore, the toxicity results are based on the mean measured concentrations.

Morphological effects: No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-10: Effects of metabolite NC 8493 on the green algae *Desmodesmus subspicatus* - growth rate

NC 8493 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
Control	567900	1.577	-
1.54	563300	1.575	0.2
2.92	200800	1.231	22.0 *
5.54	50000	0.764	51.6 *
10.5	30000	0.591	62.5 *
20.0	14200	0.346	78.1 *

* Statistically significant compared to the pooled control, Welch t-Test, $\alpha \leq 0.05$, one-sided

Table B. 9.2.7-11: Effects of metabolite NC 8493 on the green algae *Desmodesmus subspicatus*- yield

NC 8493 [mg/L] (nominal)	Yield (0-72 h)	% inhibition of growth rate compared to control
Control	562900	-
1.54	558300	0.8
2.92	195800	65.2 *
5.54	45000	92.0 *
10.5	25000	95.6 *
20.0	9200	98.4 *

* Statistically significant compared to the pooled control, Williams t-test, $\alpha \leq 0.05$, one-sided

The sensitivity of the test system was verified by testing the reference item potassium dichromate. The EC₅₀ values for the growth rate and yield were determined to be 1.16 mg/L (growth rate) and 0.45 mg/L (yield).

Conclusion:

72 h E_rC₅₀ = 4.83 mg/L (95% C.I. = 4.08 – 5.79 mg/L)

72 E_rC₁₀ = 1.19 mg/L (95% C.I. = 0.75 – 1.61 mg/L)

72 h E_yC_{50} = 1.87 mg/L (95% C.I. = 1.81 – 1.94 mg/L)

72 h E_yC_{10} = 1.43 mg/L (95% C.I. = 1.32 – 1.51 mg/L)

72 h NOEC = 1.33 mg/L (yield and growth rate)

based on mean measured concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006).

In general the study is in line with the stated test guidelines and all validity criteria are met.

The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the cell density increased by a factor of 114 which is in line with the OECD test guideline.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 18.2%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 0.5%.

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:	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with BCS-AV65501 – limit test
Author(s), year:	Bruns, E., 2012b
Report/Doc. number:	Study no. E 323 4036-1, Reference no. M-437568-02-1
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

<u>Material and methods:</u>	
Test substance:	BCS-CU88901, technical sodium-salt of BCS-AV65501 (= NC 20645), batch no. : SES 11754-3-8, purity : 69.2% (analysed)
Test species:	Green algae, <i>Pseudokirchneriella subcapitata</i>
Number of organisms:	1 x 10 ⁴ cells/mL; 6 replicates per treatment group and per control group
Type of test, duration:	Static test, 72 hours
<u>Applied concentrations:</u>	
Nominal:	0 (control and solvent control) and 10.0 mg/L
Mean measured:	- (control and solvent control) and 9.675 mg/L
Solvent:	Dimethylformamid (DMF)
<u>Test conditions:</u>	
Water quality:	Nutrient medium according to the OECD guideline
Temperature:	19.7 – 21.5 °C (mean: 20 °C)
pH:	7.8 – 7.9
Conductivity:	Not given
Incubation:	Continuous lightning, 6860 - 7540 lux (mean: 7206 lux)
Test parameters:	Morphological examinations of cells using a microscope were made over the exposure period on each study day. Cell numbers per were estimated photometrically. For this purpose, small samples were taken on day 1, day 2, and day 3 of the exposure period. The extinctions were determined at a wave length of 578 nm. The pH was measured at each observation time in all test levels and the control. The temperature was measured continuously. Samples were analysed for the actual concentration of the test substance present in the test medium of all treatment levels and the control on day 0 and 3.
Statistics:	Shown are rounded values, but all calculations were carried out using Microsoft Excel®. All further statistical evaluations were done using the commercial program ToxRat Professional.

Findings:

Analytical data: The analytical finding of BCS-AV65501 in the treatment level found on day 0 and 3 was 97 % of nominal. All results are based on nominal test concentrations of the metabolite.

Morphological effects: No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-12: Effects of the metabolite NC 20645 on the green algae *Pseudokirchneriella subcapitata*

NC 20645 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
Control	483210	1.292	-
Solvent control	479390	1.289	-
Pooled control	481300	1.291	-
10.0	466080	1.280	0.8
72 h EC ₅₀ > 10 mg/L (yield and growth rate) NOEC = 10.0 mg/L (yield and growth rate)			

* Statistically significant compared to the pooled control, William multiple sequential t-Test, $\alpha \leq 0.05$, one-sided smaller

Conclusion:

72 h EC₅₀ > 10 mg/L (yield and growth rate)
72 h NOEC = 10 mg/L (yield and growth rate)
based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006).

In general the study is in line with the stated test guidelines and all validity criteria are met.

The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the cell density increased by a factor of 48 which is in line with the OECD test guideline.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 20.9%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 1.4%.

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:	Effects of NC 20645 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test under static conditions
Author(s), year:	Juckeland, D., 2013d
Report/Doc. number:	Study no. 13 10 48 029 W, Reference no. IDD00075
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	NC20645, batch no. : EPP/RH1079.1, purity : 95.6% (analysed)
Test species:	Green algae, <i>Desmodesmus subspicatus</i>
Number of organisms:	5 x 10 ³ cells/mL; 3 replicates per treatment group and 6 replicates per control group, 1 additional vessel for analysis and the retained specimen (control and treatment group)
Type of test, duration:	Static test, 72 hours

Applied concentrations:

Nominal:	0 (control), 1.79, 4.11, 9.45, 21.7 and 50.0 mg/L
Mean measured:	0 (control), 1.25, 3.02, 8.01, 20.8 and 48.5 mg/L
Solvent:	None

Test conditions:

Water quality:	Nutrient medium according to the OECD guideline
Temperature:	21.8 – 24.0 °C
pH:	8.04 – 8.76
Conductivity:	Not given
Incubation:	Continuous lightning, average 108 µE/m ² /s
Test parameters:	At 24, 48 and 72 hours after the start of the test, the biomass (number of cells per mL) in all control and treatment vessels were determined by direct counting (actual microscopic cell count). The pH was measured at the beginning and at the end of the test. Measurement of temperature was recorded continuously. Concentrations of the metabolite NC 8493 test item in the test solutions at the test start (0 h) and at the test end (72 h) were analysed using a HPLC method with UV-detection.
Statistics:	Statistical analysis was performed using the software ToxRat Professional 2.10.06.

Findings:

Analytical data:	The measured concentrations of NC 20645 in the test solution remained within a
------------------	--

range of 72 – 101% of the nominal values at the start of the test and within a range of 74-102% of the nominal values at the end of the test. Therefore, the toxicity results are based on the mean measured concentrations.

Morphological effects: No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-13: Effects of metabolite NC 8493 on the green algae *Desmodesmus subspicatus* - growth rate

NC 20645 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
Control	479600	1.521	-
1.79	491700	1.529	- 0.5
4.11	338300	1.404	7.7 *
9.45	256700	1.311	13.8 *
21.7	151700	1.137	25.3 *
50.0	49200	0.759	50.1 *

* Statistically significant compared to the pooled control, Welch t-Test, $\alpha \leq 0.05$, one-sided

Negative values for % inhibition indicate an increase in growth relative to the control

Table B. 9.2.7-14: Effects of metabolite NC 8493 on the green algae *Desmodesmus subspicatus*- yield

NC 20645 [mg/L] (nominal)	Yield (0-72 h)	% inhibition of growth rate compared to control
Control	474600	-
1.79	486700	- 2.5
4.11	333300	29.8 *
9.45	251700	47.0 *
21.7	146700	69.1 *
50.0	44200	90.7 *

* Statistically significant compared to the pooled control, Williams t-test, $\alpha \leq 0.05$, one-sided

Negative values for % inhibition indicate an increase in growth relative to the control

The sensitivity of the test system was verified by testing the reference item potassium dichromate. The EC_{50} values for the growth rate and yield were determined to be 1.16 mg/L (growth rate) and 0.45 mg/L (yield).

Conclusion:

72 h E_rC_{50} = 52.4 mg/L (95% C.I. = 37.7 – 95.3 mg/L)

72 E_rC_{10} = 6.22 mg/L (95% C.I. = 2.24 – 10.1 mg/L)

72 h E_yC_{50} = 8.83 mg/L (95% C.I. = 5.08 – 15.3 mg/L)

72 E_yC_{10} = 1.43 mg/L (95% C.I. = 0.21 – 2.95 mg/L)

72 h NOEC = 1.25 mg/L (yield and growth rate)

based on mean measured concentrations

<u>Comment RMS:</u>	<p>The study was conducted according to the OECD test guideline (OECD 201, 2006).</p> <p>In general the study is in line with the stated test guidelines and all validity criteria are met.</p> <p>The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the cell density increased by a factor of 96 which is in line with the OECD test guideline.</p> <p>The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 23.7%.</p> <p>The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 0.6%.</p> <p>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.</p>
----------------------------	---

Reference:	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with BCS-CW35117
Author(s), year:	Sobczyk, H., 2013
Report/Doc. number:	Study no. E 323 4457-8, Reference no. M-459906-01-1
Guideline(s):	OECD 201 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	BCS-CW35117, batch no. : SES 12013-7-3, purity : 91% (analysed)
Test species:	Green algae, <i>Pseudokirchneriella subcapitata</i>
Number of organisms:	1 x 10 ⁴ cells/mL; 3 replicates per treatment group and 6 replicates per control group
Type of test, duration:	Static test, 72 hours
<u>Applied concentrations:</u>	
Nominal:	0 (control), 6.25, 12.5, 25.0, 50.0 and 100 mg/L
Mean measured:	-(control), 6.795, 13.4, 26.75, 53.25 and 106 mg/L
Solvent:	None

Test conditions:

Water quality:	Nutrient medium according to the OECD guideline
Temperature:	21.5 – 22.7 °C (mean: 21.7 °C)
pH:	7.4 – 8.2
Conductivity:	Not given
Incubation:	Continuous lightning, 5790 - 6220 lux (mean: 5972 lux)
Test parameters:	Morphological examinations of cells using a microscope were made over the exposure period on each study day. Cell numbers per were estimated photometrically. For this purpose, small samples were taken on day 1, day 2, and day 3 of the exposure period. The extinctions were determined at a wave length of 578 nm. The pH was measured at each observation time in all test levels and the control. The temperature was measured continuously. Samples were analysed for the actual concentration of the test substance present in the test medium of all treatment levels and the control on day 0 and 3.
Statistics:	Shown are rounded values, but all calculations were carried out using Microsoft Excel®. All further statistical evaluations were done using the commercial program ToxRat Professional.

Findings:

Analytical data:	The analytical findings of BCS-CW35117 in the treatment levels found on day 0 were 106 % to 108 % of nominal (average 107 %). On day 3 analytical findings of 106 % to 110 % of nominal (average 108 %) were found. All results are based on nominal test concentrations of the metabolite.
Morphological effects:	No physical abnormalities were observed in the controls or treatment groups during the study.

Table B. 9.2.7-15: Effects of the metabolite BCS-CW35117 on the green algae *Pseudokirchneriella subcapitata*

BCS-CW35117 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
Control	867000	1.487	-
6.25	942000	1.515	-1.9
12.5	932000	1.511	-1.6
25.0	904000	1.501	-1.0
50.0	744000	1.436	3.4*
100.0	434000	1.257	15.5*
72 h $E_rC_{50} > 100$ mg/L 72 h $E_rC_{10} = 79.9$ mg/L (95% C.I. = 77.9 – 81.7 mg/L) 72 $E_yC_{50} = 98.98$ mg/L (95% C.I. = 96.22 – 102.06 mg/L) 72 $E_yC_{10} = 44.2$ mg/L (95% C.I. = 41.4 – 46.7 mg/L)			

BCS-CW35117 [mg/L] (nominal)	Mean cell number per mL (72 h)	Average specific growth rates per day (0-72h)	% inhibition of growth rate compared to control
NOEC = 25.0 mg/L (yield and growth rate)			

* Statistically significant compared to the pooled control, William multiple sequential t-Test, $\alpha \leq 0.05$, one-sided smaller

Conclusion:

72 h $E_rC_{50} > 100$ mg/L

72 $E_yC_{50} = 98.98$ mg/L (95% C.I. = 96.22 - 102.06 mg/L)

72 h NOEC = 25 mg/L (yield and growth rate)

based on nominal concentrations

Comment RMS:

The study was conducted according to the OECD test guideline (OECD 201, 2006).

In general the study is in line with the stated test guidelines and all validity criteria are met.

The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. This corresponds to a specific growth rate of 0.92 per day. In the study the cell density increased by a factor of 87 which is in line with the OECD test guideline.

The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 30.3%.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 1.9%.

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.

B.9.2.8. Effects on aquatic macrophytes

For the first EU approval of the active substance ethofumesate laboratory studies with the aquatic macrophyte *Lemna sp.* were submitted. In addition to the *Lemna* studies a second aquatic macrophyte has to be tested according to the new data requirement for active substances (Commission Regulation (EU) 283/2013). Hence, laboratory studies with the aquatic macrophytes *Myriophyllum spicatum* were submitted.

The study summaries are given below.

Reference:	<i>Lemna minor</i>: Semi static phytotoxicity test
Author(s), year:	Scheerbaum, D., 1998
Report/Doc. number:	Study no.: A91865, Reference no.: M-168516-01-1
Guideline(s):	ASTM guideline E 1415-91 (1991)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate technical, batch no.: 28/02/98, purity: 98.8% (analysed), CAS no.: 26225-79-6
Test species:	<i>Lemna minor</i> , duckweed (floating aquatic plant)
Number of organisms:	3 replicates per controls and treatments, 3 uniform healthy looking plants with 4 fronds each per replicate
Type of test, duration:	Semi-static with renewal of the test media on days 0, 3, 5, 7, 10 and 12, duration of the test 14 days

Applied concentrations:

Nominal:	Not given
Measured (mean):	0 (control), 0.76, 1.9, 4.3, 10.0, 22.3 and 52.8 mg ai/L
Solvent:	None

Test conditions:

Water quality:	20X-AAP medium according to the guideline, pH 7.5 ± 0.1
Temperature:	25 ± 2 °C
pH:	7.21 – 8.82
O ₂ content:	Not given
Light regime:	Continuous light, mean light intensity 7348 lux (range: 6379 – 8302 lux)
Test parameters:	The amounts of plants (day 0) and fronds, respectively were determined on days 0, 3, 5, 7, 10, 12 and 14 days. Every frond that visibly projected beyond the edge of a parent frond was counted as a separate frond. Fronds that lost their pigmentation were not counted. Observations of change in colour, break-up of plants and destructions of roots

were made on days 3, 5, 7, 10, 12 and 14.

pH-values were measured on days 3, 5, 7, 10, 12 and 14. The room temperature in the test chamber was measured and recorded continuously.

Light intensity was determined before the test started.

Analytical measurements: Sampling and analysis of test concentration were carried out on days 0, 3 and 7 (freshly prepared media) and on days 3, 5 and 10 (2 and 3 d old test media). All test concentrations and control replicates were analysed (HPLC analyses).

Statistics: EC₅₀-value of biomass inhibition after 14 days was calculated by probit analysis. NOEC-values were determined by calculation of statistical analyses significance using one way analysis of variance (ANOVA) and Dunnett's test for biomass areas and growth rates, respectively.

When running a one way analysis of variance a normality test and an equal variance test were done first. The Kolgomorov-Smirnov-Test was used to test for normally distributed populations.

Findings:

Morphological findings: At test concentrations between 10 and 58.2 mg ai/L morphological effects were observed, i.e. smaller fronds and roots as well as clumpy fronds.

Recovery: After 14 d plants were transferred from any treatment where growth was inhibited by more than 50 % to fresh medium and allowed for growth for a further 7 d to determine whether the effect of the substance was reversible.

After 14 d the Lemna plants were transferred from the highest tested concentration level (52.8 mg/L) and control replicates to untreated test medium and allowed to grow for a further 7 d under test conditions. The test substance effect was observed to be reversible. A distinct increase of frond number was observed. Fronds and roots were similar to control plants.

Table B. 9.2.8-1: Mean yield for plant shoots, wet and dry weights

Ethofumesate [mg/L] (mean measured)	Frond number at day 14	Mean growth rate		Mean biomass integrals	
		Per day (at Day 14)	% inhibition	At day 14	% inhibition
Control	540	0.27	-	1974.5	-
0.76	522	0.27	0.89 (± 3.28)	1874.5	5.06 (± 11.9)
1.9	483	0.26	2.93 (±1.84)	1704.5 *	13.67 (± 5.0)
4.3	473	0.26	3.48 (± 1.37)	1719.0	12.94 (± 4.6)
10.0	415	0.25 *	6.92 (± 0.29)	1534.0 *	22.31 (± 1.89)
22.3	374	0.25 *	9.65 (± 1.35)	1374.5 *	30.39 (± 3.16)
52.8	199	0.20 *	26.22 (± 1.96)	920.5 *	53.38 (± 3.35)

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

Conclusion:

14 d $E_rC_{50} > 52.8$ mg ai/L
 14 d $E_bC_{50} = 50.4$ mg ai/L (95% C.I. = 8.3 – 306.4 mg ai/L)
 14 d NOEC = 4.3 mg ai/L (biomass and growth rate)
 Based on mean measured concentrations

Comment RMS:

The study was conducted according to the ASTM guideline E 1415-91 (1991). The study was conducted according to the validity criteria given in the ASTM guideline. The number of fronds should increase 5-fold within 7 days. In the study the frond number increased 7.7 fold within days 0 to 7.

The test temperature was stable (did not vary more than 4 °C). The frond and plant numbers were the same in all replicates at the beginning of the test.

The study also fulfils the validity criteria given in the current valid test guideline, OECD test guideline 221 (2006).

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

The mean growth rate in the control was determined to be 0.29 after 7 days. The factor of frond number, measured in the control between 0 and 7 days, was 45.

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:	A 7-Day aquatic plant toxicity test using <i>Lemna minor</i> with ethofumesate
Author(s), year:	Bogers, M., 2001
Report/Doc. number:	Study no.: 324078, Reference no.: IDD00077
Guideline(s):	ISO guideline (2000) and draft OECD guideline (1999)
GLP:	Yes
Deviations:	- On day 6 temperature in the incubator peaked during a short period to 32°C, but this had no effect on the temperature in the medium.
Validity:	Acceptable

Material and methods:

Test substance: Ethofumesate technical, batch no.: EFS-106, purity: 98.93% (analysed), CAS no.: 26225-79-6

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

Number of organisms: 3 replicates per controls and treatments, 4 plants with a total of 10 fronds per vessel

Type of test, duration: Semi-static with renewal of the test media on days 2 and 5, duration of the test 7 days

Applied concentrations:

Nominal: 0 (control and solvent control), 10, 18, 32, 56 and 100 mg ai/L

Measured (mean): 0 (control and solvent control), 14, 17, 26, 39 and 42 mg ai/L

Solvent: Acetone, 0.1 mL/L

Test conditions:

Water quality: SIS-medium according to the OECD guideline

Temperature: 24.5 – 25 °C

pH: 6.1 – 7.1

O₂ content: Not given

Light regime: Continuous light, light intensity 93 – 115 µE/m²/s

Test parameters: Frond numbers were counted at the start, after 2 and 5 days, and at the end of the 7-day test. Fronds were observed for lesions, chlorosis, gibbosity or necrosis at the start, after 2 and 5 days and at the end of the test.

After completing the weighting, fronds were homogenized using liquid nitrogen. Extraction of chlorophyll was performed and the filtrates were measured at 470, 646 and 663 nm using a spectrophotometer.

pH was measured at the beginning, at each renewal and at the end of the test in all vessels per concentration. The temperature was measured every day in a vessel without plants.

Analytical measurements: Samples for analytical measurements were taken at the start of the test and at the end of a 48 h (day 2, spent and fresh) and a 72 h (day 5, spent) period between the renewals. Singular samples were taken from three concentrations, i.e. 10, 32 and 100 mg ai/L, and the control for analyses.

Statistics: The results for the most sensitive parameter were tested for significance using the ANOCA-Tukey HSD and Dunnet t-test (software: SAS v. 6.12).

Findings:

Analytical data: The mean measured concentrations were in range of 13 – 144%. The initial concentrations were only incidentally in agreement with the nominal prepared concentrations. At nominal concentrations of 32, 56 and 100 mg ai/L, the initial concentrations were significantly below the nominal concentrations. The concentrations measured at 100 and 56 mg ai/L did not exceed 50 mg/L due to the low solubility of the active substance.

Hence, the results of the study are based on mean measured concentrations.

Morphological findings: Morphological effects (discoloured fronds) were observed at the two highest test concentrations, i.e. 39 and 42 mg ai/L.

Photosynthetic pigments: The contents of the pigments were equal or higher in the test substance treated solutions up to and including 17 mg ai/L. The test concentrations related decrease

in pigment contents at the higher levels follows the same trend for each of the different pigments (chlorophyll a and b, carotene and xanthophyll). Reduction of pigments remained between 30 and 40% at the highest test concentration.

Table B. 9.2.8-2: Mean growth rate and biomass

Ethofumesate [mg/L] (mean measured)	FronD number at day 7	Mean growth rate		Biomass (wet weight)	
		0-7 d	% inhibition relative to the control ^b	Mean wet weight [mg]	% inhibition relative to the control ^b
Control	127	0.3612	-0.2	0.2250	91
Solvent control	126	0.3605	-	0.2467	-
14	112 ^a	0.3445	4.4	0.2008	81
17	103	0.3303	8.8	0.1826	74
26	95	0.3215	10.8	0.1446 *	59
39	76 ^a	0.2873 *	20.3	0.0934 *	38
42	70 ^a	0.2780 *	22.9	0.1100 *	45

* Statistically significant difference from control, ANOVA –Tukey HSD and Dunnett's t-test, $p \leq 0.05$

^a Less than 3% of the total number of fronds was discoloured at the end of the test

^b Compared to the mean value of the treatment control (solvent control).

Conclusion:

7 d $E_t C_{50} > 42$ mg ai/L

7 d $E_t C_{10} = 20$ mg ai/L (95% C.I. = 8.6 – 48 mg ai/L)

$NOE_t C = 26$ mg ai/L

7 d $E_b C_{50} = 35$ mg ai/L (95% C.I. = 14 – 86 mg ai/L)

7 d $E_b C_{10} = 8.8$ mg ai/L (95% C.I. = 3.1 – 25 mg ai/L)

$NOE_b C = 17$ mg ai/L

Comment RMS:

The study was conducted according to the ISO test guideline (2000) and the draft OECD test guideline 221 (1999).

The validity criteria stated in the ISO and draft OECD guideline are in line with the current valid OECD test guideline 221 (2006).

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

The mean growth rate in the controls was determined to be 0.36 after 7 days. The factor of frond number, measured in the control between 0 and 7 days, was 12.

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 ethofumesate technical to the aquatic macrophytes, <i>Myriophyllum spicatum</i> (amended final report)
Author(s), year:	Banman, C.S., 2013
Report/Doc. number:	Study no.: EBADL019-1, Reference no.: M-411454-02-1
Guideline(s):	Higher tier study based on OECD 221 (2006)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate technical, batch no.: ABJJETN023, purity: 98.3%, CAS no.: 26225-79-6
Test species:	<i>Myriophyllum spicatum</i> (Eurasian water milfoil), rooted macrophytes
Number of organisms:	3 replicates per controls and treatments, 4 plants per replicate
Type of test, duration:	Static, 14 days
<u>Applied concentrations:</u>	
Nominal:	0 (control and solvent control), 0.048, 0.153, 0.488, 1.56 and 5.0 mg ai/L
Measured (mean):	0 (control and solvent control), 0.036, 0.115, 0.375, 1.28 and 4.13 mg ai/L
Solvent:	Acetone, CAS no.: 0.025 mL/L

Test conditions:

Water quality:	Hard processed water (spring water blended with reverse osmosis water)
Sediment:	Sediment comprised of clay, sand and peat moss according OECD 218. Instead of adding distilled water to wet the sediment, 20 XAAP media was used to provide shoots with a fertiliser source.
Temperature:	19.95 – 21.07°C
pH:	8.2 - 8.7 (Day 0), 9.5 - 9.8 (Day 14)
O ₂ content:	10.6 – 11.0 (Day 0), 11.2 – 11.5 (Day 14)
Light regime:	16 hours light, 8 hours dark, light intensity 10030 – 12490 lux (mean = 11460 lux)
Methods:	Shoots (length of 7 cm) within a replicate are planted in sediment within a 650 mL borosilicate glass crystallisation dish housed in a 4-L glass beaker. After an acclimation period of 7 days, the plants were exposed to the test solution for 14 days. All test vessels were contained in an environmentally controlled study area.
Test parameters:	Temperature was measured hourly via a calibrated probe and daily manual records via a calibrated thermometer. pH and dissolved oxygen were measured at Day -7, 0, 7 and 14. Wet and dry weight as well as shoot length was measured on Day 0 and Day 14.
Analytical measurements:	On Days 0, 7 and 14 samples for analytical verification were taken. The samples were analysed using LC-MS/MS.
Statistics:	The statistical analyses were conducted using the software CETIS. The following

statistical tests were used:

Normality: Shapiro-Wilks Test

Homogeneity of variance: Bartlett Equality of Variance

NOEC determination: ANOVA followed by the Dunnett's Test

EC_x estimates: Linear interpolation (ICPIN) and nonlinear regression

Findings:

Analytical data: The mean measured concentrations were determined to be between 74 and 83% of the nominal test concentrations. Hence, the effect levels are based on mean measured test concentrations.

Table B. 9.2.8-3: Mean yield for plant shoots, wet and dry weights

Mean measured concentration [mg ai/L]	Length (Day 14)		Wet weight (Day 14)		Dry weight (Day 14)	
	[cm]	% inhibition ¹	[g]	% inhibition ²	[g]	% inhibition ³
Control	22.8	-	0.8405	-	0.1403	-
Solvent control	23.5	-	0.9792	-	0.1264	-
Pooled control	23.2	-	0.9098	-	0.1333	-
0.036	22.3	3.6	1.0366	-13.9	0.1276	4.3
0.115	14.9	35.8*	0.7459	18.0	0.0976	26.8* ⁴
0.375	9.0	61.2*	0.7350	19.2	0.1342	-0.7
1.28	4.6	80.0*	0.6980	23.3	0.1333	0.0
4.13	2.9	87.6*	0.6489	28.7	0.1352	-1.4
Length: E _y C ₅₀ = 0.25 mg ai/L (95% CI: 0.128-0.348 mg ai/L), NOEC = 0.036 mg ai/L Wet weight: E _y C ₅₀ > 4.13 mg ai/L, NOEC = 4.13 mg ai/L Dry weight: E _y C ₅₀ > 4.13 mg ai/L, NOEC = 4.13 mg ai/L						

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

¹ Based on a mean shoot length of 10.5 cm at the start of the test (Day 0)

² Based on a mean wet weight of 0.5009 g at the start of the test (Day 0)

³ Based on a mean dry weight of 0.1261 g at the start of the test (Day 0)

⁴ For the dry weight endpoint, the data did not follow a monotonic dose response trend. The statistically significant effect at the 0.115 mg ai/L test level is not considered to be biologically significant.

Table B. 9.2.8-4: Growth rates for plant shoots, wet and dry weights

Mean measured concentration [mg ai/L]	Length (Day 14)		Wet weight (Day 14)		Dry weight (Day 14)	
	[cm ⁻¹]	% inhibition ¹	[g ⁻¹]	% inhibition ²	[g ⁻¹]	% inhibition ³
Control	0.0825	-	0.0695	-	0.0532	-
Solvent control	0.0841	-	0.0773	-	0.0496	-
Pooled control	0.0833	-	0.0734	-	0.0514	-
0.036	0.0814	2.2	0.0799	-8.8	0.0499	2.9
0.115	0.0629	24.4*	0.0637	13.2	0.0403	21.6* ⁴
0.375	0.0443	46.9*	0.0644	12.2	0.0517	-0.6
1.28	0.0262	68.6*	0.0622	15.3	0.0514	-0.1
4.13	0.0173	79.2*	0.0613	16.6	0.0520	-1.3

Mean measured concentration	Length (Day 14)	Wet weight (Day 14)	Dry weight (Day 14)
Length: $E_rC_{50} = 0.479$ mg ai/L (95% CI: 0.249-0.642 mg ai/L), NOEC = 0.036 mg ai/L Wet weight: $E_rC_{50} > 4.13$ mg ai/L, NOEC = 4.13 mg ai/L Dry weight: $E_rC_{50} > 4.13$ mg ai/L, NOEC = 4.13 mg ai/L			

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

¹ Based on a mean shoot length of 10.5 cm at the start of the test (Day 0)

² Based on a mean wet weight of 0.509 g at the start of the test (Day 0)

³ Based on a mean dry weight of 0.1261 g at the start of the test (Day 0)

⁴ For the dry weight endpoint, the data did not follow a monotonic dose response trend. The statistically significant effect at the 0.115 mg ai/L test level is not considered to be biologically significant.

Conclusion:

The lowest E_yC_{50} and E_rC_{50} in the 14 d exposure of ethofumesate technical to the rooted macrophytes *Myriophyllum spicatum* was shoot length. The statistical EC_{50} for this endpoint was 0.25 mg ai/L (based on yield) and 0.479 mg ai/L (based on growth rate).

Comment RMS:

The study was conducted according to the OECD test guideline 221 (*Lemma* growth inhibition test).

A draft OECD test guideline "Water-sediment *Myriophyllum spicatum* toxicity test" was published in 2013. Even though the test guideline is available as a draft version only, the given validity criteria were used for the evolution of the study. According to the draft OECD guideline the study is considered valid if the following points are met:

- The mean total shoot length and mean shoot fresh weight in control plants must at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures must not exceed 35% between replicates.

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

B.9.2.9. Further testing on aquatic organisms

Reference:	Effects of ethofumesate technical on new shell growth in the Eastern oyster (<i>Crassostrea virginica</i>) under flow-through test conditions
Author(s), year:	Yurk, J.J. and Ache, B.W., 1992
Report/Doc. number:	Study no. A83386, Reference no. M-155654-01-1
Guideline(s):	USE EPA - FIRA CFR 40 – Series 72-3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., CAS no. 26225-79-6, batch no. CR19291/2, purity: 97.0%
Test species:	Eastern oyster (<i>Crassostrea virginica</i>), 25 – 50 mm in length at test start
Number of organisms:	20 organisms per treatment and control groups
Type of test, duration:	Flow-through, 96 hours
Feeding:	Natural algal supplement, four times per day

Applied concentrations:

Nominal:	0 (control and solvent control), 1.3, 2.2, 3.6, 6.0 and 10 mg ai/L
Mean measured:	- (control and solvent control), 0.81, 2.0, 3.1, 5.6 and 9.0 mg ai/L
Solvent:	Dimethylformamid (DMF)

Test conditions:

Water quality:	Unfiltered seawater, pH = 8.14, alkalinity: 111 mg/L as CaCO ₃
Temperature:	24 ± 1 °C (range: 23.6 – 24.9 °C)
pH:	8.0 – 8.1
O ₂ content:	5.5 – 6.8 mg/L (> 60% air saturation)
Salinity:	32 – 35 ‰
Light regime:	16 hours light / 8 hours darkness
Test system:	<p>The test vessels were 15.4 L rectangular, glass chambers filled to a depth of 7.6 cm with approximately 7.8 L of dilution water or test solution. The exposure system was a continuous flow diluter system, with regulated dilution water and test solution flows adjusted to achieve the desired test concentrations.</p> <p>Prior to test start, the periphery of the shell margin of each oyster was ground (approximately 1-2 mm) with a fine grit grinder in order to establish a baseline for new shell growth.</p>
Test parameter:	<p>Test organisms were observed daily for mortality and any behavioural changes. Mortality was defined as the inability to close the shell on gentle prodding. An additional effect criterion determined was new shell growth, measured with vernier calipers at test termination. New shell growth was defined as the length of</p>

the longest finger of growth on the peripheral shell margin.

During the test, monitoring of water quality parameters included: daily measurement of temperature, pH and dissolved oxygen concentrations in the control and each test solution until test termination or until 100% mortality had occurred.

Analytical measurements: On days 0 and 4, concentrations of the test material were determined in samples collected from all test vessels by liquid chromatography.

Statistics: Statistical analyses of the shell growth data were performed using the mean measured concentrations of test material in the test solutions. A 96 hour EC₅₀ value and 95% confidence limits were determined by a computer program, using the following statistical methods: moving average angle, Probit analysis and non-linear interpolation.

To determine a no observed effect concentration (NOEC), statistical significance ($p < 0.05$) between the combined control and treatment new shell growth data were also evaluated using the Dunnett's test.

Findings:

Analytical measurements: Mean measured concentrations of ethofumesate were determined to be between 62 and 93% of nominal concentrations. Hence, the results of the study are based on mean measured concentrations.

Biological effects: No mortalities of eastern oyster exposed to the active substance ethofumesate were observed throughout the test duration. Only at the highest test concentration (9.0 mg ai/L) 2 animals out of 20 died (corresponding to 10%).

Table B. 9.2.9-1: New shell growth in eastern oyster

Ethofumesate [mg ai/L] (mean measured)	Mean new shell growth [mm] ^a	% reduction relative to the pooled control
Control	2.06 ± 0.74	-
Solvent control	1.81 ± 0.67	-
0.81	1.51 ± 0.38	22 *
2.0	0.68 ± 0.71 ^b	65 *
3.1	1.28 ± 0.63 ^b	34 *
5.6	0.18 ± 0.37 ^b	91 *
9.0	0.0 ± 0.0 ^b	100 *

* Statistically significant compared to the controls based on Dunnett's test ($p < 0.05$)

^a Only those organisms with discernible new shell growth (≥ 0.1 mm) are listed. All 20 values were used to calculate the mean shell growth and the standard deviation values.

^b Not all of the 20 organisms show a new shell growth.

Conclusion:

96 EC₅₀ = 1.7 mg ai/L (95% C.I. = 0.81 – 5.6 mg ai/L) based on new shell growth

96 h LC₅₀ > 9.0 mg ai/L

NOEC < 0.81 mg ai/L based on new shell growth

NOEC = 5.6 mg ai/L based on survival

Comment RMS:

The study was conducted according to the US EPA test guideline, series 72-3.

The study protocol is in line with the draft test guideline according US EPA (OPPTS 850.1025, 1996). The validity criteria outlined in the draft test guideline US EPA (1996) were considered to evaluate the validity of the results of the study.

The mortality in the controls should not exceed 10% at the end of the test. During the whole study period no mortality in the controls was observed.

The dissolved oxygen concentration should be at least 60% (being: > 60%).

No information on spawning was given in the study report. Hence, it can be assumed that no spawning was observed during the whole study period.

The concentration of the test substance was maintained over the test period.

The environmental conditions (temperature, dissolved oxygen, salinity and pH) were measured at the beginning and at the end of the test in each replicate.

In the controls a minimum of 2 mm of new shell growth should be observed (being: 1.1 – 4.0 mm).

The last validity criterion was not met in the study. The new shell growth in the controls was between 1.1 and 4 mm with mean values of 2.06 mm (control) and 1.81 mm (solvent control).

Even though the validity criterion consider new shell growth was not met in the study, the results of the study are considered acceptable.

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

From searching peer-reviewed literature published over the last 10 years prior to submission one publication was obtained, which needs further consideration (exposure monitoring including an aquatic risk assessment relating the determined concentration in the environmental compartment with a laboratory measured effect concentration). A summary is provided below.

Reference:	Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in northeastern Greece.
Author(s), year:	Papadopoulou-Mourkidou, E., Vassiliou, G., Vryzas, Z., Alexoudis, C. and Galanis, K., 2010
Report/Doc. number:	Reference no. M-458635-01-1
GLP:	No

Executive summary:

Monitoring data is presented for pesticide loading in the drainage canals of two transboundary rivers of northeastern Greece near the Greek/Bulgarian/Turkish borders during 1999 and 2008.

Ethofumesate was found at low concentrations ranging around a median of 0.0936 µg/L (extreme residue level of 0.336 µg/L) during the monitoring period from 1999 through 2008. In these years, totally 12 herbicides, 14 insecticides and 7 fungicides were measured at highest median concentrations of up to 0.286 µg/L for atrazine (highest extreme residue level of 9.8 µg/L for cypermethrin).

In addition, an environmental risk assessment was conducted on the detected pesticides based on ecotoxicological endpoints derived from secondary sources (i.e. the FOOTPRINT PPDB database). The reported endpoints for algae, aquatic invertebrates and fish correspond to EU-agreed endpoints according to EFSA review report SANCO/6503/VI/99-final (2002). Ethofumesate risk assessment was conducted with a toxicity level to aquatic invertebrates of 320 µg/L and an assessment factor of 10 resulting in a safe risk quotient (RQ) well below the trigger of 1 (RQ_{median} = 0.003 and RQ_{extreme} = 0.01).

The Risk Quotients (RQ = residue level / lowest ecotoxicological endpoint) show that ethofumesate poses no risk to aquatic organisms neither at median nor at extreme concentrations.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute toxicity to adult honeybees

Studies on the acute oral and contact toxicity of the ethofumesate were already submitted for the first EU approval of the active substance. In addition, new acute oral and contact toxicity studies were submitted addressing the acute risk to adult honey-bees.

Reference:	The acute oral and topical toxicities of ethofumesate to worker honeybees (<i>Apis mellifera</i> L.)
Author(s), year:	Barrett, K.L., 1991
Report/Doc. number:	Study no.: A83374, Reference no.: M-155642-01-1
Guideline(s):	EPA guideline (EPA-540/9-85-002, 1985)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate tech., Batch no.: R000047, Content : 99.9% w/w
Reference:	None
Solvent:	Acetone
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Acute oral and contact toxicity test
Number of organisms:	Five replicates with 10 bees for control and the test item treatment groups The study (acute oral and contact) was run twice using worker honeybees from different hives to verify the results obtained.
Food:	50% sucrose solution
Oral toxicity test:	
Applied concentrations:	Control: 50% (w/w) aqueous sucrose solution with solvent Test item: 0.05, 0.5, 5.0 and 50 µg ai/bee
Exposure route:	The test item was dissolved in acetone and a 50% sucrose solution and offered to the bees. Once the treatment had been consumed (after approximately 2 hours), the feeder was removed and untreated 50% sucrose solution fed ad libitum for the duration of the study.
Test conditions:	Temperature: 25 °C ± 2°C, Relative humidity: 43 - 64 %, Darkness (except during observation)
Test parameter:	Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive

cleaning, vomiting) were made after exposure for 18, 24, 36 and 48 h.

Contact toxicity test:

Applied concentrations: Control: 50% (w/w) aqueous sucrose solution with solvent

Test item: 0.05, 0.5, 5.0 and 50 µg ai/bee

Test parameter: A 1.0 µL droplet of the appropriate solution of the test item dissolved in acetone was administered to the thoracic surface of CO₂-anaesthetised bees with a hand-held applicator. Solvent control bees were similarly dosed with pure acetone. After application the bees were returned to the test cages and feed with 50% solution of sucrose *ad libitum*.

Test conditions: Temperature: 25 °C ± 2°C, Relative humidity: 43 - 64 %, Darkness (except during observation)

Test parameter: Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive cleaning, vomiting) were made after exposure for 18, 24, 36 and 48 h.

Findings:

Oral 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 B.9.3.1.1-1

Table B. 9.3.1.1-1: Effects of ethofumesate tech. on *Apis mellifera* following 48-h oral exposure in an acute toxicity test

Nominal dose [µg ai/bee]	Mortality [%] (no. dead bees/no. treated bees)			
	18 h	24 h	36 h	48 h
Study 1				
Control (sugar solution + solvent)	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
Treatment				
0.05	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
0.50	2 (1/50)	4 (2/50)	4 (2/50)	14 (7/50)
5.00	6 (3/50)	8 (4/50)	8 (4/50)	8 (4/50)
50.0	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
Study 2				
Control (sugar solution + solvent)	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Treatment				
0.05	6 (3/50)	8 (4/50)	12 (6/50)	12 (6/50)
0.50	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
5.00	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
50.0	2 (1/50)	2 (1/50)	4 (2/50)	4 (2/50)
Test substance: 24h / 48h LD ₅₀ > 50 µg ai/bee				

Contact toxicity test: In neither study at any treatment did mortality exceed 8%. no sub-lethal effects were observed during the whole test period. Effects on the survival of the honeybees see Table B.9.3.1.1-2.

Table B. 9.3.1.1-2: Effects of ethofumesate tech. 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 [%] (no. dead bees/no. treated bees)			
	18 h	24 h	36 h	48 h
Study 1				
Control (sugar solution + solvent)	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
Treatment				
0.05	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
0.50	6 (3/50)	6 (3/50)	6 (3/50)	6 (3/50)
5.00	0 (0/50)	0 (0/50)	2 (1/50)	2 (1/50)
50.0	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Study 2				
Control (sugar solution + solvent)	0 (0/50)	0 (0/50)	6 (3/50)	6 (3/50)
Treatment				
0.05	2 (1/50)	2 (1/50)	2 (1/50)	2 (1/50)
0.50	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
5.00	0 (0/50)	0 (0/50)	0 (0/50)	2 (1/50)
50.0	2 (1/50)	2 (1/50)	8 (4/50)	8 (4/50)
Test substance: 24h / 48h LD ₅₀ > 50 $\mu\text{g ai/bee}$				

Conclusions:

48 h LD₅₀ > 50 $\mu\text{g ai/bee}$ (oral toxicity)

48 h LD₅₀ > 50 $\mu\text{g ai/bee}$ (contact toxicity)

Comment RMS:

The study was conducted according to the US EPA test guideline (1985). According to the US EPA guideline (1985) no validity criteria are stated. Hence, the validity criteria given in the current valid guidelines according OECD 213 and 214 (19948 and US EPA were used to validate the acute oral and contact toxicity study.

The mean mortality of the control 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.

However, no toxic reference was tested to address the sensitivity of the honeybees. In addition no negative control (water only) was tested. The tested negative control contained 50% sucrose solution and the solvent acetone.

In addition no information is given regarding the starving period of honeybees (oral toxicity test).

Under consideration of the deficiencies of the study the results should be taken into account with caution. However, the study was well conducted and in line with

the guidelines. Hence, the RMS is of the opinion, that the study should be used in the risk assessment.

Reference:	The acute contact and oral toxicity to honeybees of ethofumesate technical
Author(s), year:	Cole, J.H., 1990
Report/Doc. number:	Study no.: A87621, Reference no.: M-161561-01-1
Guideline(s):	EPA guideline (EPA-540/9-85-002, 1985)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate tech., Batch no. : P 004402/2, Content : 97.0% w/w
Reference:	None
Solvent:	Acetone
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Acute oral and contact limit test
Number of organisms:	Ten replicates with 10 bees for the test item treatment groups, two replicates with 10 bees for the control groups.
Food:	20% sucrose solution
Oral toxicity test:	
Applied concentrations:	Control: 20% (w/w) aqueous sucrose solution with solvent Test item: 100 µg ai/bee
Exposure route:	The test item was dissolved in acetone and a 20% sucrose solution and offered to the bees. The bees were starved for 3-4 hours before the application of the test substance. Once the treatment had been consumed (after approximately 4 hours), the feeder was removed and untreated 20% sucrose solution fed ad libitum for the duration of the study.
Test conditions:	Temperature: 24 °C ± 1°C, Darkness (except during observation)
Test parameter:	Mortality counts were made after exposure for 24 and 48 h.
Contact toxicity test:	
Applied concentrations:	Control: 20% (w/w) aqueous sucrose solution with solvent Test item: 100 µg ai/bee
Test parameter:	A 1.0 µL droplet of the appropriate solution of the test item dissolved in acetone was administered to the thoracic surface of CO ₂ -anaesthetised bees with a hand-held applicator. Solvent control bees were similarly dosed with pure acetone. After application the bees were returned to the test cages and feed with 20% solution of sucrose <i>ad libitum</i> .

Test conditions: Temperature: 24 °C ± 1°C, Darkness (except during observation)

Test parameter: Mortality counts were made after exposure for 24 and 48 h.

Findings:

Table B. 9.3.1.1-3: Effects of ethofumesate tech. on *Apis mellifera* following 48-h oral and contact exposure in an acute toxicity test

Nominal dose [µg ai/bee]	Mortality [%]			
	Oral toxicity		Contact toxicity	
	24 h	48 h	24 h	48 h
Control (sugar solution + solvent)	0.0	0.0	0.0	5.0
Treatment (100 µg ai/bee)	12.0	19.0	10.0	16.0
24h / 48h LD ₅₀ > 100 µg ai/bee (oral toxicity)				
24h / 48h LD ₅₀ > 100 µg ai/bee (contact toxicity)				

Conclusions:

48 h LD₅₀ > 100 µg ai/bee (oral toxicity)

48 h LD₅₀ > 1000 µg ai/bee (contact toxicity)

Comment RMS:

The study was conducted according to the US EPA test guideline (1985). According to the US EPA guideline (1985) no validity criteria are stated. Hence, the validity criteria given in the current valid guidelines according OECD 213 and 214 (1998) and US EPA were used to validate the acute oral and contact toxicity study.

The mean mortality of the control 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.

However, no toxic reference was tested to address the sensitivity of the honeybees. No information is given on sub-lethal effects or environmental conditions like relative humidity. The used sucrose solution was a 20% solution instead of a 50% solution. In addition, no negative control containing water only was tested. The tested negative control contained 20% sucrose solution and the solvent acetone.

Under consideration of the deficiencies of the study the results should be taken into account with caution. However, the study was well conducted and in line with the guidelines. Hence, the RMS is of the opinion, that the study should be used in the risk assessment.

Reference:	Effects of ethofumesate techn. (Acute Contact and Oral) on Honeybees (<i>Apis mellifera</i> L.) in the Laboratory
Author(s), year:	Schmitzer, S., 2011
Report/Doc. number:	IBACON project no.: 64231035, Reference no.: M-421681-01-1
Guideline(s):	OECD 213 and 214 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate tech., Batch no.: AE B049913-01-08 (ABJJETN023), Content : 98.3% w/w (analytical)
Reference:	Perfekthion (BAS 152 11 I), Batch: 90924-06, Dimethoate 414.8 g/L (analysed)
Solvent:	Acetone
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) Solvent control: 50% (w/w) sugar solution with solvent (45% tap water, 5% acetone, 50% ready-to-use syrup) Test item: 100 µg ai/bee (nominal). 106.3 µg ai/bee (measured) Reference item: 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee (nominal) 0.05, 0.08, 0.15 and 0.24 µg dimethoate/bee (measured)
Exposure route:	The test item was dissolved in acetone before the ready-to-use syrup was added and offered to the bees. The test bees were starved for 20 minutes before they were fed with the solutions. After 3 hours and 30 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: 24 - 25 °C, Relative humidity: 40 - 68 %, 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)
	Solvent control: acetone
	Test item: 100 µg ai/bee (limit test)
	Reference item: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee (nominal)
Test parameter:	A 5.0 µL droplet of the appropriate solution of the test item dissolved in acetone was administered to the thoracic surface of CO ₂ -anaesthetised bees with a hand-held applicator. Solvent control bees were similarly dosed with a) tap water containing the wetting agent Adhäsit (0.5%) and b) pure acetone. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone). After application the bees were returned to the test cages and feed with ready-to-use syrup <i>ad libitum</i> .
Test conditions:	Temperature: 24 - 25 °C, Relative humidity: 40 - 68 %, 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 106.3 µg ai/bee of technical ethofumesate. 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 B.9.3.1.1-4

Table B. 9.3.1.1-4: Effects of ethofumesate tech. 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 (sugar solution)	0.0	0.0	0.0
Solvent control (acetone)	0.0	0.0	0.0
Treatment			
100 (106.3)	0.0	0.0	0.0
Reference item			
0.05 (0.05)	0.0	2.0	2.0
0.08 (0.08)	0.0	0.0	0.0
0.15 (0.15)	0.0	64.0	68.0
0.30 (0.24)	26.0	98.0	100.0
Test substance: 24h / 48h LD ₅₀ > 106.3 µg ai/bee Reference: 24 h LD ₅₀ = 0.13 µg dimethoate/bee (95 % C.I. 0.12 – 0.15 µg ai/bee) 48 h LD ₅₀ = 0.13 µg dimethoate/bee (95 % C.I. 0.12 – 0.14 µg ai/bee)			

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 B.9.3.1.1-5

Table B. 9.3.1.1-5: Effects of ethofumesate tech. 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	2.0
Solvent control (acetone)	0.0	0.0	0.0
Treatment			
100	0.0	0.0	0.0
Reference item			
0.10	2.0	2.0	2.0
0.15	0.0	22.0	32.0
0.20	0.0	80.0	84.0
0.30	18.0	92.0	96.0
Test substance: 24 h / 48 h $\text{LD}_{50} > 100 \mu\text{g ai/bee}$			
Reference: 24 h $\text{LD}_{50} = 0.16 \mu\text{g dimethoate/bee}$ (95 % C.I. 0.16 – 0.19 $\mu\text{g ai/bee}$)			
48 h $\text{LD}_{50} = 0.15 \mu\text{g dimethoate/bee}$ (95 % C.I. 0.15 – 0.18 $\mu\text{g ai/bee}$)			

Conclusions:

48 h $\text{LD}_{50} > 106.3 \mu\text{g ai/bee}$ (oral toxicity)

48 h $\text{LD}_{50} > 100 \mu\text{g ai/bee}$ (contact toxicity)

Comment RMS:

All validity criteria according to the OECD guidelines 213 and 214 are met.

The mean mortality of the controls (water and solvent) 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 LD_{50} values of the reference item (dimethoate) in the oral (24 h $\text{LD}_{50} = 0.13 \mu\text{g ai/bee}$) and contact (24 h $\text{LD}_{50} = 0.16 \mu\text{g ai/bee}$) 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.

The relative humidity in the oral and contact toxicity test is between 40 and 68% which is below the recommendations given in the OECD guidelines (relative humidity between 50 and 70%). Based on the results of the oral and contact toxicity test the deviation of relative humidity is considered to have no impact on the honeybees.

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.

The study is considered acceptable and the RMS is of the opinion that the results of the study should be used in the risk assessment.

B.9.3.1.2. Chronic toxicity to adult honeybees

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).

Reference:	Ethofumesate (tech.) – Assessment of Chronic Effects to the Honeybee, <i>Apis mellifera</i> L., in a 10 Days Continuous Laboratory Feeding Limit Test
Author(s), year:	Kling, A., 2013
Report/Doc. number:	Study no.: S13-00144, Reference no.: M-469458-01-1
Guideline(s):	None
GLP:	Yes (certified laboratory)
Deviations:	None
Validity:	Acceptable

Material and Methods:

Test substance:	Ethofumesate tech., Batch no.: AE B049913-01-08 (ABJJETN023), Content : 98.3% w/w (analytical)
Reference:	None (no validated reference substance available for this type of study)
Solvent:	Acetone
Test species:	<i>Apis mellifera</i> L., adult worker honeybees (1 to 4 days old)
Type of test:	Chronic 10 days continuous feeding test (limit test)
Number of organisms:	Ten replicates with 10 bees for controls and the test item treatment groups

Applied concentrations:	Control: 50% (w/v) aqueous sucrose solution containing 3% acetone. Test item: 120 mg ai/kg
Exposure route:	Over a period of 10 days, honeybees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 120 mg ai/kg of the test item ethofumesate by continuous and ad libitum feeding. Because the test item was first dissolved in acetone and then diluted with aqueous sucrose solution, the final test item application (feeding) solution contained 3 % acetone. The application (feeding) solutions were offered ad libitum to each cage of 10 bees in plastic syringes. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new syringes, filled with freshly prepared application solution over a period of 10 days. The weight of the syringes was determined before and after feeding to determine the mean food consumption of the bees per replicate.
Test conditions:	Temperature: 31.9 - 33.2 °C, Relative humidity: 54.4 – 69.9 %, Darkness (except during observation)
Test parameter:	Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive cleaning, vomiting) were made every 24 hour during the 10 days test period..
Statistics:	Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group and to determine the NOEC based on mortality. For the statistical comparison of the food consumption, non-rounded mean values per replicate over the entire test period were taken. Data of food consumption were statistically analysed using the Mann-Whitney, t-Test Satterthwaite or t-Test pooled (left-sided, $p \leq 0.05$) depending on the results of the pre-tests of Shapiro Wilks and F-Test ($p \leq 0.05$). Statistical calculations were made using the statistical program SAS release Version 9.2.

Findings:

After 10 days of continuous exposure, mortality at the test item treatment level (120 mg ai/kg) was not statistically significantly different compared to the control.

The overall mean daily consumption of the feeding solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different when compared to the untreated control group (36.3 mg/bee at 120 mg ai/kg, compared to 38.3 mg/bee in the control group). However on day 1 of the exposure period the mean consumption of feeding solution was significantly lower compared to the control group. On the following days the mean feeding consumption was comparable to the control group.

After 10 days of continuous exposure, the accumulated nominal intake of the test item at the treatment level of 120 mg ai/kg was 43.56 µg ai/bee, the corresponding average daily dose was therefore 4.4 µg ai/bee.

no behavioural effects on the honeybees were observed in all the replicates of the control groups and in most of

the replicates of the treatment group. In two of the replicates of the treatment group (replicate 6 and 7) one honeybee showed behavioural effects (affected, bees still upright and attempting to walk but showing signs of reduced coordination).

Table B. 9.3.1.2-1: Effects of ethofumesate tech. on *Apis mellifera* following 10 days oral exposure in a chronic toxicity test

Nominal dose [mg ai/kg]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Cumulative mortality [%]										
Control (sugar solution + acetone)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Treatment 120 mg ai/kg	0.0	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mean consumption of feeding solution per day [mg/bee]										
Control (sugar solution + acetone)	41	34	35	41	37	38	38	40	38	42
Treatment 120 mg ai/kg	34 *	35	36	36	37	33	38	38	37	39
Mean accumulated nominal intake of active substance [µg ai/bee]										
Control (sugar solution + acetone)	-	-	-	-	-	-	-	-	-	-
Treatment 120 mg ai/kg	4.08	8.28	12.60	16.92	21.36	25.32	29.88	34.44	38.88	43.56

* Statistically significantly different compared to the control according to t-Test, pooled, $p \leq 0.05$

Conclusions:

It can be concluded that the continuous *ad libitum* feeding of honeybees in the laboratory over a period of 10 consecutive days with the test item ethofumesate (tech.) at the treatment level of 120 mg ai/kg caused no adverse effects regarding mortality, and only slight effects on the behaviour.

The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different when compared to the untreated control group. Further, on every single day during the 10 day continuous exposure period the mean food consumption per bee was not statistically significantly different (lower) in the test item treatment group compared to the control group except for the first day of exposure.

As the overall mean daily food uptake in the test item treatment group was not significantly lower compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 120 mg ai/kg.

The NOEC for mortality was determined at the end of the test period to be 120 mg ai/kg (nominal).

The LC₅₀ was determined to be > 120 mg ai/kg (nominal).

Comment RMS:	No test guideline is available to address the chronic risk to adult honeybees. Hence, no validity criteria can be met. However, the study is considered valid
---------------------	---

because the mean mortality in the control was $\leq 15\%$ at the end of the test. The criterion of 15% is taken from the acute toxicity study outlined in EPPO 170.

The study protocol given in the draft EFSA Guidance Document on honeybees (EFSA Journal 2013;11(7):3295) was used to evaluate the chronic oral toxicity test.

For the most part the study protocol provided by the applicant is in line with the study protocol given in the draft EFSA guidance document (2013). However, some aspects were not considered in the study:

- Newly emerged honeybees should be used to ensure that the age of the test bees is homogenous.
- During the test pollen should be available ad libitum.
- Groups of honeybees should be consists of at least 20 bees.
- Effects on the hypopharyngeal glands (HPG) should be determined.
- The endpoints should be expressed in $\mu\text{g}/\text{bee}/\text{day}$.

Even though, some short-comings were identified the chronic oral toxicity study is considered to be valid and acceptable to be used for the chronic risk assessment for honeybees.

B.9.3.1.3. Effects on honeybee development and other honeybee life stages

No bee brood feeding test with the active substance was submitted. Please refer to the RAR, Volume 3, B.9 (representative formulations).

B.9.3.1.4. 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.9.3.1.5. Field 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.9.3.1.6. Investigation of special effects

Based on the results on honey-bees (adults and larvae) and non-target arthropods it was demonstrated that the active substance shows no insecticidal activity. Hence, no further data are required.

B.9.3.2. Effects on non-target arthropods other than bees

Please refer to the RAR, Volume 3, B.9 (representative formulations). No studies with the active substance ethofumesate were submitted addressing the risk to non-target arthropods other than bees.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA**B.9.4.1. Earthworm – sub-lethal effects**

For the first EU approval of the active substance one earthworm reproduction study with the formulated active substance (Ethofumesate 500 SC) has been evaluated in the Addendum to the DAR (2000) on ethofumesate. In addition, new earthworm reproduction studies with the representative formulations were submitted addressing the risk to soil organisms from exposure to the formulated active substance (please refer to Volume 3 – B.9. (PPP)).

For the renewal of the EU approval of the active substance ethofumesate earthworm reproduction studies conducted with the major soil metabolites were submitted. The study summaries are given below.

Metabolites:

Reference:	AE C508493: Sublethal toxicity to the earthworm <i>Eisenia fetida</i> in artificial soil with 5% peat
Author(s), year:	Friedrich, S., 2012a
Report/Doc. number:	Study no.: 11 10 48 063 S, Reference no.: M-435163-01-1
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	AE C508493 (metabolite of ethofumesate), Batch no.: SES 10116-5-2, Purity: 99.8% (analysed), CAS no.: 26322-82-7
Test species:	Earthworm <i>Eisenia fetida</i> (<i>Eisenia fetida andrei</i> , det. Bouché 1972)
Number of organisms:	8 replicates per treatment group and control group, each with 10 individuals.
Weight, age:	Mean: 259 - 456 mg/worm, adults with clitellum, approximately 3 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), 100 mg test substance/kg soil dw
Solvent:	None
Toxic standard:	Nutdazim 50 FLOW (Carbendazim, SC 500), tested at concentrations of 5 and 10 mg prod./kg soil dw
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 73.7 % industrial quartz sand, 0.3% calcium carbonate, 1% horse manure (food)
Substrate/test vessel:	600 g dry weight/test container

Temperature:	18.0 – 21.7 °C
Light regime:	16 hours light (630 lx) / 8 hours dark
Water content:	Test start: 25% (equivalent to 58.1% of WHC) Test end: 24.5-24.7% (equivalent of 57.0-57.4% of WHC)
pH:	Test start: 5.96 – 5.98 Test end: 5.68 – 5.73
Feeding:	Air-dried and finely ground horse manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.05 (RATTE 2010). Shapiro-Wilk's Test and Levene's test were used, respectively, to test the data for normality and homogeneity of variance. Student-t-test was used to compare the control with the independent test item group. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.
<u>Findings:</u>	
Effects test item:	No effects of the test item on growth and reproduction of earthworms were determined at a concentration of 100 mg test item/kg soil dw. no pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

Table B. 9.4.1-1: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	AE C508495 [mg test item/kg soil dw]
Exposure	-	100
Mortality of adult earthworms [%] after 28 d	0	0
Mean change of body weight of the adults from day 0 to day 28 [mg] (Standard deviation)	+ 205.5 (46.4)	+ 192.4 (45.4)
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	+ 60.2 (14.3)	+ 56.0 (12.7)
Mean number of offspring per treatment group after 56 d (Standard deviation)	63.3 (8.3)	68.6 (17.2)
Reproduction compared to control [%]	-	108.5

In the positive control (Carbendazim) the number of juveniles was reduced by 77.7 and 100% at concentrations of 5 and 10 mg prod./kg soil dw (mean number of juveniles = 20.8 and 0) after 8 weeks of test duration when compared to the control (mean number of juveniles = 93).

Conclusion: NOEC = 100 mg test item/kg soil dw (adult mortality, body weight, reproduction)
 LOEC > 100 mg test item/kg soil dw
 EC₅₀ > 100 mg test item/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 56-76 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 13.1%).

Based on the results of the study the NOEC was determined to be the highest test concentration, 100 mg test item/kg soil dw.

Reference:	Effects of NC8493 on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat
Author(s), year:	Lühns, U., 2011
Report/Doc. number:	Report no. 63561022, Reference no. IDD00078
Guideline(s):	OECD 222 (2004), ISO 11268-2 (1998)
GLP:	Yes
Deviations:	None
Validity:	Acceptable
<u>Material and methods:</u>	
Test substance:	NC8493 (metabolite of ethofumesate), Batch no.: EPP/VMV 358A, Purity: 99.8% (analysed)
Test species:	Earthworm <i>Eisenia fetida</i> (Savigny, 1826)
Number of organisms:	4 replicates per treatment group and 8 replicates per control group, each with 10 individuals.
Weight, age:	Mean: 328 – 600 mg/worm, adults with clitellum, approximately 9 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), 1.0, 2.0, 4.0, 8.0 and 16.0 mg test substance/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 prod./kg soil dw
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz sand, 0.2% calcium carbonate 40% maximum water holding capacity of artificial soil (dry weight)
Substrate/test vessel:	500 g dry weight/test container
Temperature:	18.0 – 22.0 °C
Light regime:	16 hours light (400-800 lx) / 8 hours dark
Water content:	Test start: 19.8 – 22.2% (equivalent to 49.5 – 55.5% of WHC) Test end: 21.3 – 24.0% (equivalent of 53.2 – 60.0% of WHC)
pH:	Test start: 5.8 – 5.9 Test end: 5.7 – 5.8
Feeding:	Finely ground cattle manure, 5 g per replicates was added each week for the first 4 weeks.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured

at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)

Statistics:

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

The body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk's test and the Levene's test, respectively. As the data for body weight changes and reproduction were normally distributed and homogenous in both cases, Williams t-test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).

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

Findings:

Effects test item:

No effects of the test item on growth and reproduction of earthworms were determined up to the maximal test concentration of 16 mg test item/kg soil dw. no pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

Table B. 9.4.1-2: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	NC8495 [mg test item/kg soil dw]				
Exposure	-	1.0	2.0	4.0	8.0	16.0
Mortality of adult earthworms [%] after 28 d	1.3	0.0	0.0	2.5	2.5	0.0
Mean change of body weight of the adults from day 0 to day 28 [mg] (Standard deviation)	127 (26)	134 (18)	161 (15)	120 (19)	142 (29)	146 (13)
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	28.2 (7.0)	29.5 (5.6)	35.3 (5.4)	26.4 (5.0)	31.2 (7.9)	32.0 (2.8)
Mean number of offspring per treatment group after 56 d (Standard deviation)	312 (44)	294 (43)	325 (49)	320 (20)	290 (45)	302 (43)
Reproduction compared to control [%]	-	94.2	104.3	102.6	93.1	97.0

In the positive control (Carbendazim) the number of juveniles was significantly reduced at all test concentrations (2.3 – 7.5 mg test item/kg soil dw). The mean number of juveniles was between 1 (highest test concentration) and 233 (lowest test concentration) after 8 weeks of test duration. The mean number of juveniles in the control

was 335. Based on the results of the study a NOEC (reproduction) of < 1.0 mg ai/kg soil dw was determined. The EC₅₀ was calculated to be 1.21 mg ai/kg soil dw based on effects on the reproduction of earthworms.

Conclusion: NOEC = 16 mg test item/kg soil dw (adult mortality, body weight, reproduction)
 LOEC > 16 mg test item/kg soil dw
 EC₅₀ > 16 mg test item/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 1.3%). The number of juveniles per control replicate was greater than 30 (being 256-396 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 14.1%).

Based on the results of the study the NOEC was determined to be the highest test concentration, 16 mg test item/kg soil dw.

Reference: BCS-CU88901: Sublethal toxicity to the earthworm *Eisenia fetida* in artificial soil with 5% peat

Author(s), year: Friedrich, S., 2012b

Report/Doc. number: Report no. 12 10 48 001 S, Reference no. M-435164-01-1

Guideline(s): OECD 222 (2004), ISO 11268-2 (1998)

GLP: Yes

Deviations: None

Validity: Acceptable

Material and methods:

Test substance: BCS-CU88901 (metabolite of ethofumesate), Batch no.: SES 11754-3-8, Purity: 69.2% (analysed)

Test species: Earthworm *Eisenia fetida* (*Eisenia fetida andrei*, det. Bouché 1972)

Number of organisms: 8 replicates per treatment group and control group, each with 10 individuals.

Weight, age: Mean: 283 - 452 mg/worm, adults with clitellum, approximately 3 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), 100 mg test substance/kg soil dw

Solvent: None

Toxic standard: Nutdazim 50 FLOW (Carbendazim, SC 500), tested at concentrations of 5 and 10 mg prod./kg soil dw

Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 73.7 % industrial quartz sand, 0.3% calcium carbonate, 1% horse manure (food)
Substrate/test vessel:	500 g dry weight/test container
Temperature:	18.0 – 21.8 °C
Light regime:	16 hours light (570 lx) / 8 hours dark
Water content:	Test start: 25 – 25.1% (equivalent to 58.1 – 58.4% of WHC) Test end: 24.4-24.5% (equivalent of 56.7 – 57.0% of WHC)
pH:	Test start: 5.97 – 6.00 Test end: 5.61 – 5.68
Feeding:	Air-dried and finely ground horse manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.05 (RATTE 2010). Kolmogoroff-Smirnov test and Levene's test were used, respectively, to test the data for normality and homogeneity of variance. Fisher's Exact Binomial Test and Student-t-test were used to compare the control with the independent test item group. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.
<u>Findings:</u>	
Effects test item:	no statistically significant adverse effects compared to the control were observed regarding the body weight change of adults and the mean number of offspring. no mortalities of adults (after 28 days) and offspring (after 56 days) as well as no symptoms of intoxications were observed.

Table B. 9.4.1-3: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

	Control	BCS-CU88901 [mg test item/kg soil dw]
Exposure	-	100
Mortality of adult earthworms [%] after 28 d	1.3	0
Mean change of body weight of the adults from day 0 to day 28 [mg] (Standard deviation)	+ 139.9 (23.0)	+ 144.0 (19.1)
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	+ 40.8 (7.4)	+ 41.6 (5.7)
Mean number of offspring per treatment group after 56 d (Standard deviation)	58.5 (10.6)	66.9 (10.3)
Reproduction compared to control [%]	-	114.3

In the positive control (Carbendazim) the number of juveniles was reduced by 77.7 and 100% at concentrations of 5 and 10 mg prod./kg soil dw (mean number of juveniles = 20.8 and 0) after 8 weeks of test duration when compared to the control (mean number of juveniles = 93).

Conclusion:

NOEC = 100 mg test item/kg soil dw (adult mortality, body weight, reproduction)

LOEC > 100 mg test item/kg soil dw

EC₅₀ > 100 mg test item/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 1.3%). The number of juveniles per control replicate was greater than 30 (being 42-75 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 18.1%).

Based on the results of the study the NOEC was determined to be the highest test concentration, 100 mg test item/kg soil dw.

B.9.4.1.1. Additional information

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 (Regulation No 283/2013) acute toxicity studies are no longer required. Nevertheless, the study summaries from the DAR are included in the RAR as additional information.

Hakin et al., 1991*Methods*

The acute toxicity of ethofumesate (purity 97%) to the earthworm, Eisenia foetida, was investigated in an artificial soil under laboratory conditions. Earthworms were introduced to soils treated with ethofumesate dissolved in acetone at five different rates 62.5, 125, 250, 500 and 1000 mg/kg, plus one solvent control. Four replicates were used for each treatment and for each replicate ten worms were weighted together and placed on the top of the soil in a 1 l glass container. The containers were incubated for two weeks at 23 - 24 °C and constant light. After 7 and 14 days of exposure the worms were examined and counted, and mortalities were recorded. At the end of the test the worms were weighted again.

Results

An increased mortality (93 and 98%) compared to the control (5%) was found at the two highest test concentrations. At lower test concentrations the mortality were equal to or less than that in the control. All surviving worms appeared normal. A treatment related decrease in weight was observed at 250 mg/kg and above. Calculated 7 and 14 days LC₅₀-values (with 95% confidence limits) were 420 (373 - 466) and 383 (323 - 444) mg ethofumesate/kg soil, respectively, and NOEC was found to be 125 mg/kg. All values were based on nominal concentrations.

Comments

The study was conducted in compliance with GLP and the OECD guideline 207.

Barrett and Arnold, 1986*Methods*

The acute toxicity of ethofumesate (purity 96%) to the earthworm, Eisenia andreii, was investigated in an artificial soil under laboratory conditions. Earthworms were introduced to soils treated with ethofumesate dissolved in acetone at five different rates 62.5, 125, 250, 500 and 1000 mg/kg, plus one control and one solvent control. Four replicates were used for each treatment and for each replicate ten worms were placed on the top of the soil in a 1.5 l glass jar. The jars were incubated for two weeks at 20 ± 2 °C and at constant light. After 7 and 14 days of exposure mortalities were recorded.

Results

The cumulative mortality increased with dose, from 20% at the lowest test concentration up to 100% at the two highest concentrations. The mortality in the solvent control was 7.5%. The calculated 14 days LC₅₀-value (with

95% confidence limits) was 134 (112 - 160) mg ethofumesate/kg soil. All values based on nominal concentrations.

Comments

The study was stated to be conducted in accordance with EEC PSPS recommendations. The study deviates from OECD guideline 207 in that the weight of the worms were not determined before and after the test, and that the behaviour of the worms were not reported. *Eisenia andrei* probably refers to *Eisenia foetida andrei*.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

According to the data requirements on active substances (Regulation 283/2013) 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 0 – 18) the risk to soil meso- and macrofauna from exposure to the active substance and its major soil metabolites has to be addressed. Hence, laboratory studies with the soil organisms *Folsomia candida* and *Hypoaspis aculeifer* were submitted.

Metabolites:

Reference:	AE C508493: Effects on the reproduction of the collembolans <i>Folsomia candida</i>
Author(s), year:	Friedrich, S., 2012c
Report/Doc. number:	Report no. 12 10 48 018 S, Reference no. M-435155-01-1
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	AE C508493, Batch no.: SES 10116-5-2, CAS no.: 26322-82-7, Purity: 99.8% w/w (analysed)
Test species:	Collembola <i>Folsomia candida</i> (Willem, 1902)
Number of organisms:	8 replicates per control and treatment group, each with 10 individuals. 2 additional replicates per treatment and control to check the pH and water content of the test substrate after 28 days
Life stage, age:	Juveniles / adults, 9-12 days old
Type of test, duration:	Laboratory sub-lethal limit test, 28 days
Applied concentrations:	Nominal: 0 (control) and 100 mg test item/kg soil dw

Solvent:	None
Toxic standard:	Boric acid, Purity: 100% (analysed), tested at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dw.
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate
Substrate/test vessel:	30 g wet weight/test container
Temperature:	19.4 – 21.5 °C
Light regime:	16 hours light (590 lx) / 8 hours dark
Water content:	Test start: 24.9 – 25.0% (equivalent to 57.9 – 58.1% of WHC) Test end: 24.5% (equivalent of 57.0% of WHC)
pH:	Test start: 5.95 Test end: 5.71 – 5.76
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 weekly after application. Mortality of adults, behavioural effects and number of juvenile Collembola were assessed after 28 days
Statistics:	Shapiro-Wilk's Test and Cochran's test were used, respectively, to test the data for normality and homogeneity of variance. Fisher's Exact Binomial Test and the Student-t-test were used to compare the control with the independent test item group. 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. 9.4.2-1: Effects on mortality and reproduction of *Folsomia candida* in a sub-chronic test

	Control	AE C508493 [mg test item/kg soil dw]
Exposure	-	100
Mortality of adult Collembola [%] after 28 d	1.3	0.0
Mean number of juveniles per treatment group after 28 d (± SD)	1107 (157.2)	1053 (73.6)
Reproduction compared to control [%]	-	95

SD...Standard Deviation

In the most recent study the LC₅₀ and EC₅₀ for the reference item boric acid was determined to be 193 mg test item/kg soil dw and 107 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg test item/kg soil dw, respectively.

Conclusion: NOEC (mortality, reproduction) = 100 mg test item/kg soil dw
 LC₅₀ (mortality) > 100 mg test item/kg soil dw

Comment RMS: The Collembola reproduction study was conducted according to the OECD test guideline 232 (2009). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being 1.3%). The mean number of juveniles per control replicate was greater than 100 (being 947 - 1301 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 14.2%).
 Based on results of the study a NOEC of 100 mg test item/kg soil dw based on highest tested concentration was determined.

Reference:	Effects of 2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (NC 8493) on reproduction of the collembolan <i>Folsomia candida</i>
Author(s), year:	Friedrich, S., 2013a
Report/Doc. number:	Report no. 13 10 48 062 S, Reference no. IDD00079
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance: 2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (synonym: NC 8493), Batch no. EPP/VMV 541.A, Purity: 99.9% (analysed), CAS no.: 26322-82-7

Test species: Collembola *Folsomia candida* (Willem, 1902)

Number of organisms: 8 replicates per control and 4 replicates per treatment group, 2 additional replicates per treatment and control to check the pH and water content of the test substrate after 28 days, each with 10 individuals.

Life stage, age: Juveniles / adults, 9-12 days old

Type of test, duration: Laboratory sub-lethal test, 28 days

Applied concentrations: Nominal: 0 (control), 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dw

Solvent: None

Toxic standard: Boric acid, Purity: 100% (analysed), tested at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dw.

Test substrate: Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate

Substrate/test vessel:	30 g wet weight/test container
Temperature:	18.1 – 20.8 °C
Light regime:	16 hours light (530 lx) / 8 hours dark
Water content:	Test start: 24.9 – 25.0% (equivalent to 57.2 – 57.5% of WHC) Test end: 24.4 – 24.6% (equivalent of 56.1 – 56.6% of WHC)
pH:	Test start: 6.27 – 6.30 Test end: 6.03 – 6.07
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 weekly after application. Mortality of adults, behavioural effects and number of juvenile Collembola were assessed after 28 days
Statistics:	The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH. Fisher's Exact Binomial Test with Bonferroni Correction and the Williams t-test were used to compare the control with the independent test item groups. The EC _x values were calculated by Probit analysis using the maximum likelihood method.

Findings:

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

Table B. 9.4.2-2: Effects on mortality and reproduction of *Folsomia candida* in a sub-chronic test

	Control	NC 8493 [mg test item/kg soil dw]						
Exposure	-	29	53	95	171	309	556	1000
Mortality of adult Collembola [%] after 28 d	5.0	5.0	2.5	7.5	5.0	5.0	7.5	5.0
Mean number of juveniles per treatment group after 28 d (± SD)	795 (109)	763 (92)	844 (121)	798 (111)	802 (158)	756 (34)	723 (190)	624* (113)
Reproduction compared to control [%]	-	96	106	100	101	95	91	79*

SD...Standard Deviation

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

In the most recent study the LC₅₀ and EC₅₀ for the reference item boric acid was determined to be 199 mg test item/kg soil dw and 104 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg test item/kg soil dw, respectively.

Conclusion: NOEC (reproduction) = 556 mg test item/kg soil dw
 EC₁₀ (reproduction) = 384 mg test item/kg soil dw (95% C.I. 166 – 889 mg test item/kg soil dw)
 NOEC (mortality) = 1000 mg test item/kg soil dw
 LC₁₀ (mortality) > 1000 mg test item/kg soil dw

Comment RMS: The Collembola reproduction study was conducted according to the OECD test guideline 232 (2009). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being 5.0%). The mean number of juveniles per control replicate was greater than 100 (being 627 – 931 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 13.7%).
 Based on results of the study a NOEC of 556 mg test item/kg soil dw based on reproduction was determined.

Reference: BCS-CU88901: Effects on the reproduction of the collembolan *Folsomia candida*
Author(s), year: Friedrich, S., 2013b
Report/Doc. number: Report no. 13 10 48 108 S, Reference no. M-462560-01-1
Guideline(s): OECD 232 (2009), ISO 11267 (1999)
GLP: Yes
Deviations: None
Validity: Acceptable

Material and methods:

Test substance: BCS-CU88901, Batch no.: SES 11754-01-01, Purity: 69.2% (analysed)
Test species: Collembola *Folsomia candida* (Willem, 1902)
Number of organisms: 8 replicates per control and treatment group, each with 10 individuals.
 2 additional replicates per treatment and control to check the pH and water content of the test substrate after 28 days
Life stage, age: Juveniles / adults, 9-12 days old
Type of test, duration: Laboratory sub-lethal limit test, 28 days
Applied concentrations: Nominal: 0 (control) and 100 mg pure metabolite/kg soil dw corresponding to 145 mg test item/kg soil dw based on analysed purity
Solvent: None
Toxic standard: Boric acid, Purity: 100% (analysed), tested at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dw.
Test substrate: Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz

	sand, 0.3% calcium carbonate
Substrate/test vessel:	30 g wet weight/test container
Temperature:	19.0 – 21.7 °C
Light regime:	16 hours light (540 lx) / 8 hours dark
Water content:	Test start: 25.0% (equivalent to 57.1% of WHC) Test end: 24.6% (equivalent of 56.2% of WHC)
pH:	Test start: 6.10 – 6.12 Test end: 5.89 – 5.92
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 weekly after application. Mortality of adults, behavioural effects and number of juvenile Collembola were assessed after 28 days
Statistics:	Fisher's Exact Binomial Test and the Student-t-test were used to compare the control with the independent test item group. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.06, ® ToxRat Solutions GmbH..

Findings:

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

Table B. 9.4.2-3: Effects on mortality and reproduction of *Folsomia candida* in a sub-chronic test

	Control	BCS-CU88901 [mg pure metabolite/kg soil dw]
Exposure	-	100
Mortality of adult Collembola [%] after 28 d	2.5	2.5
Mean number of juveniles per treatment group after 28 d (± SD)	1526 (170.1)	1483 (142.7)
Reproduction compared to control [%]	-	97

In the most recent study the LC₅₀ and EC₅₀ for the reference item boric acid was determined to be 192 mg test item/kg soil dw and 108 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg test item/kg soil dw, respectively.

Conclusion:

NOEC (mortality, reproduction) = 100 mg pure metabolite/kg soil dw

LC₅₀ (mortality) > 100 mg pure metabolite/kg soil dw

Comment RMS:

The Collembola reproduction study was conducted according to the OECD test guideline 232 (2009). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being 2.5%). The mean number of juveniles per control replicate was greater than 100 (being 1236 - 1771 juveniles per replicate). The coefficient of

variation of reproduction in the control was $\leq 30\%$ (being 11.1%).

Based on results of the study a NOEC of 100 mg pure metabolite/kg soil dw based on highest tested concentration was determined.

Reference:	Effects of 2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (NC 8493) on reproduction of the predatory mite <i>Hypoaspis aculeifer</i>
Author(s), year:	Schulz, L., 2013a
Report/Doc. number:	Report no. 13 10 48 063 S, Reference no. IDD00080
Guideline(s):	OECD 226 (2008)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (synonym: NC 8493), Batch no. EPP/VMV 541.A, Purity: 99.9% (analysed), CAS no.: 26322-82-7
Test species:	Predatory mites, <i>Hypoaspis aculeifer</i> (Canestrini, 1883)
Number of organisms:	8 replicates per control and 4 replicates per treatment group, 2 additional replicates per treatment and control to check the pH and water content of the test substrate after 14 days, each with 10 individuals.
Life stage:	Adult females
Type of test, duration:	Laboratory sub-lethal test, 14 days
Applied concentrations:	Nominal: 0 (control), 9.0, 16.0, 29.0, 53.0, 95.0, 172 and 309 mg test item/kg soil dw
Solvent:	None
Toxic standard:	Dimethoate EC 400, 411.7 g/L (analysed)
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate
Substrate/test vessel:	20 g dry weight/test container
Temperature:	19.5 – 21.1 °C
Light regime:	16 hours light (540 lx) / 8 hours dark
Water content:	Test start: 17.14 – 18.66% (equivalent to 42.80 – 46.58% of WHC) Test end: 15.58 – 18.52% (equivalent of 39.14 – 46.23% of WHC)
pH:	Test start: 5.5 – 5.8 Test end: 5.6 – 5.9
Feeding:	Before and during the test, the predatory mites were fed every 2 days with cheese

	mites (<i>Tyrophagus putrescentiae</i>).
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked on day 7 after application. Mortality of adults, differences in morphology, behavioural effects and number of juveniles were assessed after 14 days
Statistics:	Reproduction: Fisher's Exact Test with Bonferroni correction and Dunnett-t-test were used to compare the control with the independent test item groups. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Findings:

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

Table B. 9.4.2-4: Effects on mortality and reproduction of *Hypoaspis aculeifer* in a sub-chronic test

	Control	NC 8493 [mg test item/kg soil dw]						
Exposure	-	9.0	16.0	29.0	53.0	95.0	172	309
Mortality of adult mites [%] after 14 d	2.5	5.0	5.0	2.5	0.0	0.0	10.0	0.0
Mean number of juveniles per treatment group after 14 d (± SD)	277.6 (50.4)	259 (23.7)	243.5 (64.7)	282.3 (22.8)	241.5 (34.3)	235.8 (29.8)	288.8 (41.5)	247.8 (35.8)
Reproduction compared to control [%]	-	93	88	102	87	85	104	89

SD...Standard Deviation

To verify the sensitivity of the test system, the reference item Dimethoate EC 400 was tested at concentrations of 4.10, 5.12, 6.40, 8.0 and 10 mg ai/kg soil dw. Based on the effects on the reproduction an EC₅₀ of 6.64 mg ai/kg soil dw was determined.

Conclusion:LC₅₀ / EC₅₀ (mortality, reproduction) > 309 mg test item/kg soil dw

NOEC (mortality, reproduction) = 309 mg test item/kg soil dw

Comment RMS:

The predatory mite reproduction study was conducted according to the OECD test guideline 226 (2008). Based on the validity criteria stated in the guideline the study was considered acceptable. The mean mortality of adults in the control was below 20% (being 2.5%). The mean number of juveniles per control replicate was greater than 50 (being 180 - 345 juveniles per replicate). The coefficient of variation of reproduction in the control was ≤ 30% (being 18.2%).

Based on results of the study a NOEC of 309 mg test item/kg soil dw based on the highest tested concentrations was determined.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

In the first EU peer-review of the active substance ethofumesate nitrogen and carbon mineralisation studies were submitted addressing the risk to soil micro-flora. 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.

Hossack et al., 1991

Methods

The effect of ethofumesate (purity 97%) on soil micro-organism respiration was studied using two different soils (Table 8.8.1a) known not to have been treated with pesticides during the preceding 5 years. Ethofumesate was applied to the soils at two rates, 1.87 and 9.33 mg ai/kg soil, corresponding to field application rates of 1.4 and 7 kg ai/ha (referred to as maximum and 5 times maximum rate), respectively. Lucerne meal (N content 2.69%) was added as a substrate at a rate of approximately 150 mg N/kg soil. In addition, controls with soil plus substrate and soil only were used. Soil respiration was measured as carbon dioxide in triplicate respirometer flasks at 20°C. The trapping solution (NaOH) in the respirometer traps were analysed at 2, 7, 14, 21, 28, 35, 50 and 63 days.

Table 8.8.1.a. Soil characteristics.

Soil	Org. C (%)	pH	sand (%)	silt (%)	clay (%)	MWHC (%)	CEC (m. equ./100 g soil)
Sandy loam	2.4	5.8	60	26	14	53	16
Clay loam	3.0	7.8	28	43	29	69	7.8

Results

no significant treatment related effects were found in the sandy loam soil. In the clay loam soil the highest ethofumesate treatment had a significantly higher CO₂ level than the control on day 2, but this effect had disappeared on day 7 and thereafter.

Comments

The study was conducted in compliance with GLP. The experimental procedures was stated to follow the principles of the OECD Chemicals Testing Program UPEC/3 4th draft 1981 and BBA guideline, part VI.

Aldred, 1993

Methods

The effect of ethofumesate (purity 97%) on soil micro-organism respiration was studied using two different soils (Table 8.8.1b). Ethofumesate was applied to the soils at two rates, 1.18 and 5.98 mg ai/kg soil, corresponding to

field application rates of 0.9 and 4.5 kg ai/ha, respectively. Glucose was added as a substrate at a rate of 2 and 4 g/kg in the sandy clay loam and the loamy sand, respectively. The study was conducted with three replicates per treatment at a temperature of 22 ± 1 °C. Soil respiration was measured as carbon dioxide production by an infrared gas analyser at a flow rate of 2 l/h on day 0, 14 and 28.

Table Soil characteristics.

Soil	Org. mtr. (%)	pH	sand (%)	silt (%)	clay (%)	MWHC (%)	CEC (m. equ./ 100 g soil)
Sandy clay loam	5.8	7.2	50	14	36	71	n.r.
Loamy sand ¹	1.9	6.9	75	12	13	34	n.r.

¹Speyer standard soil 2.3

n.r. = not reported

Results

No statistically significant treatment related effects were found.

Comments

The study was conducted in compliance with GLP. no guideline was given but the methods was stated to follow Anderson and Domsch (1978): A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol. Biochem.* 10, 215 - 221.

Voets et al., 1977

Methods

The effect of ethofumesate (purity not given) on soil microbiota was studied under laboratory conditions. The following parameters were investigated; the numbers of bacteria, fungi and cellulolytic microorganisms, the phosphatase, urease and saccharase activity, and the soil respiration and nitrification rate. The study was conducted with a sandy soil with the following characteristics; sand 29%, silt 60%, clay 11%, Organic C 1.17%, pH 5.8 and CEC 10. The soil was brought to 75% of MWHC and treated with ethofumesate at rates corresponding to 0, 2.0 and 40.0 kg ai/ha. To study nitrogen transformations, 215 mg urea was added to a 0.5 kg soil sample, and the amount of NH_4^+ , NO_2^- and NO_3^- in 10 g soil samples were analysed at regular intervals. Incubation was carried out in plastic pots at room temperature.

Results

A slight but significant effect was found on the number of cellulolytic microorganisms at the high application rate and on the urease activity at the low application rate. A slight inhibition of the nitrification rate compared to the control was observed at both treatment levels. The respiration rate was also slightly inhibited at the low application rate, whereas the high application rate resulted in a pronounced increase in soil respiration.

Comments

The study does not refer to any guideline but is performed and reported in an acceptable way.

Active substance:

Reference:	Effect of Ethofumesate on Nitrogen - Transformation in Soil
Author(s), year:	Vonk, J.W., 1988
Report/Doc. number:	Report no. R88/048, Reference no. M-161564-01-1
Guideline(s):	Dutch guidelines (Form A, Commission for Registering Pesticides, 1983)
GLP:	Yes
Deviations:	None relevant
Validity:	Additional information

Material and methods:

Test substance:	Ethofumesate, Purity: 98-99%
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 42 days
Applied concentrations:	0 (control), 0.3 and 3.0 mg ai/kg soil dw
Solvent/vehicle:	None
Toxic standard:	Dinoterb
Test substrate:	Humic sandy soil and loamy soil, from two different fields located in The Netherlands. <u>Humic sandy soil</u> : C _{ORG} : 4.5 %, pH: 5.4, CaCO ₃ : 0.1%, Moisture at 0.32 bar: 13.7% Texture: 3.1 % clay, 7.9 % silt, 84.5 % sand <u>Loamy soil</u> : C _{ORG} : 2.1 %, pH: 7.3, CaCO ₃ : 7.9 %, Moisture at 0.32 bar: 24.6% Texture: 22.7 % clay, 40.6 % silt, 26.7 % sand 0.5% w/w lucerne meal
Substrate/test vessel:	100 g soil dw/cotton-plugged conical flasks
Incubation:	20 ± 1°C, darkness
Water content	Test start: 17.25 – 17.69 g/100 g soil dw (> 45% of WHC) Test end: 16.91 – 17.47 g/100 g soil dw (> 45% of WHC)
pH:	Test start: 6.2 Test end: 6.0 – 6.1
Test parameters:	Nitrogen transformation was followed by measuring the amounts of NH ₄ ⁺ -N, NO ₂ ⁻ -N and NO ₃ ⁻ -N in soil. The nitrogen transformation was determined on day 0 and at intervals of 7, 14, 28 and 42 days after application. Samples were extracted with 2 M KCl, by mechanical shaking for 1 hour on a vibrator using 2 mL of extractant for each gram of soil. The extract was filtered through a Whatman no. 1 paper and the filtrate was analysed using a Technicon Auto-Analyser.

Conclusion: Ethofumesate has no influence on soil ammonification and nitrification which is beyond the normal fluctuations in the experiments (about 20%).

Comment RMS: The nitrogen transformation test was conducted according to Dutch test guidelines. The stated test guideline was not available to the RMS. For the first EU peer-review of the active substance ethofumesate the study was considered acceptable. The study report, especially the evaluation of the results is poorly documented. No statistical analyses of the results were conducted. No information on the used batch or the history of the test soils is given.

The used test soil “humic sandy soil” is considered acceptable; however, the second test soil “loamy soil” is not considered acceptable according to the current valid test guideline (OECD 216, 2000) considering the sand content of < 50% (being: 26%).

Under consideration of the identified deficiencies of the study the RMS is of the opinion that the results of the study should be used as additional information only.

Metabolites:

Reference:	AE C508493: Effects on the activity of soil microflora (Nitrogen transformation test)
Author(s), year:	Schulz, L., 2013b
Report/Doc. number:	Report no. 13 10 48 074 N, Reference no. M-460349-01-1
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	AE C508493 (metabolite of ethofumesate), Batch no.: SES 10116-5-2, Purity: 99.8% (analysed)
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 28 days
Applied concentrations:	0 (control), 1.20 and 12.0 mg test item/kg dry soil, 3 replicates per control and treatment group
Solvent/vehicle:	None
Toxic standard:	Dinoterb, tested at concentrations of 6.8, 16 and 27 mg/kg soil dw, 3 replicates per treatment group

Test substrate:	<p>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.</p> <p>C_{ORG} 1.38 %, pH: 6.5, Humus content: 2.37%, Carbon content of microbial biomass: 37.16 mg C/100 g soil dw (corresponding to 2.69 % of C_{ORG})</p> <p>Total nitrogen content: 0.15%</p> <p>Water holding capacity (WHC): 36.61 g/100 g soil dw</p> <p>Texture according to DIN 11277: 10.3 % clay, 36.8 % silt, 52.9 % sand</p> <p>0.5% (i.e. 1.0 g/200 g soil dw) lucerne meal</p>
Substrate/test vessel:	200 g soil dw/500 mL glass flasks
Incubation:	19.4 – 21.5°C, darkness
Water content	<p>Test start: 17.23 – 17.74 g/100 g soil dw (> 45% of WHC)</p> <p>Test end: 16.89 – 17.39 g/100 g soil dw (> 45% of WHC)</p>
pH:	<p>Test start: 6.2</p> <p>Test end: 6.2</p>
Test parameters:	<p>The nitrogen transformation was determined on day 0 (after approximately 3 hours), and at intervals of 7, 14 and 28 days after application.</p> <p>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.</p> <p>For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used.</p>
Statistics:	A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level) and 2-sided Welch-t-test (for inhomogeneous variances at 5 % significance level).

Findings:

Nitrogen transformation:	<p>The test item AE C508493 caused a temporary inhibition of the daily nitrate rate at the tested concentration of 1.20 mg/kg at time interval 7-14 days after application.</p> <p>However, no adverse effects of AE C508493 on nitrogen transformation in soil could be observed at both test concentrations (1.20 mg/kg dry soil and 12.00 mg/kg dry soil) at the end of the 28-day experiment. Differences from the control of -1.4 % (test concentration 1.20 mg/kg dry soil) and +15.2 % (test concentration 12.00 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).</p>
--------------------------	---

Table B.9.5-1: Effects of AE C508493 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	3.82 (0.03)	-
	7-14	1.69 (0.15)	-
	14-28	1.05 (0.06)	-
1.2 mg/kg soil dw	0-7	3.98 (0.19)	+ 4.2
	7-14	1.25 (0.45)	-26.2
	14-28	1.04 (0.20)	-1.4
12 mg/kg soil dw	0-7	3.88 (0.13)	+ 1.5
	7-14	1.43 (0.07)	- 15.2
	14-28	1.21 (0.07)	+ 15.2 *

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

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of + 33.7 % and + 42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application.

Conclusion:

AE C508493 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 12.00 mg test item/kg soil dry weight.

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 NO₃-N were $\leq 15\%$. In this study the CV in the control were maximum 1.6% and thus fulfilled the validity criteria.

Based on results of the study a EC₂₅ of > 12.0 mg test item/kg soil dw was determined.

Reference:	BCS-CU88901: Effects on the activity of soil microflora (Nitrogen transformation test)
Author(s), year:	Schulz, L., 2013c
Report/Doc. number:	Report no.: 13 10 48 075 N, Reference no.: M-460357-01-1
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	BCS-CU88901 (metabolite of ethofumesate), Batch no.: SES 11754-3-8, Purity: 69.2% (analysed)
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 28 days
Applied concentrations:	0 (control, purified water), 1.38 and 13.8 mg pure metabolite/kg soil dw (corresponding to 1.99 and 19.94 mg test item/kg soil dw)
Solvent/vehicle:	None
Toxic standard:	Dinoterb

Test conditions:

Test substrate:	<p>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.</p> <p>C_{ORG} 1.38 %, pH: 6.5, Humus content: 2.37%, Carbon content of microbial biomass: 37.16 mg C/100 g soil dw (corresponding to 2.69 % of C_{ORG})</p> <p>Total nitrogen content: 0.15%</p> <p>Water holding capacity (WHC): 36.61 g/100 g soil dw</p> <p>Texture according to DIN 11277: 10.3 % clay, 35.4 % silt, 54.3 % sand</p> <p>0.5% (i.e. 1.0 g/200 g soil dw) lucerne meal</p>
Substrate/test vessel:	200 g soil dw/500 mL wide mouth glass flasks (treatment and control groups, 3 replicates per treatment)
Incubation:	19.4 – 21.5°C, darkness
Water content	<p>Test start: 17.25 – 17.69 g/100 g soil dw (> 45% of WHC)</p> <p>Test end: 16.91 – 17.47 g/100 g soil dw (> 45% of WHC)</p>
pH:	<p>Test start: 6.2</p> <p>Test end: 6.0 – 6.1</p>
Test parameters:	<p>The nitrogen transformation was determined on day 0 (after approximately 3 hours), and at intervals of 7, 14 and 28 days after application.</p> <p>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</p>

$\pm 5^{\circ}\text{C}$.

For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used.

Statistics: A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level).

Findings:

Nitrogen transformation: no adverse effects of BCS-CU88901 on nitrogen transformation in soil could be observed at both test concentrations (1.99 mg/kg dry soil and 19.94 mg/kg dry soil) during the 28 day experiment. Differences from the control of +6.9 % (test concentration 1.99 mg/kg dry soil) and +6.7 % (test concentration 19.94 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table B.9.5-2: Effects of BCS-CU88901 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	3.71 (0.12)	-
	7-14	1.47 (0.10)	-
	14-28	1.03 (0.04)	-
1.99 mg/kg soil dw	0-7	3.64 (0.20)	- 2.1
	7-14	1.45 (0.08)	- 1.3
	14-28	1.10 (0.09)	+ 6.9
19.94 mg/kg soil dw	0-7	3.78 (0.11)	+ 1.8
	7-14	1.44 (0.17)	- 1.9
	14-28	1.10 (0.02)	+ 6.7

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of + 33.7 % and + 42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application.

Conclusion: BCS-CU88901 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 19.94 mg test item/kg soil dry weight.

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 NO₃-N were $\leq 15\%$. In this study the CV in the control were maximum 2.3% and thus fulfilled the validity criteria.
Based on results of the study a EC₂₅ of > 19.94 mg test item/kg soil dw was determined.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

In the first EU peer review evaluation of ethofumesate a non-GLP post- and pre-emergence screening test was submitted to address the risk to non-target plants.

Reference:	Screening data for ethofumesate (NC 008438 = AE B049913)
Author(s), year:	Rosinger, C.H., 2000
Report/Doc. number:	Study no. C 048328
Guideline(s):	None
GLP:	No
Deviations:	None
Validity:	Not acceptable

Material and Methods:

Test substance: 1) Ethofumesate formulated as a solution in 75% acetone and 25% of 1% Tween 20 surfactant

2) 20% emulsifiable concentrate of ethofumesate (20 EC) in water

Type of test: Pre- and post-emergence screening test

Test species: Temperate species:

Dicotyledonous species: *Veronica persica* (buxbaums speedwell), *Stellaria media* (chickweed), *Galium aparine* (cleavers), *Chrysanthemum segetum* (corn marigold), *Viola arvensis* (field pansy), *Brassica napus* (oil-seed rape), *Polygonum lapathifolium* (pale persicaria), *Matricaria inodora* (scentless mayweed) and *Beta vulgaris* (sugar beet)

Monocotyledonous species: *Hordeum vulgare* (barley), *Bromus sterilis* (barren brome), *Alopecurus myosuroides* (blackgrass), *Elytrigia repens* (couch), *Triticum aestivum* (wheat) and *Avena fatua* (wild oats)

Non-temperate species:

Dicotyledonous species: *Solanum nigrum* (black nightshade), *Xanthium pungens* (cocklebur), *Gossypium hirsutum* (cotton), *Datura stramonium* (jimsonweed), *Ipomoea purpurea* (morningglory), *Amaranthus retroflexus* (pigweed), *Sida spinosa* (prickly sida), *Cassia obtusifolia* (sicklepod), *Glycine max* (soybean) and *Abutilon theophrasti* (velvetleaf)

Monocotyledonous species: *Echinochloa crus-galli* (barnyardgrass), *Setaria viridis* (green foxtail), *Zea mays* (maize), *Sorghum halepense* (sorghum), *Cyperus rotundus* (purple nutsedge) and *Cyperus esculentus* (yellow nutsedge)

Pre-emergence screening test, Stage 1

Applied concentrations: Control

	Test item: 125, 500, 1000 and 3000 g ai/ha
Exposure route:	Seeds of 12 weed species were sown in plastic trays on 4.5 cm deep loamy soil and covered with 5 mm of the same soil. The trays were sprayed with the test substance. After treatment the trays were placed in growth rooms.
Test conditions:	Temperature: 21 ± 1 °C (temperate species) and 25 ± 1 °C (non-temperate species), Photoperiod: 14 hour photo-period from daylight fluorescent tubes.
Test parameter:	The assessment of herbicidal effects was made 21 days after treatment. The extent of the effects was determined by comparison with untreated control plants and expressed on a scale of 0 (no injury) to 4 (90 – 100% injury).
Pre-emergence screening test, Stage 2:	
Applied concentrations:	Control Test item: 125, 250, 500, 1000 and 2000 g ai/ha
Exposure route:	Seeds of warm (non-temperate) and cool (temperate) climate weed and crop species were sown in 8.5 cm square plastic pots on 7 cm deep loamy soil and covered with 2-10 mm of the same soil. The plots were sprayed with the test substance. After treatment the plots were placed in glasshouses.
Test conditions:	Temperature: 16 °C (temperate species) and 21 °C (non-temperate species), Photoperiod: 16 hour photo-period from high pressure sodium lamps.
Test parameter:	The assessment of herbicidal effects was made 3-4 weeks after treatment. The extent of the effects was determined by comparison with untreated control plants and expressed on a scale of 0 (no injury) to 4 (90 – 100% injury).
Post-emergence screening test, Stage 1:	
Applied concentrations:	Control Test item: 250 and 1000 g ai/ha
Exposure route:	Seeds of 12 weed species were sown in plastic trays on 4.5 cm deep loamy soil and covered with 5 mm of the same soil. The trays were placed in growth rooms with a 14 hour photo-period. After 10 days plants had reached the first leaf stage and the trays were then sprayed with the test substance. After treatment the trays were placed in growth rooms.
Test conditions:	Temperature: 21 ± 1 °C (temperate species) and 25 ± 1 °C (non-temperate species), Photoperiod: 14 hour photo-period from daylight fluorescent tubes.
Test parameter:	The assessment of herbicidal effects was made 2-3 weeks after treatment. The extent of the effects was determined by comparison with untreated control plants and expressed on a scale of 0 (no injury) to 4 (90 – 100% injury).
Post-emergence screening test, Stage 2:	
Applied concentrations:	Control Test item: 63, 125, 250, 500, 1000 and 2000 g ai/ha

Exposure route:	Seeds of warm (non-temperate) and cool (temperate) climate weed and crop species were sown in 8.5 cm square plastic pots on 7 cm deep loamy soil and covered with 2-10 mm of the same soil. The plots were placed in glasshouses. After 1 to 2 weeks plants had reached the 1 to 2 leaf stage and the pots were then sprayed with the test substance. After treatment the plots were placed in glasshouses.
Test conditions:	Temperature: 16 °C (temperate species) and 21 °C (non-temperate species), Photoperiod: 16 hour photo-period from high pressure sodium lamps.
Test parameter:	The assessment of herbicidal effects was made 3-4 weeks after treatment. The extent of the effects was determined by comparison with untreated control plants and expressed on a scale of 0 (no injury) to 4 (90 – 100% injury).

Findings:

Pre-emergence test, stage 1: At the highest test concentration, i.e. 3000 g ai/ha significant effects (> 70%) against all tested grass and broad-leaved species were observed. The most sensitive species were identified to be the monocotyledonous species couch, green foxtail and blackgrass and the dicotyledonous species black nightshade and cleavers.

Table B. 9.6.1-1: Pre-emergence stage 1 screening results for ethofumesate

Test species	Test rate [g ai/ha]			
	125	500	1000	3000
Pale persicaria ^d	0	1	2	3
Cleavers ^d	2	4	4	4
Corn marigold ^d	0	0	1	3
Black nightshade ^d	1	4	3	4
Cocklebur ^d	nd	3	nd	nd
Morningglory ^d	2	2	3	3
Blackgrass ^m	2	4	4	4
Couch ^m	4	4	4	4
Wild oats ^m	0	2	nd	4
Purple nutsedge ^m	nd	0	nd	4
Green foxtail ^m	2	4	4	4
Barnyardgrass ^m	0	2	3	4

nd...not determined, m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Pre-emergence test, stage 2: At the highest test concentration (2000 g ai/ha) significant effects on all monocotyledonous species and some dicotyledonous species were observed. The most sensitive species were observed to be blackgrass, couch, wheat and sorghum (monocotyledonous) and chickweed, prickly sida, cleavers and buxbaums speedweed (dicotyledonous).

Table B. 9.6.1-2: Pre-emergence stage 2 screening results for ethofumesate, temperate plant species

Test species	Test rate [g ai/ha]				
	125	250	500	1000	2000
Wheat ^m	2	2	3	4	4
Barley ^m	1	1	2	3	4
Couch ^m	3	2	3	3	4
Blackgrass ^m	2	2	2	4	4
Wild oats ^m	0	2	2	4	4
Barren brome ^m	0	2	2	4	4
Sugar beet ^d	0	0	0	0	1
Oil-seed rape ^d	1	1	2	2	3
Field pansy ^d	0	0	2	2	2
Chickweed ^d	3	4	4	4	4
Cleavers ^d	2	2	2	3	3
Scentless mayweed ^d	0	0	1	2	1
Pale persicaria ^d	0	0	1	2	2
Buxbaums speedweed ^d	0	0	3	3	2

m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Table B. 9.6.1-3: Pre-emergence stage 2 screening results for ethofumesate, non-temperate plant species

Test species	Test rate [g ai/ha]				
	125	250	500	1000	2000
Maize ^m	0	0	1	2	4
Barnyardgrass ^m	1	2	2	3	4
Sorghum ^m	0	2	3	4	4
Green foxtail ^m	1	1	2	4	4
Yellow nutsedge ^m	0	1	1	2	4
Cotton ^d	0	0	0	2	2
Soybean ^d	0	1	2	3	3
Jimsonweed ^d	0	1	1	3	3
Morningglory ^d	0	1	2	3	4
Sicklepod ^d	nd	nd	nd	2	2
Prickly sida ^d	0	0	1	3	4
Velvetleaf ^d	1	1	1	3	4
Pigweed ^d	nd	nd	nd	nd	4
Cocklebur ^d	nd	1	2	1	2

nd...not determined, m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Post-emergence test, stage 1: At 1000 g ai/ha ethofumesate showed moderate to good activity against most of the grass and broad-leaved weeds treated.

Table B. 9.6.1-4: Post-emergence stage 1 screening results for ethofumesate,

Test species	Test rate [g ai/ha]	
	250	1000
Pale persicaria ^d	0	2
Cleavers ^d	3	3
Corn marigold ^d	3	1
Black nightshade ^d	nd	3
Cocklebur ^d	nd	nd
Morningglory ^d	3	3
Blackgrass ^m	0	2
Couch ^m	nd	2
Wild oats ^m	nd	2
Yellow nutsedge ^m	3	3
Green foxtail ^m	0	3
Barnyardgrass ^m	1	3

nd...not determined, m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Post-emergence test, stage 2: The most sensitive species were observed to be barren brome, maize and green foxtail (monocotyledonous) and chickweed, morningglory and cleavers (dicotyledonous).

Table B. 9.6.1-5: Post-emergence stage 2 screening results for ethofumesate, temperate plant species

Test species	Test rate [g ai/ha]					
	63	125	250	500	1000	2000
Wheat ^m	1	2	2	2	2	3
Barley ^m	0	2	2	2	2	3
Couch ^m	0	2	1	2	2	3
Blackgrass ^m	0	1	1	2	2	3
Wild oats ^m	0	0	1	2	2	3
Barren brome ^m	1	1	2	2	2	4
Sugar beet ^d	0	0	0	0	0	1
Oil-seed rape ^d	1	2	2	2	3	3
Field pansy ^d	0	1	1	1	1	2
Chickweed ^d	3	2	2	3	3	4
Cleavers ^d	3	3	3	3	3	4
Scentless mayweed ^d	1	2	2	2	2	3
Pale persicaria ^d	1	2	2	2	2	3
Buxbaums speedweed ^d	1	2	2	2	2	3

m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Table B. 9.6.1-6: Post-emergence stage 2 screening results for ethofumesate, non-temperate plant species

Test species	Test rate [g ai/ha]					
	63	125	250	500	1000	2000
Maize ^m	1	1	2	3	3	3
Barnyardgrass ^m	1	2	3	3	3	3
Sorghum ^m	0	3	3	3	3	2
Green foxtail ^m	2	2	3	3	3	3
Yellow nutsedge ^m	1	0	0	1	2	2
Cotton ^d	2	2	2	3	3	2
Soybean ^d	2	3	3	3	3	2
Jimsonweed ^d	2	2	2	2	3	2
Morningglory ^d	2	4	4	4	4	3
Sicklepod ^d	1	1	1	2	2	1
Prickly sida ^d	1	2	2	2	3	2
Velvetleaf ^d	1	2	2	3	3	2
Pigweed ^d	2	2	2	3	3	2
Cocklebur ^d	0	1	1	2	2	1

nd...not determined, m...monocotyledonous, d...dicotyledonous

0...no injury (equal to control), 1...1-24% injury, 2...25-69% injury, 3...70-89% injury, 4...90-100% injury (100% = complete kill of all plants of that species)

Conclusions:

Based on the results of the pre- and post-emergence screening data a high toxicity to a various dicotyledonous and monocotyledonous plant species was identified.

Comment RMS:

The pre- and post-emergence screening test is not considered acceptable as important information for the evaluation of the results is missing.

- No information is given regarding the control group (seedling emergence, phytotoxic effects, survival of the seedlings)
- No analytical measurements were conducted to confirm the concentrations of application.
- No information is given on the test substance used in the pre- and post-emergence screening tests.
- Information on the study design, statistical analysis and test conditions is poorly reported in the study summary.

B.9.6.2. Testing on non-target plants

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulation and are presented in the respective Volume 3, B.9 (PPP).

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No studies regarding effects on other terrestrial organisms were submitted for the renewal of the EU approval of the active substance.

Based on the overall risk assessment no further studies on other terrestrial organisms are required.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

For the first EU approval of the active substance ethofumesate a study on effects on biological methods for sewage treatment was submitted. For the renewal of the EU approval a new activated sludge, respiration inhibition test was submitted. The study summaries are provided below.

The study by Castillo (1979) was conducted to no given test guideline.

Castillo, 1979*Methods*

The effect of ethofumesate (purity not given) on the activated sludge process was investigated under laboratory conditions. Ethofumesate from a stock solution in acetone was added to continuous flow activated sludge units operated on a synthetic sewage, each consisting of an aeration chamber and a sedimentation basin. Two units received ethofumesate as shock and continuous loads, and two units were used as control and solvent control, respectively. The ethofumesate concentrations examined were: 0.1, 1, 10, 25, 50 and 100 mg/L under the shock mode, and 0.1, 1, 10 and 100 mg/L under the continuous mode. The sequence of dosing was from low to high concentrations. The duration of shock at each concentration was 5 hours and shock tests were performed twice a week. The duration of continuous dosing was at least one week at each concentration. The performance of the units was monitored by measurements of total suspended solids concentration (TSS), total organic carbon concentration (TOC) and pH in influent and effluent water, turbidity in effluent water, and TSS, pH, dissolved oxygen concentration (DO) oxygen uptake rate (OUR), initial settling rate (ISR) and microscopic examination in mixed liquor (aeration chamber).

Results

No permanent adverse effects of ethofumesate on the performance of the activated sludge process were found at the concentrations and treatments tested.

Comments

The study does not refer to any guideline but is performed and reported in an acceptable way. The performance of the ethofumesate treated activated sludge units generally appeared to mimic the performance of the control units, however, no replicates indicating variation within treatments were used in the study.

Reference:	Activated sludge, respiration inhibition test with ethofumesate
Author(s), year:	Neuhahn, A., 2011
Report/Doc. number:	Study no. 2011/0146/01, Reference no. M-421988-01-1
Guideline(s):	OECD 209 (1984), EC Guideline Annex V - Method C.11 (2008)
GLP:	Yes
Deviations:	None
Validity:	Acceptable

<u>Material and methods:</u>	
Test substance:	Ethofumesate techn., batch no.: ABJJETN023, purity: 98.3% w/w, CAS no.: 26225-79-6
Test species:	Activated sludge (mixed population of aquatic microorganisms) Source: aeration tank of a domestic sewage treatment plant (Cologne-Stammheim)
Type of test, duration:	Laboratory aerobic activated sludge inhibition test, 3 hours
Applied concentrations:	
Nominal:	10, 100 and 1000 mg/L
Toxic reference	3,5-dichlorophenol (purity 99.6%) tested at concentrations of 2.5, 5, 10, 20 and 40 mg/L, 3 h EC ₅₀ = 7.553 mg/L
Substrate/test vessel:	Activated sludge (solid concentration: 2 g/L) and synthetic sewage feed (according OECD guideline) treated with the test item, reference substance and inoculum control, final volume 250 mL/flask Additional vessels to determine the physico-chemical oxygen consumption were prepared containing the test item, and the synesthetic medium but no activated sludge.
Incubation:	Aerated for 3 hours at 20 ± 2°C
Test parameters:	Inhibition of respiration rate (rate of oxygen uptake) pH-value, temperature and dissolved oxygen concentration were determined at the start and at the end of the test.
Statistics:	Test item: EC ₅₀ and NOEC were determined directly from the raw data Toxic reference: EC ₅₀ was calculated based on probit analyses
<u>Findings:</u>	
Oxygen consumption	The respiratory rates of the two control groups differ less than 15% from each other. No effects on oxygen consumption were observed in all treatment groups up to a test concentration of 1000 mg a.s./L (0% inhibition).

Table B.9.8-1: Effect of ethofumesate on the respiration rate of activated sludge

Test substance	Nominal concentration [mg/L]	pH	Respiratory rate [mg O ₂ /L/min]	Oxygen concentration [mg O ₂ /L]		Inhibition [%]
				Start	End	
Control 1	-	8.0	26.00	5.4	2.8	-
Control 2	-	8.0	25.20	4.9	2.8	-
Control 3 ^a	1000	7.3	0.00	6.9	6.9	-
Ethofumesate	10	8.1	36.00	5.5	2.5	0.00
	100	7.9	48.00	3.6	2.8	0.00
	1000	7.9	26.00	4.2	2.9	0.00
Toxic reference	2.5	8.0	20.25	5.4	2.7	20.90
	5	7.9	20.00	4.7	2.7	21.88
	10	7.9	8.25	6.2	5.1	67.77
	20	7.9	3.43	6.5	6.1	86.61
	40	8.0	2.25	6.7	6.4	91.21

^a Physico-chemical oxygen-consumption control

Conclusions: EC₅₀ > 1000 mg ai/L
based on nominal concentrations.

Comment RMS: The study was conducted according to the OECD test guideline 209 (1984) and the EC test guideline. Under consideration of the validity criteria given in the test guidelines the study is considered valid.

The respiratory rates of the two control groups differ less than 15% from each other. The EC₅₀ of the reference compound 3,5-Dichlorophenol is in the range of 5 and 30 mg ai/L (being: 7.553 mg ai/L).

B.9.9. MONITORING DATA

A literature review was carried out for ethofumesate and the relevant metabolites, relevant impurities and trade names according to the requirements of the Regulation (EU) No 844/2012” (the AIR3 renewal regulation), which itself refers to Article 8(5) of Regulation (EC) No 1107/2009. The review itself is in accordance with the EFSA Guidance document as published in EFSA Journal 2011; 9(2):2092.

Search of the scientific peer reviewed open literature was conducted by both notifiers, covering a period from 2003 to 2013.

Both Task Force and UPL used different bibliographic databases but at least some of the databases were considered appropriate by RMS for search on ecotoxicology, e.g. Agricola.

While Task Force conducted the search using only the name of active substance, known metabolites and its trade names without considering any keywords, UPL included in the search only the active substance name combined by Boolean operators with some keywords which they considered relevant to address data requirements.

The RMS concluded that in case of ethofumesate, where all detected metabolites are unique ethofumesate metabolites, the metabolites would be captured in the search also by using only the key word ethofumesate. Regarding the inclusion of the trade names in the search it is considered that this should be done case by case (if it is known that the formulation has a higher toxicity than the active substance) since trade names might tremendously increase the “background noise” (amount of information not related to the topic) in the search.

After the rapid and the full-text assessment the Task Force concluded that none of the articles retrieved would change the risk assessment based on the available data.

However, three publications on monitoring data concerning potential adverse effects of the active substance to non-target aquatic organisms were obtained. Summaries of these peer-reviewed publications are provided below.

After detailed assessment of the chosen approaches for the literature search, the RMS concluded that both notifiers, although having different approaches, appropriately addressed the scientific peer reviewed open literature.

Reference:	Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination.
Author(s), year:	Berenzen, N., Kumke, T., Schulz, H., Schulz, R., 2004
Report/Doc. number:	M-458568-01-1
Guidelines:	Not applicable
GLP/GEP:	No

Executive summary:

The study aimed to assess the contribution of runoff-related pesticide entries to the macroinvertebrate community structure in small headwater streams. Amongst various selected pesticides ethofumesate was analytically measured in stream water at 6 sampling sites in middle Germany (Braunschweig) following three intense rainfall events. Event-triggered sampling systems were used. Among others, ethofumesate was detected at four sampling sites. At one sampling site, only one of the three runoff events resulted in the presence of a.s. in a concentration close to the detection limit (i.e. 0.05 µg/L). At three sampling sites, ethofumesate residues were detected after each of three runoff events with maximum concentrations of 1.0, 3.0 and 1.2 µg/L, respectively. At the remaining two sampling sites ethofumesate was not detected.

The toxicity of pesticide mixtures contained in a water sample was assessed by calculating a sum parameter based on toxic units (TU_{sum}). For this calculation acute *Daphnia magna* endpoints from secondary sources were used i.e. derived from US EPA database (endpoint used for ethofumesate was 13500 µg/L). To investigate effects of the pesticide mixtures contained in the water samples on macroinvertebrate communities in the streams, macroinvertebrates were sampled three times during the investigation period.

The community composition of three pesticide contaminated stream sites (maximal TU_{sum} values ≥ 0.02, corresponding to total pesticide concentrations ≥ 0.02 × acute toxicity to *Daphnia magna*) was clearly distinct from control sites (TU_{sum} < 0.00001). A specific statement with respect to toxicity of detected ethofumesate concentrations was not made.

Reference:	Current-use pesticides in stream water and suspended particles following runoff: exposure, effects, and mitigation requirements
Author(s), year:	Bereswill, R., Streloke, M., Schulz, R., 2013
Report/Doc. number:	M-462597-02-1
Guidelines:	Not applicable
GLP/GEP:	No

Executive summary:

The main objectives of the present study were to characterize pesticide exposure in stream water and suspended particles under current conventional agricultural farming practices following rainfall-related, edge-of-field runoff events and to detect pesticide effects on the macroinvertebrate community. Amongst various selected pesticides ethofumesate was analytically measured in stream water and suspended particles at 10 sampling sites in middle Germany (Braunschweig) following three intense rainfall events. Event-triggered sampling systems were used. Ethofumesate was detected in 13 water samples with an average concentration of $3.6 \pm 6 \mu\text{g/L}$ (range not given, maximum determined $21 \mu\text{g/L}$) and in 12 suspended particles samples with an average of $17 \pm 18 \mu\text{g/kg}$ dry weight (dw) (range not given, maximum = $51 \mu\text{g/kg}$ dw). The toxicity of measured pesticides was assessed using the toxic unit (TU) approach, relating the determined concentration in the environmental compartment with a laboratory measured effect concentration. For this calculation an acute *Daphnia magna* endpoint for ethofumesate derived from the Pesticide Properties Database developed by the Agriculture and Environment Research Unit was used ($\text{EC}_{50} = 14000 \mu\text{g ai/L}$). All reported water-phase based logTU for ethofumesate were < -3.5 . No effects were expected to occur as a direct result of these concentrations.

Macroinvertebrate community structure at the stream sites was determined at the beginning and the end of the study period (lasting from 10 May to 14 June). Generally, invertebrate fauna was dominated by pesticide-tolerant species. A shift in macroinvertebrate community structure towards more sensitive species (indicated in terms of SPEAR indices) could be observed from May to June at sites receiving highly toxic water-phase pesticide entries ($\text{logTU} > -2$). No acute significant negative effects on macroinvertebrates were observed at sites where ethofumesate accounted for the maximum TU ($\text{TU}_{\text{max}} < -3.5$) and sites where no pesticides were detected in-stream water.

Reference:	Analyzing effects of pesticides on invertebrate communities in streams.
Author(s), year:	Liess, M., Von Der Ohe, P., 2005
Report/Doc. number:	M-458575-01-1
Guidelines:	Not applicable
GLP/GEP:	No

Executive summary:

The aim of study was to find patterns in macroinvertebrate community composition that were related to the effect of pesticides. Amongst various selected pesticides ethofumesate was analytically measured in stream

water and suspended particles at 20 sampling sites in middle Germany (Braunschweig) following intense rainfall events. Event-triggered sampling systems (an automated active sampler and two passive samplers) were used. During the investigation period (1.8 years per site), pesticides (including ethofumesate) were detected at 125 runoff events at 18 of the 20 sites. The mean measured concentration of ethofumesate for all stream sites was reported to be $8.66 \pm 23.35 \mu\text{g/L}$. The highest measured concentration was $129 \mu\text{g/L}$ corresponding to a logTU of -2.02.

Streams were grouped according to their TU, and differences between groups were analysed. Comparing the mean percent abundance of species at risk (SPEAR) in June, revealed that the mean values of the groups of streams with logTU values of -3 to -2, -2 to -1 and -1 to 0 were statistically significant lower compared to the mean of unpolluted sites (logTU < -4). The stream site receiving $129 \mu\text{g/L}$ ethofumesate (on 14-06-99) belongs to the group of streams with logTU in the range of -3 to -2. However, the article contains no information if at this site with the maximum ethofumesate peak concentration any difference to unpolluted streams sites was observed.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

There are no concerns for groundwater from the use of ethofumesate in accordance with the use pattern for the current formulation.

PEC_{GW} values were calculated for the active substance and the metabolites NC 8493 and NC20645 considering the representative GAP uses (pre- and post-emergence). The PEC_{GW} were calculated using the model FOCUS PEARL and FOCUS PELMO. For details of the calculation please refer to Section B.8 (PPP).

Based on the FOCUS modelling no PEC_{GW} values (for all FOCUS scenarios) greater than $0.1 \mu\text{g/L}$ were determined for the active substance and its metabolites considering all FOCUS scenarios.

Hence, no assessment of the biological activity of these metabolites is considered necessary.

B.9.11. 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
KCA 8.1.1.1	[REDACTED]	1990a	THE ACUTE ORAL TOXICITY (LD50) OF ETHOFUMESATE TO THE MALLARD DUCK [REDACTED] Bayer CropScience, Report no.: A87610, Edition Number: M-161543-01-1 Date: 1990-11-15 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.1	[REDACTED]	1990b	THE ACUTE ORAL TOXICITY (LD50) OF ETHOFUMESATE TO THE BOBWHITE QUAIL [REDACTED] Bayer CropScience, Report no.: A87612, Edition Number: M-161547-01-1 Date: 1990-11-30 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.1	[REDACTED]	1977a	THE ACUTE ORAL TOXICITY (LD50) OF NC 8438 TO THE MALLARD DUCK [REDACTED] Bayer CropScience, Report no.: A83331, Report includes Trial Nos.: FPL 245 WL/77937 Edition Number: M-155600-01-1 Date: 1977-12-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.1	[REDACTED]	1977b	ACUTE ORAL TOXICITY (LD50) OF NC8438 TO THE BOBWHITE QUAIL [REDACTED] Bayer CropScience, Report no.: A83330, Report includes Trial Nos.: FPL 245 WL/77934 Edition Number: M-155599-01-1 Date: 1977-12-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.2	[REDACTED]	1994a	Short term toxicity, 8d study on birds [REDACTED] TF- Ethofumesate, Report no.: M-468479-01-1, Edition Number: M-468479-01-1 Date: 1994-12-01 GLP/GEP: no, unpublished ...also filed: KCA 8.1.1.1 /06	Y	N	-	Adama (formerly Feinchemie Schwebda)	In the DAR (1998)
KCA 8.1.1.2	[REDACTED]	1991a	TECHNICAL ETHOFUMESATE: SUBACUTE DIETARY TOXICITY (LC 50) TO MALLARD DUCK [REDACTED] Bayer CropScience, Report no.: A83367, Report includes Trial Nos.: SMS 269/91303	Y	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
			TOX 90539 Edition Number: M-155635-01-1 EPA MRID no.: 41949202 Date: 1991-05-15 GLP/GEP: yes, unpublished					
KCA 8.1.1.2		1990a	THE DIETARY TOXICITY (LC50) OF ETHOFUMESATE TO THE MALLARD DUCK Bayer CropScience, Report no.: A87611, Edition Number: M-161545-01-1 Date: 1990-11-30 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.2		1991b	TECHNICAL ETHOFUMESATE: SUBACUTE DIETARY TOXICITY (LC 50) TO BOBWHITE QUAIL Bayer CropScience, Report no.: A83369, Report includes Trial Nos.: SMS 268/91302 TOX 90538 Edition Number: M-155637-01-1 EPA MRID no.: 41949201 Date: 1991-05-15 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.2		1990b	THE DIETARY TOXICITY (LC50) OF ETHOFUMESATE TO THE BOBWHITE QUAIL Bayer CropScience, Report no.: A87613, Edition Number: M-161549-01-1 Date: 1990-11-30 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.1.1.3		1994b	Effects on reproduction TF- Ethofumesate, Report no.: M-468481-01-1, Edition Number: M-468481-01-1 Date: 1994-12-01 GLP/GEP: no, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In the DAR (1998)
KCA 8.1.1.3		2001	Bobwhite quail dietary reproduction study Ethofumesate Code: AE B049913 00 1D97 0002 Bayer CropScience, Report no.: C013708, Report includes Trial Nos.: 1999.0060 Edition Number: M-205119-01-1 Date: 2001-05-15 GLP/GEP: yes, unpublished	Y	Y	Required for risk assessment	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 8.1.1.3		2000	Mallard duck dietary reproduction study Ethofumesate Code: AE B049913 00 1D97 002 Bayer CropScience,	Y	N	-	Bayer CropScience	In the Addendum to the DAR (2000)

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
			Report no.: C008193, Report includes Trial Nos.: TOX99077 Edition Number: M-197270-01-1 Date: 2000-08-25 GLP/GEP: yes, unpublished					
KCA 8.2.1		1991b	THE ACUTE TOXICITY OF [14C]- ETHOFUMESATE TO BLUEGILL SUNFISH (<i>Lepomis macrochirus</i>) UNDER SEMI-STATIC CONDITIONS Bayer CropScience, Report no.: A83373, Report includes Trial Nos.: 86B Edition Number: M-155641-01-1 EPA MRID no.: 42015501 Date: 1991-08-09 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.1		1990a	DETERMINATION OF ACUTE TOXICITY (LC50) TO RAINBOW TROUT (96H, SEMI-STATIC) Ethofumesate Bayer CropScience, Report no.: A87614, Report includes Trial Nos.: 141714 Edition Number: M-161551-01-1 EPA MRID no.: 46546301 Date: 1990-11-06 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.1		1990b	DETERMINATION OF ACUTE TOXICITY (LC50) TO BLUEGILL SUNFISH (96H, SEMI-STATIC) Ethofumesate Bayer CropScience, Report no.: A87615, Report includes Trial Nos.: 141709 Edition Number: M-161552-01-1 Date: 1990-11-06 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.1		1989	TECHNICAL ETHOFUMESATE - DETERMINATION OF ACUTE TOXICITY (LC50) TO MIRROR CARP (96 HOURS, SEMISTATIC) AND THE ANALYSIS OF ETHOFUMESATE IN WATER SAMPLES Bayer CropScience, Report no.: A83349, Report includes Trial Nos.: 140438 79B Edition Number: M-155618-01-1 Date: 1989-10-12 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.1		1992a	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL	Y	Y	Needed for risk	Bayer CropScience	Submitted for the

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
	B. J.		TO THE SHEEPSHEAD MINNOW (<i>Cyprinodon variegatus</i>) IN A STATIC SYSTEM [REDACTED] Bayer CropScience, Report no.: A83384, Edition Number: M-155652-01-1 EPA MRID no.: 42409301 Date: 1992-06-12 GLP/GEP: yes, unpublished			assessment		purpose of renewal (2014)
KCA 8.2.1	[REDACTED]	1991a	Acute toxicity in rainbow trout (<i>Salmo Gairdneri</i>) test article: Ethofumesate [REDACTED] Feinchemie Schebda , Report no.: OFC00004887, Edition Number: M-352116-01-1 Date: 1991-09-12 GLP/GEP: yes, unpublished	Y	N	-	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 8.2.1	[REDACTED]	1993a	Acute toxicity in golden orfe (<i>Leuciscus Idus</i>) - Test article: Ethofumesate techn. [REDACTED] Feinchemie Schebda , Report no.: OFC00004888, Edition Number: M-352126-01-1 Date: 1993-03-20 GLP/GEP: yes, unpublished	Y	N	-	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 8.2.2	[REDACTED]	1990	A STUDY OF THE PROLONGED TOXICITY TO FISH (<i>Salmo gairdneri</i>) OF ETHOFUMESATE TECHNICAL [REDACTED] Bayer CropScience, Report no.: A83355, Report includes Trial Nos.: 78B BE-ET-12-89-02-FIP-2 Edition Number: M-155624-01-1 Date: 1990-05-29 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.2	[REDACTED]	1991b	Prolonged toxicity test in rainbow trout (<i>Salmo Gairdneri</i>) - Test article: Ethomumesate [REDACTED] Feinchemie Schebda , Report no.: OFC00004889, Edition Number: M-352123-01-1 Date: 1991-09-12 GLP/GEP: yes, unpublished	Y	N	-	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 8.2.2	[REDACTED]	1993	21-DAY PROLONGED TOXICITY STUDY IN THE RAINBOW TROUT UNDER FLOW-THROUGH CONDITIONS Ethofumesate [REDACTED] Bayer CropScience, Report no.: A87616, Edition Number: M-161553-01-1	Y	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: 1993-04-27 GLP/GEP: yes, unpublished					
KCA 8.2.2.1	[REDACTED]	2013	Ethofumesate technical: Statistical Re-evaluation of the fish early life stage toxicity study with fathead Minnow (<i>Pimephales promelas</i>) by [REDACTED] 1991 Bayer CropScience, Bayer CropScience, Report no.: M-470756-01-1, Edition Number: M-470756-01-1 GLP/GEP: n.a., unpublished	Y	Y	Needed for risk assessment	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.2.1	[REDACTED]	1991	ETHOFUMESATE - FATHEAD MINNOW (<i>Pimephales promelas</i>) EARLY LIFE STAGE TOXICITY TEST [REDACTED] Bayer CropScience, Report no.: A83372, Edition Number: M-155640-01-1 EPA MRID no.: 42008901 Date: 1991-07-08 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.2.2	[REDACTED]	2013	Zebra fish (<i>Danio rerio</i>), life cycle test, flow through conditions - Ethofumesate [REDACTED] Bayer CropScience, Report no.: BAY-035/4-60/A, Edition Number: M-464613-01-1 Date: 2013-08-20 GLP/GEP: yes, unpublished	Y	Y	New data requirement	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.2.3	[REDACTED]	1991	DETERMINATION OF THE ACCUMULATION AND ELIMINATION OF [14C]-ETHOFUMESATE IN BLUEGILL SUNFISH (<i>Lepomis macrochirus</i> L.) [REDACTED] Bayer CropScience, Report no.: A83371, Report includes Trial Nos.: 83B Edition Number: M-155639-01-1 EPA MRID no.: 41970704 Date: 1991-07-11 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.2.3	[REDACTED]	1992	BIOACCUMULATION TEST IN BLUEGILL SUNFISH 14C-Ethofumesate [REDACTED] Bayer CropScience, Report no.: A87617, Report includes Trial Nos.: 141541 Edition Number: M-161555-01-1 Date: 1992-05-29 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.4.1	Juckeland, D.	2013a	Acute toxicity of NC 20645 to <i>Daphnia magna</i> in a 48-hour static test United Phosphorus Ltd., 13 10 48 030 W BioChem Agrar, Gerichshain,	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)

Annex point	Author(s)	Year	Title Source (where different from company) Company name, Report no., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Germany GLP: yes Published: no					
KCA 8.2.4.1	Juckeland, D.	2013b	Acute toxicity of NC 8493 to <i>Daphnia magna</i> in a 48-hour static test United Phosphorus Ltd., 13 10 48 015 W BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.2.4.1	Koenig, N.	2013	Acute toxicity of ethofumesate acetic acid to the waterflea <i>Daphnia magna</i> in a static laboratory test system - Limit test Bayer CropScience, Report no.: EBADN008, Edition Number: M-444843-01-1 Date: 2013-01-10 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.4.1	Riebschlaeger, T.	2012	Acute toxicity of BCS-BB94377 (tech.) to the waterflea <i>Daphnia magna</i> in a static-renewal laboratory test system - Limit test - Amendment 1 to report Bayer CropScience, Report no.: EBADL039, Edition Number: M-434284-02-1 Date: 2012-06-27 ...Amended: 2013-03-18 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.4.1	Riebschlaeger, T.	2012	Acute toxicity of BCS-AV65501 (tech.) to the waterflea <i>Daphnia magna</i> in a static-renewal laboratory test system - Limit test - Amendment 1 to report Bayer CropScience, Report no.: EBADL038, Edition Number: M-434289-02-1 Date: 2012-06-27 ...Amended: 2013-03-18 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.4.1	Thun, S.	1993b	Acute toxicity in <i>Daphnia Magna</i> - Test article: Ethofumesate techn. IBR Forschungs GmbH, Walsrode, Germany Feinchemie Schebda, Report no.: 80-91-2312-02-93, Edition Number: M-352128-01-1 Date: 1993-03-15 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 8.2.4.2	Schupner, J. K.; Stachura, B. J.	1992b	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL TO THE MYSID SHRIMP <i>Mysidopsis bahia</i> IN A STATIC SYSTEM Nor-Am Chemical Company, Pikeville, NC, USA Bayer CropScience, Report no.: A83389, Edition Number: M-155657-01-1 EPA MRID no.: 42364502 Date: 1992-06-12 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 8.2.5.1	Adema, D. M. M., de Ruiter,	1989	THE CHRONIC TOXICITY OF ETHOFUMESATE TO <i>Daphnia</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
	A.		<i>magna</i> TNO; Bayer CropScience, Report no.: A83345, Report includes Trial Nos.: 70B Edition Number: M-155614-01-1 EPA MRID no.: 41554103 Date: 1989-10-04 GLP/GEP: yes, unpublished					
KCA 8.2.5.1	Bellmann, W.	1992a	21 d Daphnia-reproduction test according to OECD guideline 202, part II - Test article ethofumesate Technischer Ueberwachungsverein, Filderstadt, Germany Feinchemie Schwebda, Report no.: OFC00004891, Edition Number: M-352134-01-1 Date: 1992-09-21 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 8.2.5.1	Douglas, M. T.; James, C. M.; McDonald, I. A.	1990a	AN ASSESSMENT OF THE EFFECTS OF ETHOFUMESATE ON THE REPRODUCTION OF <i>Daphnia magna</i> Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87619, Edition Number: M-161558-01-1 Date: 1990-10-26 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.5.4	Desmares-Koopmans, M.J.E.	2002	Sediment-Water Chironomid Toxicity Test using water spiked with Ethofumesate AgriChem B.V., 324089 Notox B.V, 5231 DD 's-Hertogenbosch, The Netherlands GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 8.2.5.4	Mattock, S. D.	1998	Chronic toxicity to the sediment dwelling organism <i>Chironomus riparius</i> (BBA method) Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report no.: A91783, Report includes Trial Nos.: 194/183 Envir 208B Edition Number: M-168438-01-1 Date: 1998-03-30 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.5.4	Stäbler, D.	2003	Assessment of side effects of Ethofumesate Technical on the larvae of the midge, <i>Chironomus riparius</i> with the Laboratory Test Method United Phosphorus Ltd., 20021050/01-ASCr GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.2.6.1	Bruns, E.	2008	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with	N	Y	Needed for risk	TaskForce Ethofumesate	Submitted for the

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
			ethofumesate (techn.) Bayer CropScience, Report no.: EBADL004, Edition Number: M-302092-03-1 Date: 2008-06-04 ... Amended: 2010-02-16 GLP/GEP: yes, unpublished			assessment		purpose of renewal (2014)
KCA 8.2.6.1	Bruns, E.	2012a	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with ethofumesate-NC8493 (AE C508493) Bayer CropScience, Report no.: EBADL032, Edition Number: M-436372-01-1 Date: 2012-08-03 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.6.1	Bruns, E.	2012b	Amendment no.1- <i>Pseudokirchneriella subcapitata</i> growth inhibition test with BCS-AV65501 - limit test Bayer CropScience, Report no.: EBADL035, Edition Number: M-437568-02-1 Date: 2012-08-27 ... Amended: 2012-10-08 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.6.1	Juckeland, D.	2013c	Effects of NC 20645 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test under static conditions United Phosphorus Ltd., 13 10 48 029 W BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.2.6.1	Juckeland, D.	2013d	Effects of NC 8493 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test under static conditions (including amendment) United Phosphorus Ltd., 13 10 48 014 W BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.2.6.1	Sobczyk, H.	2013	<i>Pseudokirchneriella subcapitata</i> growth inhibition test with BCS-CW35117 Bayer CropScience, Report no.: E 323 4457-8, Edition Number: M-459906-01-1 Date: 2013-06-27 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.6.2	Banman, C. S.; Daly, R. A.; Lam, C. V.	2009a	Toxicity of ethofumesate technical to the blue green algae <i>Anabaena flos-aquae</i> Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL008, Edition Number: M-349150-01-1 Date: 2009-06-10 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.6.2	Banman, C. S.; Daly, R. A.;	2009b	Toxicity of ethofumesate technical to the saltwater diatom	N	Y	Needed for risk	TaskForce Ethofumesate	Submitted for the

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
	Lam, C. V.		<i>Skeletonema costatum</i> Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL009, Edition Number: M-347965-01-1 Date: 2009-05-19 GLP/GEP: yes, unpublished			assessment		purpose of renewal (2014)
KCA 8.2.7	Banman, C. S.	2011	Toxicity of ethofumesate technical to the aquatic macrophyte, <i>Myriophyllum spicatum</i> (amended final report) Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL019-1, Edition Number: M-411454-02-1 Date: 2011-07-25 ...Amended: 2013-05-22 GLP/GEP: yes, unpublished	N	Y	Not a formal data requirement for herbicides according to EU Regulations for chemical active substances no 283/2013 under 1107/2009, however considered to be relevant for risk assessment as this species was found to be the new most sensitive species.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.2.7	Bogers, M.	2001	A 7-Day Aquatic Plant Toxicity Test using <i>Lemna minor</i> with Ethofumesate AgriChem B.V., 324078 Notox B.V, 5231 DD 's-Hertogenbosch, The Netherlands GLP: yes Published: no	N	N	-	ACM*	Submitted for the purpose of renewal (2014)
KCA 8.2.7	Scheerbaum, D.	1998b	Ethofumesate - Substance technical 98.8 percent w/w - <i>Lemna minor</i> : Semi static phytotoxicity test - Code: AE B049913 00 1D97 0002 Dr. U. Noack-Laboratorium fuer Angewandte Biologie, Sarstedt, Germany Bayer CropScience, Report no.: A91865, Report includes Trial Nos.: ENVIR/211B TLA5699- TLA56991 Edition Number: M-168516-01-1 Date: 1998-05-28 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.2.8	Papadopoulou-Mourkidou, E.; Vassiliou, G.; Vryzas, Z.; Alexoudis, C.; Galanis, K.	2011	Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in northeastern Greece. Journal: Ecotoxicol. Environ. Saf., Volume:74, Issue:2, Pages:174-181, Year:2011, Report no.: M-458635-01-1, Edition Number: M-458635-01-1	N	N	-	LIT	Submitted for the purpose of renewal (2014) (Used as additional information)

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: 2011-12-31 GLP/GEP: no, published ...also filed: KCA 7.5 /07					
KCA 8.2.8	Yurk, J. J.; Ache, B. W.	1992	EFFECT OF ETHOFUMESATE TECHNICAL ON NEW SHELL GROWTH IN THE EASTERN OYSTER (<i>Crassostrea virginica</i>) UNDER FLOW-THROUGH TEST CONDITIONS Environmental Science and Engineering, Inc., Gainesville, FL, USA Bayer CropScience, Report no.: A83386, Report includes Trial Nos.: 507B Edition Number: M-155654-01-1 EPA MRID no.: 42388101 Date: 1992-05-28 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 8.3.1.1.1	Barrett, K. L.	1991a	THE ACUTE ORAL AND TOPICAL TOXICITIES OF ETHOFUMESATE TO WORKER HONEYBEES (<i>Apis mellifera</i> L) Schering AG, Berlin, Germany Bayer CropScience, Report no.: A83374, Report includes Trial Nos.: 87B Edition Number: M-155642-01-1 EPA MRID no.: 41970703 Date: 1991-07-09 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.2 /01	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.3.1.1.1	Cole, J. H.	1990	THE ACUTE CONTACT AND ORAL TOXICITY TO HONEY BEES OF ETHOFUMESATE TECHNICAL Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87621, Edition Number: M-161561-01-1 Date: 1990-02-19 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.2 /02	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.3.1.1.1	Schmitzer, S.	2011a	Effects of ethofumesate tech. (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report no.: 64231035, Edition Number: M-421681-01-1 Date: 2011-12-19 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.2 /03	N	Y	Required due to deficiencies in previous studies	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.3.1.1.2	Barrett, K. L.	1991b	THE ACUTE ORAL AND TOPICAL TOXICITIES OF ETHOFUMESATE TO WORKER HONEYBEES (<i>Apis mellifera</i> L) Schering AG, Berlin, Germany Bayer CropScience, Report no.: A83374, Report includes Trial Nos.: 87B	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
			Edition Number: M-155642-01-1 EPA MRID no.: 41970703 Date: 1991-07-09 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.1 /01					
KCA 8.3.1.1.2	Cole, J. H.	1990	THE ACUTE CONTACT AND ORAL TOXICITY TO HONEY BEES OF ETHOFUMESATE TECHNICAL Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87621, Edition Number: M-161561-01-1 Date: 1990-02-19 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.1 /02	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.3.1.1.2	Schmitzer, S.	2011b	Effects of ethofumesate tech. (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report no.: 64231035, Edition Number: M-421681-01-1 Date: 2011-12-19 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.1 /03	N	Y	Required due to deficiencies in previous studies	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.3.1.2	Kling, A.	2013	Ethofumesate (tech.) - Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days continuous laboratory feeding limit test eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report no.: S13-00144, Edition Number: M-469458-01-1 Date: 2013-11-04 GLP/GEP: yes, unpublished	N	Y	New regulatory requirement	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.4.1	Barrett, K. L.; Arnold, D. J.	1986	DETERMINATION OF THE TOXICITY OF ETHOFUMESATE IN EARTHWORMS (<i>Eisenia andrei</i>) USING AN ARTIFICIAL SOIL TEST FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report no.: A83286, Report includes Trial Nos.: 61B Edition Number: M-155555-01-1 Date: 1986-02-18 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.4.1	Friedrich, S.	2012a	AE C508493: Sublethal toxicity to the earthworm <i>Eisenia fetida</i> in artificial soil with 5 percent peat BioChem agrar GmbH, Gerichshain, Germany TF- Ethofumesate, Report no.: 11 10 48 063 S, Edition Number: M-435163-01-1 Date: 2012-04-05 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA	Hakin, B.;	1991	THE ACUTE TOXICITY (LC50)	N	N	-	Bayer	In the DAR

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
8.4.1	Rodgers, M. H.; Johnson, A. J.		OF ETHOFUMESATE TO THE EARTHWORM (<i>Eisenia foetida</i>) Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87622, Edition Number: M-161563-01-1 Date: 1991-03-27 GLP/GEP: yes, unpublished ...also filed: KCA 8.7 /01				CropScience	(1998)
KCA 8.4.1	Lührs, U.	2011a	Effects of NC8493 on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat United Phosphorus Ltd., 63561022 IBACON GmbH, Rossdorf Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.4.2.1	Friedrich, S.	2012c	AE C508493: Effects on the reproduction of the collembolans <i>Folsomia candida</i> BioChem agrar GmbH, Gerichshain, Germany TF- Ethofumesate, Report no.: 12 10 48 018 S, Edition Number: M-435155-01-1 Date: 2012-04-05 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.4.2.1	Friedrich, S.	2013b	Effects of 2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (NC 8493) on the reproduction of the collembolan <i>Folsomia candida</i> United Phosphorus Ltd., 13 10 48 062 S BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.4.2.1	Schulz, L.	2013a	Effects of 2,3-Dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate (NC 8493) on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> United Phosphorus Ltd., 13 10 48 063 S BioChem Agrar, Gerichshain, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 8.5	Aldred, D.	1993	A LABORATORY ASSESSMENT OF THE EFFECTS OF ETHOFUMESATE ON SOIL MICROFLORA RESPIRATION Euro Laboratories Ltd., Buckinghamshire, United Kingdom Bayer CropScience, Report no.: A83392, Report includes Trial Nos.: 96B Edition Number: M-155660-01-1 Date: 1993-06-07 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA	Hossack, D. J.	1991	THE EFFECT OF	N	N		Bayer	In the DAR

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
8.5	N.; Thomas, F. J.; Chanter, D. O.		ETHOFUMESATE ON SOIL MICRO-FLORA RESPIRATION Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report no.: A87660, Edition Number: M-161631-01-1 Date: 1991-01-03 GLP/GEP: yes, unpublished				CropScience	(1998)
KCA 8.5	Schulz, L.	2013b	AE C508493: Effects on the activity of soil microflora (Nitrogen transformation test) BioChem Agrar GmbH, Gerichshain, Germany TF- Ethofumesate, Report no.: 13 10 48 074 N, Edition Number: M-460349-01-1 Date: 2013-07-05 GLP/GEP: yes, unpublished	N	Y	Necessary due to change in residue definition for risk assessment.	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 8.5	Voets, J. P.; Angerosa, I. M. O.; Goddeeris, H.; Verstraete, W.	1977	The influence of pyrazon, ethofumesate and metamilon on the soil microbiota Publisher: Magyar Tudományok Akadémiája, Location: Hungary, Journal: Acta Phytopathologica Academiae Scientiarum Hungaricae, Volume: 12, Issue: 1-2, Pages: 31-39, Year: 1977, Report no.: Lit. 2128, Edition Number: M-155551-01-2 Date: 1977-01-01 GLP/GEP: n.a., published	N	N	-		In the DAR (1998)
KCA 8.5	Vonk, J. W.	1988	EFFECT OF ETHOFUMESATE ON NITROGEN TRANSFORMATIONS IN SOIL TNO Division of Technology for Society, Delft, Netherlands Bayer CropScience, Report no.: A87623, Edition Number: M-161564-01-1 Date: 1988-02-22 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.8	Castillo, M. J.	1979	EFFECTS OF TECHNICAL ETHOFUMESATE ON THE PERFORMANCE OF ACTIVATED SLUDGE PROCESS Union Carbide Corporation, USA Bayer CropScience, Report no.: A83275, Edition Number: M-155544-01-1 Date: 1979-02-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In the DAR (1998)
KCA 8.8	Neuhahn, A.	2011	Activated sludge, respiration inhibition test with Ethofumesate Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report no.: 2011/0146/01, Edition Number: M-421988-01-1 Date: 2011-12-14	N	Y	Required as old study does not comply with new data requirements	TaskForce Ethofumesate	Submitted for the purpose of renewal (2014)

Annex point	Author(s)	Year	Title Source (where different from company) Company name, Report no., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			GLP/GEP: yes, unpublished					
KCA 8.9	Berenzen, N.; Kumke, T.; Schulz, H.; Schulz, R.	2004	Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. Journal:Ecotoxicol. Environ. Saf., Volume:60, Issue:1, Pages:37-46, Year:2004, Report no.: M-458568-01-1, Edition Number: M-458568-01-1 Date: 2004-12-31 GLP/GEP: no, published ...also filed: KCA 7.5 /11	N	N	-	LIT	Submitted for the purpose of renewal (2014) (Used as additional information)
KCA 8.9	Bereswill, R.; Streloke, M.; Schulz, R.	2013	Current-use pesticides in stream water and suspended particles following runoff: exposure, effects, and mitigation requirements Journal:Environmental Toxicology and Chemistry, Volume:32, Issue:6, Pages:1254-1263, Year:2013, Report no.: M-462597-02-1, Edition Number: M-462597-02-1 Date: 2013-08-01 GLP/GEP: no, published ...also filed: KCA 7.5 /10	N	N	-	LIT	Submitted for the purpose of renewal (2014) (Used as additional information)
KCA 8.9	Liess, M.; Von Der Ohe, P.	2005	Analyzing effects of pesticides on invertebrate communities in streams. Journal:Environ. Toxicol. Chem., Volume:24, Issue:4, Pages:954-965, Year:2005, Report no.: M-458575-01-1, Edition Number: M-458575-01-1 Date: 2005-12-31 GLP/GEP: no, published ...also filed: KCA 7.5 /12	N	N	-	LIT	Submitted for the purpose of renewal (2014) (Used as additional information)

* AgriChem B.V. is part of United Phosphorus Ltd since the summer of 2012. Studies performed for Agrichem B.V. are therefore now fully owned by United Phosphorus Ltd

LIT: public literature