Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

ethofumesate (ISO); (*RS*)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate

EC Number: 247-525-3
CAS Number: 26225-79-6

CLH-O-0000001412-86-196/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 March 2018

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Ethofumesate

(±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate

EC Number: **247-525-3**

CAS Number: **26225-79-6**

Index Number: **607-314-00-2**

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CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1-1: Substance identity

Substance name:	***Ethofumesate (ISO);*** ***(±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate***
EC number:	***247-525-3***
CAS number:	***26225-79-6***
Annex VI Index number:	***607-314-00-2***
Degree of purity:	***Minimum purity 970 g/kg***
Impurities:	***Relevant impurities:*** ***Methane sulfonic acid ethyl ester (EMS)*** ***Ethyl, 1,1-dimethyl-2-[(methylsulfonyl)oxy]- (IBMS)***

1.2 Harmonised classification and labelling proposal

Table 1-2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Aquatic Chronic 2, H411 (Reg. 1272/2008)	Not relevant
Current proposal for consideration by RAC	Aquatic Acute 1, H400, M = 1 Aquatic Chronic 1, H410, M = 1 (based on new endpoints)	Not relevant
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1, H400, M = 1 Aquatic Chronic 1, H410, M = 1 (based on new endpoints)	Not relevant

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 1-3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.2.	Flammable gases	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.4.	Oxidising gases	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.5.	Gases under pressure	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.6.	Flammable liquids	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.7.	Flammable solids	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	No classification	Not applicable	None	Conclusive but not sufficient for classification

2.14.	Oxidising solids	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	No classification	Not applicable	None	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	No classification	Not applicable	None	Data lacking
3.1.	Acute toxicity - oral	No classification	Not applicable	None	Conclusive but not sufficient for classification
	Acute toxicity - dermal	No classification	Not applicable	None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	No classification	Not applicable	None	Data lacking
3.4.	Skin sensitisation	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	No classification	Not applicable	None	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	No classification	Not applicable	None	Conclusive but not sufficient for

					classification
4.1.	Hazardous to the aquatic environment	H400, H410,	M = 1 M = 1	H411	-
5.1.	Hazardous to the ozone layer	No classification	Not applicable	None	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning
Pictogram: GHS9
Hazard statements: H410

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Ethofumesate has been previously discussed in the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances Pesticides, ECB Ispra, 19-21 May 1999 (ECBI/43/99 Rev. 2) and 17.19 November 1999 (ECBI/07/00 Rev.3). In the Minutes of the meetings it was agreed on the following classification.

The Group agreed not to classify ethofumesate for health effects. It was agreed to classify ethofumesate with N; R51-53 for environmental effects.

2.2 Short summary of the scientific justification for the CLH proposal

For the re-newal of the active substance ethofumesate, according to the Regulation 1107/2009 new studies with the active substance were submitted by the applicants. In addition, existing studies were re-evaluated taking into account the current valid test guidelines.

Therefore, the classification and labelling of ethofumesate was evaluated based on the new information on the active substance.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Index Number	EC Number	CAS Number	International Chemical Identification
607-314-00-2	247-525-3	26225-79-6	ethofumesate (ISO) (±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate

ATP Inserted / Updated: CLP00
CLP Classification (Table 3.1)

Classification		Labelling			Specific Concentration limits, M-Factors	Notes
Hazard Class and Category Code (s)	Hazard Statement Code (s)	Hazard Statement Code (s)	Supplementary Hazard Statement Code (s)	Pictograms, Signal Word Code (s)		
Aquatic Chronic 2	H411	H411		GHS09		

Signal Words	Pictograms
No signal word	 Environment

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification according directive 67/548/EEC is no longer relevant.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

No information provided by the notifier.

2.4.2 Current self-classification and labelling based on DSD criteria

No information provided by the notifier.

RAC general comment
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 where performed using the racemic mixture.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification for pesticides.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

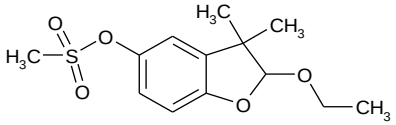
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1-5: Substance identity

EC number:	247-525-3
EC name:	Ethofumesate (ISO); (±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate
CAS number (EC inventory):	26225-79-6
CAS number:	26225-79-6
CAS name:	Ethofumesate
IUPAC name:	(*RS*)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate
CLP Annex VI Index number:	607-314-00-2
Molecular formula:	$C_{13}H_{18}O_5S$
Molecular weight range:	286.3 g/mol

Structural formula:



1.2 Composition of the substance

Table 1-6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Ethofumesate	Min. purities: 970 g/kg	-	-

Current Annex VI entry:

Table 1-7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Relevant impurities: Methane sulfonic acid ethyl ester (EMS) Ethyl, 1,1-dimethyl-2-[(methylsulfonyl)oxy]- (IBMS)	 Max. 0.1 mg/kg Max. 0.1 mg/kg	-	-

Current Annex VI entry:

Table 1-8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives				

Current Annex VI entry: -

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 1- 9: Summary of physico - chemical properties

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Melting point and boiling point						
Melting, freezing or solidification point **B.2.1/01**	EC A.1, OECD 102 DSC	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	The melting point of ethofumesate at atmospheric pressure (1013.3 hPa) is 70.7 °C.	**Acceptable** New study was performed since a melting range was stated in the DAR	Y	**Taskforce:** Smeykal, H.; 2008 M-299734-01-1
	OECD 102	Ethofumesate technical concentrate Content: 99.9% capillary method with photocell detection	Melting range: 69.6 - 70.7°C	EU agreed endpoint DAR 1998	Y	**UPL:** Ward, 1990 (see DAR)
Boiling point **B.2.1/02**	EC A.2, OECD 103 DSC	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	At 280 to 290 °C the colour of Ethofumesate turns from white to brown under evolution of bubbles. This behavior has to be interpreted as a decomposition process. Therefore Ethofumesate has no boiling point.	**Acceptable** New study was performed as the former was not according to GLP.	Y	**Taskforce:** Smeykal, H.; 2008 M-299734-01-1
	OECD 113 CIPAC MT 113 DSC and TGA	Ethofumesate technical concentrate F/97/010 Content: not stated	Ethofumesate decomposes before reaching the boiling point. Temperatures of decomposition are: 285°C (Differential Scanning Calorimetrie) and 224°C (Thermogravimetric Analysis).	EU agreed endpoint DAR 1998	Y	**UPL:** Werle, 1997 (see DAR)
Decomposition / Sublimation temperature **B.2.1/03**	OECD 113	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	DSC: Ethofumesate showed an endothermic effect in the temperature range 65 – 90 °C (melting) and an exothermal decomposition in the temperature range 290 – 405 °C with a mean energy of 375 J/g. Capillary method: Decomposition at 280 to 290 °C.	**Acceptable** New study was performed as the former was not according to GLP.	Y	**Taskforce:** Smeykal, H.; 2008 M-299734-01-1

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Vapour pressure, volatility						
Vapour pressure **B.2.2/01**	EC A.4, OECD 104 Gas saturation method	Ethofumesate Batch n° C66/87 purity 99.9 %	Extrapolated: 3.6 × 10-4 Pa for 20 °C 6.5 x 10-4 Pa for 25 °C Measured : 4.0 x 10-3 Pa for 40 °C	EU agreed endpoint DAR 1998	Y	**Taskforce and UPL:** Bright, A.A.S. 1988 M-155198-01-1
Volatility (Henry's Law constant) **B.2.2/02**	Calculation	Ethofumesate Batch n° C66/87 purity 99.9 %	Henry's law constant at 25 °C at different pH values: 3.72 x 10^{-3} Pa x m^3 x mol^{-1} No pH effect because the active substance is not ionisable.	EU agreed endpoint DAR 1998	N	**Taskforce and UPL:** Bright, A.S. Stalker, A.M. 1994 M-158022-01-1
			The Henry's law constants at 20°C were found to be: Milli RO water: K = 1.73 x 10^{-3} Pa•m^3•mol^{-1} pH 4 buffer solution: K = 1.77 x 10^{-3} Pa•m^3•mol^{-1} pH 7 buffer solution: K = 1.78 x 10^{-3} Pa•m^3•mol^{-1} pH 9 buffer solution: K = 1.66 x 10^{-3} Pa•m^3•mol^{-1} <u>parameter used for calculation:</u> Vapour pressure at 20 °C (extrapolated): 3.6 × 10^{-4} Pa. Water solubility at 20 °C in Milli RO water 59.59 mg • L^{-1} ≙ 59.59 g • m^{-3} pH 4 buffer solution 58.22 mg • L^{-1} ≙ 58.22 g • m^{-3} pH 7 buffer solution 57.83 mg • L^{-1} ≙ 57.83 g • m^{-3} pH 9 buffer solution 61.92 mg • L^{-1} ≙ 61.92 g • m^{-3}	**Acceptable**	N	**Taskforce and UPL:** Ziemer, F. (2015) M-521293-01-1
Appearance (physical state, colour)						
Physical state and colour **B.2.3/01**	OPPTS 830.6302, OPPTS 830.6303	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	Purified active substance : odorless white powder	**Acceptable** New study was performed as the former was not according to GLP.	Y	**Taskforce:** Ziemer, F. Strunk, B. 2012 M-431327-01-1

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
		Ethofumesate TGAI Batch n° AE B049913-01-08 purity 98.5 %	Active substance as manufactured : Beige platelets intensive odor (not characteristic)		Y	Ziemer, F. Strunk, B. 2012 M-431325-01-1
	visual assessment	Ethofumesate TGAI Content: 99.1%	White crystalline powder	**Acceptable** No information on the pure active substance is required since the purity of TGAI is > 98%.	Y	**UPL:** Diepenhorst, P.C. 2011
Spectra (UV/Vis, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity						
Ultraviolet/visible (UV/VIS) **B.2.4/01**	OECD 101 OPPTS 830.7050	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	UV/VIS (methanol) **Wavelength [nm]** / **Molar extinction coefficient [L/mol x cm]** 203 / 19441 228 / 7228 281 / 2797 291 / 1412 UV/VIS (methanol + HCl c_{HCl} = 0.1 mol/L) **Wavelength [nm]** / **Molar extinction coefficient [L/mol x cm]** 202 / 24122 227 / 7339 280 / 2797 291 / 1357 UV/VIS (methanol + NaOH c_{NaOH} = 0.1 mol/L)	**Acceptable** New study was performed as the former was not according to GLP and acetonitrile and chloroform, respectively was used as solvent. According to OECD 101 a suitable organic solvent should be used (methanol preferred) if it is not possible to obtain sufficient concentrations in any of the aqueous media.	Y	**Taskforce:** Wiche, A. Bogdoll, B 2012 M-435863-01-1

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results		Comments (Acceptable / Non acceptable)	GLP	Reference
			Wavelength [nm]	**Molar extinction coefficient [L/mol x cm]**			
			227	7339			
			280	2853			
			291	1357			
			At 290 nm Ɛ > 1000 L/mol x cm				
		Ethofumesate standard: 99.6%	Neutral in methanol		**Acceptable**	Y	**UPL:** Bhandari, N.M. (2013) Document provided in the confidential part Volume 4 since information on purity of batches and impurities is provided as well.
			Wavelength [nm]	**Molar extinction coefficient [L/mol x cm]**			
			202.5	17362.4			
			227	6716.4			
			280.5	2545.6			
			Acidic (methanol + HCl)				
			Wavelength [nm]	**Molar extinction coefficient [L/mol x cm]**			
			202.0	18872.3			
			227	6663.4			
			280.5	2529.7			
			Basic (methanol + NaOH)				
			Wavelength [nm]	**Molar extinction coefficient [L/mol x cm]**			
			212	6434.0			
			227	6549.6			
			280.0	2557.0			
			at 290 nm Ɛ>1000 L/mol x cm				
Infrared (IR)	OECD 101	Ethofumesate pure Batch n°	IR (attenuated total reflection diamond single reflection unit) The results demonstrate agreement with the proposed		**Acceptable**	Y	**Taskforce:**

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.4/02	OPPTS 830.7050	AE B049913 00 1B99 0002 purity 99.9 %	structure of the test item as demonstrated by the assignments of major absorption signals.	New study was performed as the former was not according to GLP.		Wiche, A. Bogdoll, B 2012 M-435863-01-1
		Ethofumesate pure purity 99.9 %	IR spectrum confirmed the structure of Ethofumesate. IR spectrum confirmed the structure of Ethofumesate.	EU agreed endpoint DAR 1998 **Acceptable** New study was performed as the former was not according to GLP	N Y	**UPL:** Audus, 1994, Anonymous, 1995 Patel, A.H. (2013c) Documents provided in the confidential part Volume 4 since information on purity of batches is contained as well.
Nuclear magnetic resonance (NMR) **B.2.4/03**	OECD 101 OPPTS 830.7050	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	^{1}H-NMR ^{13}C-NMR The spectra confirmed the structure	**Acceptable** New study was performed as the former was not according to GLP.	Y	**Taskforce:** Wiche, A. Bogdoll, B 2012 M-435863-01-1
		Ethofumesate pure purity 99.9 %	^{1}H-NMR and ^{13}C-NMR spectra confirmed the structure ^{1}H-NMR and ^{13}C-NMR spectra confirmed the structure	EU agreed endpoint DAR 1998 **Acceptable** New study was performed as the former was not according to GLP	N Y	**UPL:** Audus, 1994, Anonymous, 1995 Patel, A.H. (2013c) Documents provided in the confidential part Volume 4 since information on purity of batches is contained as well.
Mass spectra (MS) **B.2.4/04**	OECD 101 OPPTS 830.7050	Ethofumesate pure Batch n° AE B049913 00 1B99 0002 purity 99.9 %	Mass spectrum (LC-MS/ESI^{+}-spectrum) The spectrum confirmed the structure	**Acceptable** New study was performed as the former was not according to GLP.	Y	**Taskforce:** Wiche, A. Bogdoll, B 2012 M-435863-01-1
		Ethofumesate pure purity 99.9 %	Mass spectrum (EI-spectrum) The spectrum confirmed the structure	EU agreed endpoint DAR 1998	N	**UPL:** Audus, 1994, Anonymous, 1995

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
			Mass spectrum (EI-spectrum) The spectrum confirmed the structure	**Acceptable** New study was performed as the former was not according to GLP	Y	Patel, A.H. (2013c) Documents provided in the confidential part Volume 4 since information on purity of batches is contained as well.
Spectra for impurities **EMS (ethyl methane sulfonate)** **B.2.4/05a**	OECD 101 OPPTS 830.7050	Ethyl methanesulfonate (EMS ; AE C639174) Batch n° AE C639174-PU-01 / 1292222 purity 97.8 %	**EMS (AE C639174)** UV/VIS-, IR-, ^{1}HNMR-, ^{13}C-NMR and MS-spectra are provided to confirm the chemical structure. UV/VIS: Measurements at 1 g/L were performed in neutral medium but no meaningful spectra could be achieved. Therefore the molar extinction coefficients are ultimately not calculable but extinction/absorption coefficient can be anticipated to be < 10 L/mol x cm at wavelength > 290 nm. Furthermore, no measurements were performed at even higher concentrations and in acidic and alkaline medium.	**Acceptable** This impurity is considered to be relevant. It was not considered to be relevant for the first Annex I inclusion. However, according to the FAO specification for ethofumesate (2007) a remark that ethyl methane sulfonate can occur as a result of certain manufacturing processes and if it occurs at ≥0.1 mg/kg (relative to ethofumesate) it would be designated as relevant impurity.	Y	**Taskforce:** Selzer, J. 2013 M-465124-01-1
		Ethyl methanesulfonate (EMS) purity 99.99 %	It is demonstrated that there is no UV/VIS absorption even not at higher concentration. MS-EI-, IR- and NMR-spectra are included in study Patel, A.H. (2013c)	**Acceptable**	Y Y	**UPL:** Bhandari, N.M. (2013) Patel, A.H. (2013c) Documents provided in the confidential part Volume 4 since information on purity of batches is contained as well.

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Spectra for impurities **iBMS (isobutyl-methane sulfonate)** **B.2.4/05b**	OECD 101 OPPTS 830.7050	Isobutyl-methane sulfonate (iBMS ; AE C639170) Batch n° AE C639170 00 1B99 0002 / MD2082 purity 98.7 %	**iBMS (AE C639170)** UV/VIS-, IR-, ^{1}HNMR-, ^{13}C-NMR and MS-spectra are provided to confirm the chemical structure. UV/VIS: neutral medium (water) **Wavelength [nm]** / **Molar extinction coefficient [L/mol x cm]** 200 / 6 291 / 0 acidic medium (HCl) **Wavelength [nm]** / **Molar extinction coefficient [L/mol x cm]** 203 / 6 291 / 0 basic medium (NaOH) **Wavelength [nm]** / **Molar extinction coefficient [L/mol x cm]** 220 / 3 291 / 0	**Acceptable** This impurity is considered to be relevant. It was not considered to be relevant for the first Annex I inclusion. However, according to the FAO specification for ethofumesate (2007) a remark that ethyl methane sulfonate can occur as a result of certain manufacturing processes and if it occurs at ≥0.1 mg/kg (relative to ethofumesate) it would be designated as relevant impurity.	Y	**Taskforce:** Selzer, J. 2013 M-465124-01-1
		Isobutyl-methane sulfonate (iBMS) purity 99.32 %	It is demonstrated that there is no UV/VIS absorption even not at higher concentration.	**Acceptable**	Y	**UPL:** Bhandari, N.M. (2013)
			MS-EI-, IR- and NMR-spectra confirm the chemical structure.	**Acceptable**	Y	**UPL:** Patel, A.H. (2013c) Documents provided in the confidential part Volume 4 since information on purity of batches is contained as well.

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Solubility in water						
Solubility in water **B.2.5/01**	EC A.6 OECD 105 OPPTS 830.7840	Ethofumesate pure Batch n° C66/87 purity 99.9 %	No pH effect because the active substance is not ionisable. Solubility = 50 mg/L at 25 °C and pH 7.7	EU agreed endpoint DAR 1998	Y	**Taskforce:** Bright, A.A.S. 1988 M-155193-02-1
	EC A.6 OECD 105	Ethofumesate lot no.: 234-57A Content: 99%	At 20°C: Milli RO Water 59.59 mg/L pH 7 buffer 57.83 mg/L pH 4 buffer 58.22 mg/L pH 9 buffer 61.92 mg/L	**Acceptable**	Y	**UPL:** Macdonald & Craig, 2002
	EC A.6 OECD 105	Ethofumesate technical Content: 98.59%	At pH 4: 8°C 26.3 mg/L, 20°C 41.1 mg/L, 30°C 66.0 mg/L At pH 6.5: 8°C 27.1 mg/L, 20°C 40.0 mg/L, 30°C 68.8 mg/L At pH 9: 8°C 27.0 mg/L, 20°C 43.5 mg/L, 30°C 65.7 mg/L	**Acceptable** Although technical Ethofumesate was used the purity is > 98%.	Y	Walter, D., 2003 (KCA 2.5/01)
Solubility in organic solvents						
Solubility in organic solvents **B.2.6/01**	EC A.6 OECD 105	Ethofumesate TGAI Batch n° AE B049913-01-08 purity 98.3 %	**solvent** / **solubility [g/L] at 20 °C** methanol: 119 n-heptane: 3.4 xylene: > 260 1,2 dichloroethane: > 260 acetone: > 260 ethyl acetate: > 260 dimethyl sulfoxide: > 260	**Acceptable** The notifier justified the new study that the old study was not sufficiently precise.	Y	**Taskforce:** Eyrich, U Ziemer, F. 2012 M-430903-01-1
	CIPAC MT 181 OECD 105	Ethofumesate lot no.: 234-57A Content: 99%	**solvent** / **solubility [g/L] at 20 °C** xylene: 250 - 500 acetone: 1250 - 1429 dichloroethane: 1429 - 1667 ethyl acetate: 714 - 1000	**Acceptable**	Y	**UPL:** Macdonald & Craig, 2002

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results		Comments (Acceptable / Non acceptable)	GLP	Reference
			methanol	114 - 133			
			heptane	3.042			
Partition coefficient n-octanol/water							
Partition coefficient n-octanol/water **B.2.7/01**	EC A.8 OECD 117 (shake flask method)	Ethofumesate pure Batch n° C66/87 purity 99.9 %	At 25 °C Pow log Pow pH 6.44 486 2.7 No pH effect because the active substance ethofumesate is not ionisable.		EU agreed endpoint DAR 1998	Y	**Taskforce and UPL:** Bright, A.A.S. Stalker, A.M. 1990 M-155196-01-1
of metabolite : Ethofumesate-NC20645 (BCS-CU88901)	EC A.8 OECD 117 (shake flask method)	Ethofumesate-NC20645 sodium salt (BCS-CU88901) purity 69.2 %	Pow log Pow pH 5 2.4 0.4 pH 7 0.042 -1.4 pH 9 0.0038 -2.4 at room temperature (mean: 22 °C)		**Acceptable** No surface tension is provided for this compound. However, a centrifuge (15 min at 3000 rpm) is used for phase separation. This study is evaluated and considered as information for other sections.	Y	**Taskforce:** Ziemer, F Kloeckner, C. 2012 M-428034-01-1
of metabolite : Ethofumesate-acetic acid (BCS-CW35117)	EC A.8, OECD 117 (shake flask method)	Ethofumesate-acetic acid (BCS-CW35117) purity 91 %	Pow log Pow pH 5 1.5 0.2 pH 7 0.049 -1.3 pH 9 0.025 -1.6 at room temperature (mean: 23 °C)		**Acceptable** No surface tension is provided for this compound. However, a centrifuge (15 min at 3000 rpm) is used for phase separation. This study is evaluated and considered as information for other sections.	Y	**Taskforce:** Eyrich, U. Ziemer, F 2013 M-451360-01-1
of metabolite : Ethofumesate-NC9607 (AE C509607)	EC A.8, OECD 117 (HPLC-method)	Ethofumesate-NC9607 (AE C509607) purity 99.8 %	Pow log Pow pH 5 158 2.2 pH 7 158 2.2 pH 9 158 2.2 at 25 °C		**Acceptable** This study is evaluated and considered as information for other sections.	Y	**Taskforce:** Bogdoll, B Peschke, C. 2012 M-427346-01-1
of metabolite : Ethofumesate-NC8493 (AE C508493)	EC A.8, OECD 117 (HPLC-method)	Ethofumesate-NC8493 (AE C508493) purity 99.8 %	Pow log Pow pH 5 32 1.5 pH 7 32 1.5 pH 9 32 1.5 at 25 °C		**Acceptable** This study is evaluated and considered as information for other sections.	Y	**Taskforce:** Bogdoll, B Peschke, C. 2012 M-427348-01-1

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Dissociation in water						
Dissociation constant **B.2.8/01**	OECD 112 (statement)	Ethofumesate	Dissociation constant is not applicable to ethofumesate in consideration of the molecular structure.	EU agreed endpoint DAR 1998	Y	**Taskforce and UPL:** Ward, J.C. Stalker, A.M. 1990 M-155681
Flammability and shelf-heating						
Flammability **B.2.9/01**	EC A.10	Ethofumesate TGAI Batch n° AE B049913-01-08 purity 98.3 %	Ethofumesate is not a highly flammable solid in the sense of EC guideline A.10.	**Acceptable**	Y	**Taskforce:** Winkler, S. 2012 M-425937-01-1
		Ethofumesate technical concentrate Content: 96.3%	Ethofumesate is not flammable,	EU agreed endpoint DAR 1998	N	**UPL:** Barker, 1991
Self heating **B.2.9/02**	EC A.16	Ethofumesate TTGAI Batch n° AE B049913-01-08 purity 98.3	No self-ignition temperature of ethofumesate was observed up to the maximum test temperature of 401°C.	**Acceptable**	Y	**Taskforce:** Winkler, S. 2012 M-425939-01-1
		Ethofumesate technical concentrate Content: 96.3%	Ethofumesate is not autoflammable.	EU agreed endpoint DAR 1998	N	**UPL:** Barker, 1991
Flash point						
Flash point **B.2.10/01**			Not applicable. The active substance is a solid; its melting point is > 40 °C			

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Explosive properties						
Explosive properties **B.2.11/01**	EC A.14	Ethofumesate TGAI Batch n° AE B049913-01-08 purity 98.3 %	Ethofumesate has no explosive properties in the sense of EC guideline A.14.	**Acceptable** A statement based on the chemical structure was given in the DAR 1998.	Y	**Taskforce:** Winkler, S. 2012 M-425938-01-1
		Ethofumesate technical concentrate Content: 96.3%	Ethofumesate is not explosive.	EU agreed endpoint DAR 1998	N	**UPL:** Barker, 1991
Surface tension						
Surface tension **B.2.12/01**	EC A.5 OECD 115	Ethofumesate TGAI Batch n° 9728 purity 98.5 %	68.3 mN/m at 20 °C (saturated aqueous solution)	EU agreed endpoint DAR 1998 Active substance is classified to be non-surface active according to EC Guideline A.5. **Acceptable** According to the new requirement the study has to be performed with the analytical standard but can be accepted, since the purity is > 98 %.	Y	**Taskforce:** Walter, D. 1999 M-249651-01-1
		Ethofumesate TGAI Batch n°1997/1 purity 98.5 9%	63.9 mN/m at 20 °C (saturated aqueous solution)	**Acceptable** According to the new requirement the study has to be performed with the analytical standard but can be accepted, since the purity is > 98 %.	Y	**UPL:** Walter, D., 2002

Test or Study Annex Point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
Oxidising properties						
Oxidizing properties **B.2.13/01**	EC A.17	Ethofumesate TGAI Batch n° AE B049913-01-08 purity 98.3 %	Ethofumesate has no oxidizing properties in the sense of EC guideline A.17.	**Acceptable** In the DAR 1998 a statement based on the chemical structure was given which was acceptable as well.	Y	**Taskforce:** Winkler, S. 2012 M-425948-01-1
	statement		Not oxidative based on chemical structure	EU agreed endpoint DAR 1998	N	**UPL:** Barker, 1991 Schnell, 1993 (see DAR)
Other studies						
Relative density of purified active substance	EC A.3 OECD 109 OPPTS 830.7300	Ethofumesate pure Batch n° R000047 purity 99.9 %	D_4^{20} = 1.29	EU agreed endpoint DAR 1998	Y	**Taskforce:** Stalker, A.M. Ward, J.C. 1990 M-155675-01-1

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Herbicide (Inhibitor of cell division)

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification required.

4 HUMAN HEALTH HAZARD ASSESSMENT

Ethofumesate has been previously discussed in the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances Pesticides, ECB Ispra, 19-21 May 1999 (ECBI/43/99 Rev. 2). In that meeting, it was decided not to propose any classification for human health.
During the re-evaluation of the active substance as active ingredient in PPPs, no new toxicological studies were provided by the notifier which would change the conclusion. Therefore, the conclusion from 1999 is considered still valid.

New (CLP) classification categories:

No non-lethal effects in acute oral toxicity studies were observed which would warrant the classification as STOT SE (specific target organ toxicity - single exposure) for ethofumesate.
No effects on rats in 28 days oral toxicity studies were observed below the value of 300 mg/kg bw/d which is considered as guidance value for potential classification of substances as STOT-RE 2 (specific target organ toxicity – repeated exposure). Similarly, no effects on rats and mice were observed in 90 days oral toxicity studies below the value of 100 mg/kg bw/d which is considered as guidance value for potential classification of substances as STOT-RE 2 after 90 days exposure period. According to Regulation (EC) No 1272/2008 no guidance values are set for effects observed in dog studies, however, ethofumesate did not cause any effects in dogs which would trigger classification as STOT-RE at tested doses.
No effects on rodents were observed below the values of 25 mg/kg bw/d (chronic studies) and 12.5 mg/kg bw/d (carcinogenicity studies) which are considered as guidance values for potential classification of substances as STOT-RE 2 (specific target organ toxicity – repeated exposure). According to Regulation (EC) No 1272/2008 no guidance values are set for effects observed in dog studies, however, ethofumesate did not cause any effects in dogs which would trigger classification as STOT-RE at tested doses. No treatment related non-neoplastic or neoplastic findings were observed in any of the studies. Therefore, ethofumesate is considered not to be potentially carcinogenic substance.
No effects on rodents were observed which are considered relevant for potential classification of substance as reproductive toxicant. Therefore, ethofumesate is considered not to be potentially reprotoxic substance with regard to effects observed in multigeneration studies.
In the European peer review (2015) no proposal for classification of ethofumesate for human health was made by EFSA or by Member States.

4.1 Acute toxicity - oral route

Hazard class not assessed in this dossier

4.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier

4.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier

4.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

4.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

4.6 Respiratory sensitisation

Hazard class not assessed in this dossier

4.7 Skin sensitisation

Hazard class not assessed in this dossier

4.8 Germ cell mutagenicity

Hazard class not assessed in this dossier

4.9 Carcinogenicity

Hazard class not assessed in this dossier

4.10 Reproductive toxicity

Hazard class not assessed in this dossier

4.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier

4.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier

4.13 Aspiration hazard

Hazard class not assessed in this dossier

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Summary of relevant information on degradation

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>Hydrolysis

OECD 111</td><td>DT50 (pH 4, 20 °C): stable to hydrolysis
DT50 (pH 7, 20 °C, extrapolated): stable to hydrolysis
DT50 (pH 9, 20 °C, extrapolated): stable to hydrolysis</td><td>none</td><td>Macdonald E., Craig, W.B.; 2002

Howarth, R.; Tremain, S. P.; Bartlett, A. J.;1991</td></tr>
<tr><td>Aqueous Photolysis

OECD 316
US EPA OCSPP Test Guideline No. 835.2240
Japanese MAFF New Test Guidelines Annex No. 2-6-2;

USEPA Subdivision N - Chemistry: Environmental Fate, NTIS PB83-153973</td><td>Environmental DT50: 53.2 d (Phoenix, Arizona, USA)

Environmental DT50:
Summer 20°N: 37 d
Summer 40°N: 43 d
Summer 60°N: 62 d</td><td>none</td><td>Weuthen, M.; Stupp, H. P.;2013

Brehm, M.; 1989</td></tr>
<tr><td>Biological degradation

OECD 301 D</td><td><table><tr><th>Test substance</th><th>O_2 consumption, mg/L
(28 days)</th><th>% of ThOD degraded
(28 days)</th></tr><tr><td>Ethofumesate 1 mg/L</td><td>-0.26</td><td>-14</td></tr><tr><td>Ethofumesate 3 mg/L</td><td>-0.23</td><td>-4</td></tr><tr><td>Sodium acetate 2 mg/L</td><td>1.01</td><td>65</td></tr></table></td><td>none</td><td>Bogers, M.;1993</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td>Sodium acetate 2 mg/L
+
ethofumesate
1 mg/L</td><td>0.94</td><td>-</td></tr>
</table>
Not readily biodegradable
</td><td></td><td></td></tr>
<tr><td>OECD 301 D</td><td>
<table>
<tr><th>Substance</th><th>O_2 depletion (mg O_2/l)</th><th>Degradation (% of ThOD)</th></tr>
<tr><td>Ethofumesate (3 mg/l)</td><td>0.575</td><td>10</td></tr>
<tr><td>Sodium benzoate (3 mg/l)</td><td>4.40</td><td>88</td></tr>
<tr><td>Aniline (2 mg/l)</td><td>4.17</td><td>68</td></tr>
</table>
Not readily biodegradable
</td><td></td><td>Douglas, M. T.; Sewell, I. G.;1989</td></tr>
<tr><td>DIN: 38409, H-41; EU (=EEC): 79/831, Annex V, Part C, Sect. C.5 + C.6</td><td>BOD 1.6
-------- = ------ = 0.010
COD 157.3

Not readily biodegradable</td><td></td><td>Wuethrich, V.;1993</td></tr>
<tr><td>Aerobic mineralisation in surface water

OECD 309

OECD 309</td><td>

No degradation observed

DT50: 331 d</td><td></td><td>

Caviezel, A., 2013

Fahrbach, M. (2012)</td></tr>
<tr><td>Water/Sediment Study

BBA: IV, 5-1

BBA: IV, 5-1</td><td>

Water:
DT50: 13.3 – 23 d (DFOP)
DT90: 94 – 155 d (DFOP)
Whole system:
DT50: 103 – 164 d
DT90: 342 – 543 d

Water:
DT50: 7.8 – 52 d (DFOP)
DT90: 101 – 457 d (DFOP)
Whole system:</td><td>

none

</td><td>

Kellner, G.; 1995 (kinetic evaluation in Schmitt, W.; 2008)

Blech, S.;1996</td></tr>
</table>

Method	Results	Remarks	Reference
	DT50: 250 – 294 d DT90: 830 – 976 d		
OECD 308	Water: DT50: 9.9 – 43 d (DFOP) DT90: 130 – 187 d (DFOP) Whole system: DT50: 89 – 141 d DT90: 296 – 469 d		Stupp, H. P. Weuthen, M.;2012 (kinetic evaluation in Chapple, A.C.; 2013)
BBA: IV, 5-1	Water: DT50: 37 – 141 d (DFOP) DT90: 343 – 804 d (DFOP) Whole system: DT50: 209 – 217 d DT90: 693 – 722 d		Heintze, A.; 2003 (kinetic evaluation in Stangelj, A.; 2014)

5.1.1 Stability

Hydrolysis:

Reference:	**Ethofumesate Determination of the Physico-Chemical Properties of Ethofumesate**
Notifier:	UPL/Agrichem
Author(s), year:	Macdonald E., Craig, W.B.; 2002
Report/Doc. number:	Report No. 21131
Guideline(s):	OECD 111
GLP:	yes
Deviations:	
Validity:	Valid
Status:	New study
Justification:	Since the summary of the hydrolysis study included in the Monograph is insufficiently detailed to fully determine its acceptability, an existing study conducted according to the OECD Guideline 111 is submitted to cover this point and is summarised below.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** Ethofumesate
   Batch No.: EFS-116
   Purity: 99%
   CAS No.: 26225-79-6

2. Buffers: 0.1 buffer solutions in Milli-RO water were prepared at pH 4 using mono-potassium citrate and sodium hydroxide, pH 7 using mono-potassium phosphate and sodium hydroxide, pH 9 using boric acid, potassium chloride and sodium hydroxide.

B. STUDY DESIGN

1. Experimental conditions

The hydrolysis of Ethofumesate was studied at pH 4, pH 7 and pH 9 buffers at 50°C ± 0.5°C for 5 days. The buffers were autoclaved prior to use. Ethofumesate (ca 100 mg) was weighted into a 10 mL volumetric flask and adjusted to volume with methanol. 0.5 mL of Ethofumesate was then transferred to 100 mL amber

volumetric flasks and made to volume with buffer. Samples were prepared in triplicate at each pH and were taken for analysis at 0 h, 2.4 h, 24 h and 5 days.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

At each sampling point (2.4 h, 24 h, and 5 days) the recovery levels for each media did not vary by more than ± 10% from the recovery value obtained at t_0.

B. FINDINGS

Ethofumesate was found to be hydrolytically stable at pH 4, 7 and 9 over a period of 5 days.

Table 5-1: Hydrolysis of Ethofumesate at pH 4, 7 and 9

Sample No.	Concentration [µg/mL]		% Recovery from $T_{2.4h}$		% Recovery from T_{24h}		% Recovery from $T_{5\ day}$	
	Nominal	T_0	Nominal	T_0	Nominal	T_0	Nominal	T_0
pH 4-1 pH 4-2 pH 4-3	49.75	47.82	96.8	100.7	94.9	98.7	91.0	94.7
pH 7-1 pH 7-2 pH 7-3	49.75	49.64	99.1	99.3	99.7	99.9	107.4	107.6
pH 9-1 pH 9-2 pH 9-3	49.75	49.55	98.6	99.0	99.3	99.7	106.8	107.3

Table 5- 1

III. CONCLUSION

In the aqueous hydrolysis study Ethofumesate was found to be hydrolytically stable at pH 4, 7 and 9 over a period of 5 days.

Comments RMS

The study is conducted according to OECD 111 and shows that ethofumesate is hydrolytically stable at pH 4, 7 and 9 over a period of 5 days.

Reference:	**TECHNICAL ETHOFUMESATE: DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES**
Notifier:	Taskforce
Author(s), year:	Howarth, R.; Tremain, S. P.; Bartlett, A. J.;1991
Report/Doc. number:	A87526 / C 500-1 / M-161417-01-1
Guideline(s):	For the hydrolysis part OECD 111 For the photostability study: EPA, Pesticide assessment Guidelines, Subdivision N, Chemistry: Environmental fate For the auto-flammability study: The Official Journal of the European Communities, L251, Vol 27, 19 September 1984.
GLP:	yes
Deviations:	
Validity:	Valid
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods
A hydrolysis test was carried out in accordance with OECD 111 with ethofumesate technical (purity > 98% w/w).

Result:
Ethofumesate is stable to hydrolysis at pH 4, 7 and 9 at 50°C during a test period of 5 days.

Table 5-2: Concentrations of ethofumesate at various pH values over time

	pH 4.0	pH 7.0	pH 9.0
Concentration of 104619 found initially (g/l)	1.98×10^{-2}	2.02×10^{-2}	1.93×10^{-2}
Concentration of 104619 found at 2.4 hours (g/l)	1.96×10^{-2}	2.01×10^{-2}	1.91×10^{-2}
Expressed as % of initial	99.0	99.5	99.0
Concentration of 104619 found at 24 hours (g/l)	1.88×10^{-2}	2.02×10^{-2}	1.94×10^{-2}
Expressed as % of initial	94.9	100.0	100.5
Concentration of 104619 found at 48 hours (g/l)	1.81×10^{-2}	2.01×10^{-2}	1.98×10^{-2}
Expressed as % of initial	91.4	99.5	102.6
Concentration of 104619 found at 72 hours (g/l)	1.83×10^{-2}	1.95×10^{-2}	1.97×10^{-2}
Expressed as % of initial	92.4	96.5	102.1
Concentration of 104619 found at 96 hours (g/l)	1.77×10^{-2}	1.93×10^{-2}	2.06×10^{-2}
Expressed as % of initial	89.4	95.5	106.7
Concentration of 104619 found at 120 hours (g/l)	1.85×10^{-2}	1.94×10^{-2}	2.01×10^{-2}
Expressed as % of initial	93.4	96.0	104.1

Comments RMS

The study shows that ethofumesate is hydrolytically stable.

The photodegradation part of the study is superseded by new aqueous photolysis studies by both notifiers.

Photolysis:
Direct photochemical degradation

Reference:	**THE PHOTOLYSIS OF ETHOFUMESATE (SCHERING CODE NO. ZK 49913) IN AQUEOUS SOLUTION**
Notifier:	Taskforce
Author(s), year:	Brehm, M.;1989
Report/Doc. number:	A83339 / W 81 / M-155608-01-1
Guideline(s):	USEPA Subdivision N - Chemistry: Environmental Fate, NTIS PB83-153973
GLP:	yes
Deviations:	
Validity:	Valid with respect to direct photchemical degradation of the active substance ethofumesate
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods

Aqueous photolysis of [benzene ring-U-^{14}C] ethofumesate was studied in a solution containing ≈50 and 10 mg as/l. Acetonitrile, 1%, was used as organic co-solvent. The solution was buffered (double distilled water) and held at pH of 7.0. The solutions were irradiated using a "merry go round" photoreactor and with filtered light from a Hg-arc lamp, resulting in wavelengths >290 nm,. The light intensity, measured by chemical actinometry, was in the wavelength range of 290-320 nm. The intensity was increased by a factor of about 3-5 compared to natural light in summer, 40°N, midday and at cloudless sky. A mixture of [^{14}C]-ethofumesate, with a radiochemical purity of 98%, and unlabelled ethofumesate, with a chemical purity of 99.9% W/W, was used. Nine samples of 50 mg as/l, two samples of 10 mg as/L and one sample of double distilled water were continuously irradiated in quartz cuvettes for 71 hours. Test solutions and double distilled water were kept in the dark for 71 hours. The temperature in the irradiated solutions were kept at 27.8±0.8°C and in the dark samples at 26.5±1.6°C. Aliquots of 200 µL were withdrawn after 2.0, 4.0, 8.0, 23, 30, 50 and 71 hours from the irradiated cuvettes and after 0.0, 30 and 71 hours from the cuvettes kept in the dark. The samples were analysed by HPLC/UV (230 nm) to determine the rate of reaction.

Additionally, for the determination of material balance analysis was carried out by HPLC coupled with a flow through radioactivity detector. The aim was to determine the rate of photolysis and the quantum yield of ethofumesate. The calculated quantum yield was further used to calculate environmental aqueous half-life with the computer program GCSOLAR.

Results
Transformation was only observed in the irradiated samples, which indicates that ethofumesate was transformed by photolysis. The half-lives were calculated according to first order reaction:

50 ppm: $t_{½}$ = 31 h
10 ppm: $t_{½}$ = 28 h

These half-lives correspond to a continuos irradiation coupled with a 3-5 fold intensity compared to natural sunlight (290-320 nm). Considering this, half-life was calculated by the investigator to be 8-13 days under natural conditions.

The results from the material balance showed one main peak in the analysis by HPLC. The recovery of radioactivity was about 100% after 30 hours of irradiation. Thereafter, the recovery decreased to 93% at the end of the test. The decrease was accounted to result from secondary reactions, but was not possible to explain. Standards of four possible metabolites, NC 8493, NC 9607, NC 10458 and NC 1790 were used to identify the peak detected in the HPLC chromatogram, but it was not identical to any of these. The radioactive peak early in the HPLC chromatogram, which amounted to ≈41% of the applied activity after 71

hours of illumination, was not identified. It was concluded that the peak probably contained non-specific polar products. No other products were detected.

The quantum yield, Φ, was calculated from the rate of initial transformation of ethofumesate under well defined irradiation conditions and the UV-spectrum of the substance.

$\Phi = 9.54 \cdot 10^{-2}$

Table 5-3: GCSOLAR estimated environmental half-lives of ethofumesate in days

Latitude Season	20°N days	40°N days	60°N days
spring	41	60	120
summer	37	43	62
fall	53	111	560

Comments RMS

The study was conducted in agreement to US EPA Guideline, Subdivision N, § 161-2 (1982) and was well performed and reported. The estimated range of environmental aqueous photolysis half-lives of ethofumesate in Europe (40-60°N) under summer conditions are approximately 37-62 days according to the used computer program GCSOLAR.In this study, 41% of the radioactivity remain unidentified, the study is considered valid with respect to direct photchemical degradation of the active substance ethofumesate.

Reference:	**Aqueous photolysis (14C)-ethofumesate**
Notifier:	Taskforce
Author(s), year:	Keirs, D. C.; 2000
Report/Doc. number:	C009667 / M-199018-01-1
Guideline(s):	SETAC: Proc. Env. Fate & Ecotox. (1995)
GLP:	yes
Deviations:	
Validity:	Valid (quantum yield determination part)
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and methods

An aqueous photolysis study in accordance with the SETAC guideline (1995) was conducted with [Benzene ring-U-^{14}]-ethofumesate (specific activity and radiochemical activity were 214 µCi·mg^{-1} and 98%, respectively, and ethofumesate chemical purity was 99.9% w/w). The study was conducted with a p-nitroacetophenone/pyridine actinometer.

Results

A quantum yield of 1.92 x 10^{-4} molecules degraded per photon absorbed was determined.

Comments RMS*:*

Study is of acceptable quality and the quantum yield value (1,92 x 10^{-4} molecules degraded/photon absorbed) can be used for further calculations. The photodegradation part of the study is superseded by two new studies provided by the notifiers as it was characterized by unknown radioactivity of up to 18% in the irradiated samples.

Reference:	**[Phenyl-UL-14C]Ethofumesate: Phototransformation in water**
Notifier:	Taskforce
Author(s), year:	Weuthen, M.; Stupp, H. P.;2013
Report/Doc. number:	EnSa-12-0228 / M-453458-01-1
Guideline(s):	OECD 316 US EPA OCSPP Test Guideline No. 835.2240 Japanese MAFF New Test Guidelines Annex No. 2-6-2;
GLP:	yes
Deviations:	
Validity:	Valid
Status:	New study

MATERIALS AND METHODS

Materials :

Test Material : [Phenyl-UL-^{14}C]Ethofumesate

Specific activity: 3.78 MBq/mg

Test system :

Sterile phosphate buffer solution (pH 7), 10 mM

Methods :

Study design :

The photochemical reaction was studied in aqueous solution. Samples were continuously exposed to a xenon lamp with < 290 nm cut-off filter (Suntest equipment) for 10 days equivalent to e.g. 34 days under environmental conditions (Phoenix, Arizona, USA). For comparison, control samples were incubated in the dark.

The individual test vessels for irradiated and dark samples contained 10 mL of test solution. The irradiated vessels were individually connected to traps for the collection of CO_2 and organic volatiles while the dark control samples were closed with glass stoppers.

Experimental Conditions :

The photochemical reaction was studied in a sterile phosphate buffer solution (pH 7) at 25 ± 2 °C and an initial nominal concentration of 1 mg/L. Samples were continuously exposed to a xenon lamp with < 290 nm cut-off filter (Suntest equipment) for 10 days equivalent to e.g. 34 days under environmental conditions (Phoenix, Arizona, USA). For comparison, control samples were incubated in the dark.

Sampling :

Test solutions were analyzed 0, 1, 2, 3, 7, 9 and 10 days after application

Analytical Procedures :

Test solutions were analyzed in duplicate 0, 1, 2, 3, 7, 9 and 10 days after application by LSC and reversed phase HPLC with radio-detection to determine the degradation of [phenyl-UL-^{14}C]Ethofumesate as well as the formation and decline of transformation products. Representative samples were additionally analyzed and the result was confirmed with a second separation method (HPLC). The test item in the stock solution was identified by spectroscopic methods.

RESULT AND DISCUSSION

Mass balance and distribution of radioactivity:

Table 5-4: Distribution of residues in % of AR in irradiated samples

		Sampling Times [days]						
	Environment[1)]	**0**	**3**	**7**	**10**	**24**	**31**	**34**
Compound	**Experiment**	**0**	**1**	**2**	**3**	**7**	**9**	**10**
Ethofumesate	Mean	100.0	96.1	95.9	88.5	73.5	68.8	64.2
A	Mean	n.d.	0.4	0.9	1.4	2.9	3.9	4.3
B	Mean	n.d.	0.3	n.d.	n.d.	n.d.	n.d.	n.d.
C	Mean	n.d.	0.3	0.5	0.9	n.d.	1.2	n.d.
D	Mean	n.d.	2.7	4.1	5.2	4.9	4.7	4.2
E	Mean	n.d.	n.d.	0.5	1.3	2.7	3.9	4.5
F	Mean	n.d.	n.d.	0.5	1.1	1.4	2.5	2.8
G	Mean	n.d.	n.d.	0.3	0.9	1.5	2.2	2.6
H	Mean	n.d.	n.d.	0.3	0.4	n.d.	0.9	n.d.
I	Mean	n.d.	n.d.	n.d.	n.d.	3.6	3.5	5.9
J	Mean	n.d.	n.d.	n.d.	n.d.	1.7	2.3	2.7
K	Mean	n.d.	n.d.	n.d.	n.d.	1.0	1.1	1.2
L	Mean	n.d.	n.d.	n.d.	n.d.	0.9	0.9	2.0
M	Mean	n.d.	n.d.	n.d.	n.d.	1.0	0.8	2.2
N	Mean	n.d.	n.d.	n.d.	n.d.	0.9	0.8	n.d.
O	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	1.4
P	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.
Q	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	n.d.
R	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	n.d.
Total [2)]	Mean	100.0	99.3	102.7	99.5	95.5	96.3	94.6
$^{14}CO_2$	Mean	n.a.	0.2	0.2	0.7	3.3	3.8	5.4
Volatile organics	Mean	n.a.	0.0	0.1	0.1	0.1	0.1	0.1
Total recovery % [2)]	Mean	100.0	99.6	103.0	100.2	98.9	100.2	100.2

n.d.: not detected

[1)] Irradiation equivalent to environmental conditions in Phoenix, Arizona, USA

[2)] Values were taken from Material Balance

Table 5-5: Distribution of residues in % of AR in non-irradiated samples

		Sampling Times [days]						
Compound	**Experiment**	**0**	**1**	**2**	**3**	**7**	**9**	**10**
Ethofumesate	Mean	100.0	99.5	103.5	100.5	100.1	99.6	100.0
Total recovery %	Mean	100.0	99.5	103.5	100.5	100.1	99.6	100.0

Non-extractable and Extractable Residues :
The test was performed in aqueous solution. All residues were extractable.

Mineralization:
Mineralization was at maximum 5.4% of applied radioactivity in the irradiated samples. The mineralization was not determined in dark samples.

Transformation of Test material and Transformation Products :
In the test solutions of the irradiated test systems, the amounts of [phenyl-UL-^{14}C] Ethofumesate declined from 100.0% at time zero to 64.2% of AR after 10 days of continuous irradiation. No degradation was detected under dark conditions. Under irradiated conditions a multitude of transformation products was detected in the test solutions and all of them were characterized according to their retention times. The maximum amount of a single transformation product was 5.9% of

AR (I, DAT-10). Due to the low amounts of each single transformation product, identification procedures for transformation products were not performed.

Table 5-6: Result synopsis

Test medium	Sterile aqueous buffer solution at pH 7
Source of irradiation	Xenon lamp with cut-off filter < 290 nm
Experimental DT_{50} / DT_{90} [days] in Suntest®	15.6 / 51.8
Environmental DT_{50} [days]: Phoenix, Arizona, USA	53.2
Environmental DT_{50} [days]: Tokyo, Japan	112.9
Dark control DT_{50} / DT_{90} [days]	718.9 / 2388.2
Net Experimental DT_{50} [days][1)]	16.0
Transformation products	
- Exposure to light	a) Numerous minor transformation products in solution (Maximum of a single product: 5.9% of AR, DAT-10) b) Max. 5.4% of CO_2, DAT-10
- Dark	None

[1)] Calculated from net rate constant (rate constant of irradiated samples – rate constant of dark samples

Conclusion :
A multitude of minor metabolites are formed under irradiated conditions.

Comments RMS

The study shows that phototransformation of [phenyl-UL-^{14}C]Ethofumesate in water systems is a relevant process. A multitude of minor metabolites are formed under irradiated conditions. Among these metabolites, compound I occurs at >5%AR at study end.

The study is acceptable.

The relevant endpoints are:
Experimental DT50 15.6 d
Environmental DT50 53.2 d (Phoenix, Arizona, USA)

Reference:	**Ethofumesate: Assessment of the environmental half-life of the direct photo-degradation in water**
Notifier:	Taskforce
Author(s), year:	Hellpointner, E.; 2013
Report/Doc. number:	EnSa-13-0355 / M-461408-01-1
Guideline(s):	COMMISSION REGULATION (EU) No 283/2013: Data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament OECD Test Guideline 101, 1981 OECD Test Guideline 316, 2008
GLP:	yes
Deviations:	
Validity:	Valid
Status:	New study

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ETHOFUMESATE (ISO); (RS)-2-ETHOXY-2,3-DIHYDRO-3,3-DIMETHYLBENZOFURAN-5-YL METHANESULFONATE

MATERIALS AND METHODS

Materials :
Test Material : ethofumesate
Certificate No. : AZ 16166

Test system :
The test substance was dissolved in pure water (22.3 mg/L)

Methods :
The UV-VIS absorption properties of ethofumesate were characterized by the extinction in steps of 1 nm from 200 – 800 nm.

The environmental half live was calculated using two different models :

The arithmetic model developed by Zepp and Cline allows for a transfer of laboratory data concerning the direct photo-transformation in water to field conditions. The model estimates on the basis of a clear summer sky with no influence of clouds. The half-lives calculated therefore may be regarded as minimum half-lives depending on frequency and extent of cover of sky by clouds.

In contrast to the model approach by Zepp and Cline, the arithmetic model developed by Frank and Kloepffer considers the influence of clouded sky for the region Central Europe, i.e. Germany.

RESULT AND DISCUSSION

The absorption spectrum of ethofumesate in pure water shows two absorption maxima at 225 nm (ε = 6527 L mol-1 cm-1) and at 278 nm (ε = 2427 L mol-1 cm-1).
No absorption was measured from 306 to 800 nm. Therefore, the overlap with the environmentally relevant range of wavelength is weak, and significant light absorption ends at 306 nm with ε < 10 L mol-1 cm-1, already.
The molar extinction coefficient (ε) at 295 nm is 144 L mol-1 cm-1. Since the old cut-off wavelength for the tiered evaluation of photo-transformation in the EU was 290 nm, this value with ε = 744 L mol-1 cm-1 was calculated.
Based on the known quantum yield of Φ = 0.0001 and the molar extinction coefficients determined for the wavelengths of 297.5 to 305 nm, environmental half-lives were calculated:

Table 5-7: Environmental half-lives calculated according to Zepp & Cline

Season	**Environmental DT_{50} of Direct Photo-Transformation of Ethofumesate in Pure Water**			
	30th degree lat.	**40th degree lat.**	**50th degree lat.**	**60th degree lat.**
Spring	> 1 year	> 1 year	> 1 year	> 1 year
Summer	> 1 year	> 1 year	> 1 year	> 1 year
Fall	> 1 year	> 1 year	> 1 year	> 1 year
Winter	> 1 year	> 1 year	> 1 year	> 1 year

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day.
The column of the 50th degree of latitude is more or less relevant to the conditions of Central Europe.

Table 5-8: Environmental half-lives calculated according to Frank & Kloepffer

Month	**Photolysis Constant**	**Environmental DT_{50} of Direct Photo-Transformation of Ethofumesate in Pure Water**		
	[1/sec]	**Minimum**	**Mean**	**Maximum**
April	0.922×10^{-11}	> 1 year	> 1 year	> 1 year
May	0.183×10^{-10}	> 1 year	> 1 year	> 1 year
June	0.272×10^{-10}	> 1 year	> 1 year	> 1 year
July	0.280×10^{-10}	> 1 year	> 1 year	> 1 year

August	0.243×10^{-10}	> 1 year	> 1 year	> 1 year
September	0.936×10^{-11}	> 1 year	> 1 year	> 1 year
October	0.247×10^{-11}	> 1 year	> 1 year	> 1 year

(50th degree lat.), no contribution of another mono- or bimolecular elimination process.
Minimum = clear sky
Maximum = clouded sky

Conclusion:
From this investigation it is evident that direct photo-transformation of ethofumesate in water does not contribute significantly to the elimination of this compound from the environment.

Comments RMS

The study shows that the environmental half-life relevant to Central Europe is above 1 year and that therefore photochemical degradation of ethofumesate might play a minor role under such conditions. The study is valid.

Reference:	**Photodegradation of [14C]Ethofumesate in Water, Based on the OECD 316 Direct Photolysis Guideline Tier II – Generation and Characterization of Photoproducts**
Notifier:	UPL
Author(s), year:	Peizhi, L.; 2013
Report/Doc. number:	13485.6132
Guideline(s):	OECD 316
GLP:	yes
Deviations:	
Validity:	Valid
Status:	New study

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** [^{14}C U-ring]Ethofumesate
   Lot No.: 11BLY014
   Radiochemical Purity: > 98%
   Specific activity: 55 mCi/mmol (426,476 dpm/µg)

2. **Test material (reference):** Ethofumesate
   Synonym: PESTANAL®
   Batch No.: SZE6128X
   CAS No.: 26225-79-6
   Purity: 99.5%
   Expiry Date: 8 May 2013

2. **Test material (reference):** NC8493 (a metabolite of Ethofumesate)
   Chemical Name: 2,3-dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methanesulphonate
   Batch No.: EEP/VMV 358A
   CAS No.: 26322-82-7
   Purity: 99.8%
   Expiry Date: 11 May 2012

B. STUDY DESIGN

1. Experimental conditions

Test samples were prepared by placing 5.0 mL of sterile purified reagent water in each of the ten 7-mL quartz photolysis tubes for each test substance. A 28-µL aliquot of the secondary radiolabelled stock solution was added to each tube and

tube was closed with a sterile cap. Light samples were continuously irradiated under the Suntest sunlight simulator with a xenon arc lamp, which had been filtered to remove wavelengths less than 290 nm. Samples were irradiated continuously for up to 2 days at 25 ± 1°C and analysed at 0 hours and after 2, 4, 8 and 12 hours of irradiation. Additional samples were taken after 1 and 2 days of irradiation.

The Ethofumesate test solution was scanned from 250 to 800 nm in order to determine absorbance at appropriate wavelength intervals to calculate the direct photolysis rate constant.

2. Description of analytical procedures

At selected time intervals, samples were analysed directly by LSC and by HPLC/RAM to determine the recovery and distribution of radioactivity in the solution.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Recoveries of applied radioactivity ranged from 92.78% to 99.30%. Negligible quantities of volatile organic compounds were detected.

B. FINDINGS

The average amount of [^{14}C]Ethofumesate declined in sterile water irradiated samples from 97.25% AR at 0 hours to 41.27% AR after 12 hours of irradiation. Ethofumesate was photodegraded to numerous photoproducts and one significant photoproduct was detected, Degradate 1 (13.5 minutes), first detected at 2 hours at an average of 1% AR and increased to a maximum of 9.57% AR at 12 hours. Several minor regions of radioactivity were observed and were less than 5% AR and therefore not considered further. The HPLC fractionation and further analysis of Degradate 1 by TLC showed that it contains at least six components. The proposed molecular structure for the major component of this mixture was 2,3,5-trihydroxy-4-(1-hydroxyethyl)-hexanedioic acid.

Table 5-9: Distribution of radioactivity in the irradiated sterile water samples treated with (^{14}C)ethofumesate as percent of applied radioactivity and as concentration

Time (hours)	(^{14}C)Ethofumesate ~ 18.3-min % AR	Deg-1 ~13.5-min % AR	Deg-2 ~15.2-min % AR	Deg-3 ~ 16.9-min % AR	Others in AQ (% AR)	Total (% AR)	Total Conc. (µg/mL)
0	96.98	ND	1	ND	0.78	98.76	10.2
0	94.53	ND	0.73	ND	1.04	99.3	10.26
Average	97.25	ND	0.87	ND	0.91	99.03	10.23
2	83.68	1.06	1.89	4.63	5.97	97.22	10.05
2	85.7	0.94	1.54	2.75	7.41	98.34	10.16
Average	84.69	1	1.72	3.69	6.69	97.78	10.1
4	73.28	3.69	3.03	4.89	12.96	97.85	10.11
4	77.68	2.44	1.74	4.99	11.41	98.26	10.15
Average	75.48	3.06	2.39	4.94	12.18	98.05	10.13
8	55.78	6.47	3.37	3.89	26.82	96.34	9.95
8	55.97	7.17	4.19	5.09	25.46	97.88	10.11
Average	55.87	6.82	3.78	4.49	26.14	97.11	10.03
12	39.31	9.18	4.63	4.03	34.63	91.77	9.48
12	43.22	9.97	4.84	4.04	32.23	94.29	9.74
Average	41.27	9.57	4.74	4.03	33.43	93.03	9.61

III. CONCLUSION

The study demonstrated that Ethofumesate degrades quickly by photolysis in sterile water at 25 ± 1°C. Ethofumesate was photodegraded to numerous photoproducts and one significant photoproduct was detected, Degradate 1, peaking with a maximum of 9.57% AR at 12 hours. The HPLC fractionation and further analysis of Degradate 1 by TLC showed that it contains at least six components. The proposed structure for the major component of this mixture was 2,3,5-trihydroxy-4-(1-hydroxyethyl)-hexanedioic acid.

Comment RMS
The study shows that a multitude of minor metabolites are formed under irradiated conditions.

One of the degradates (Degradate 1) occurs at 9.57% after 12 hours. The HPLC fractionation and further analysis of Degradate 1 by TLC showed that it contains at least six components. The proposed molecular structure for the major component of this mixture was 2,3,5-trihydroxy-4-(1-hydroxyethyl)-hexanedioic acid.
The study is valid.

In the course of the peer-review, the notifier UPL/Agrichem was requested to provide an assessment of the metabolite fractions isolated in the study with the respective chromatograms and their interpretation.

The notifier provided the following statement.
All the peaks were integrated and none of them was >10%AR when considering the percentage of radioactivity in the water.

Comment RMS
The statement provided by the notifier is conclusive. None of the metabolites requires characterization since only metabolites have to be analyzed which exceed 10%AR.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

5.1.2.2 Screening tests

Reference:	**READY BIODEGRADABILITY: 28 DAYS CLOSED BOTTLE TEST WITH ETHOFUMESATE**
Notifier:	Taskforce
Author(s), year:	Bogers, M.;1993
Report/Doc. number:	A87607 / W 507-1 / M-161538-01-1
Guideline(s):	OECD 301D EU 84/449
GLP:	yes
Deviations:	
Validity:	Valid
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods
A ready biodegradability test of ethofumesate was carried out according to OECD Guidelines 301D: "Ready Biodegradability: Closed Bottle Test" (1981). The purity of ethofumesate was ≥97%. The test was carried out with a filtered and aerated inoculum from a municipal sewage treatment plant (secondary effluent). The test was carried out at ethofumesate concentrations (nominal) of 1 and 3 mg/L test solution. The following solutions were used as oxygen controls: test medium (Millipore water + nutrients) without inoculum, test medium with inoculum, 2 mg sodium acetate/L with inoculum (as positive control) and finally 2 mg/L sodium acetate + 1 mg ethofumesate/L with inoculum (as inhibition control). The test was carried out at 20±1°C and test bottles were withdrawn for O_2 determination with an oxygen electrode after 0, 5, 14 and 28 days.

Results
Theoretical oxygen demand (ThOD) for ethofumesate is calculated to 1.84 mg O_2/L and for sodium acetate 0.781 mg O_2/l.

Table 5-10: BOD (mg O_2 /L) for ethofumesate, positive control and inhibition control, related to the inoculum control after 28 days incubation at 20°C.

Test substance	O_2 consumption, mg/L (28 days)	% of ThOD degraded (28 days)
Ethofumesate 1 mg/L	-0.26	-14
Ethofumesate 3 mg/L	-0.23	-4
Sodium acetate 2 mg/L (positive control)	1.01	65
Sodium acetate 2 mg/L + ethofumesate 1 mg/L	0.94	-

Comments RMS

The study is acceptable.

The test is shortly described but seems to have been carried according to the mentioned OECD Guideline. The results indicate that ethofumesate is not readily biodegradable.

Reference:	**DETERMINATION OF BIOCHEMICAL AND CHEMICAL OXYGEN DEMAND OF ETHOFUMESATE DISPERSED IN WATER**
Notifier:	Taskforce
Author(s), year:	Wuethrich, V.;1993
Report/Doc. number:	A87608 / W 508-1 / M-161539-01-1
Guideline(s):	DIN: 38409, H-41; EU (=EEC): 79/831, Annex V, Part C, Sect. C.5 + C.6
GLP:	yes
Deviations:	
Validity:	Valid
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods

The biological and chemical demand, BOD_5 and COD, respectively, of ethofumesate were determined at RCC, Itingen, Switzerland. The tested batch of ethofumesate had a chemical purity of ≥97%. The BOD-test was carried out using inoculum from domestic waste-water, which after washing and filtering contained 4 g dry matter per litre, mixed with Sörensen buffer solution (pH 7). The test flasks were incubated in a SAPROMAT D12 (Voith GmbH), which determine the generated amount of CO_2 evolved at the consumption of O_2. Control solutions were test medium and test medium + inoculum (0.2 g/L test solution) Three test flasks with 10.3, 10.3 and 10.2 mg ethofumesate/250 test medium + 50 mg inoculum, were incubated together with the mentioned controls and the positive controls of 50 mg D(+)Glucose- and 50 mg Sodium-L-glutaminate/250 mL test medium (both with 50 mg inoculum) in the test apparatus for 5 days at 20±1°C. There was no description of the COD method used.

Results

COD of ethofumesate was determined to be 157.3 mg O_2/100 mg ethofumesate. In two of the BOD-flasks with ethofumesate no CO_2 was evolved after 5 days of incubation. In the third flask BOD was determined to 4.9 mg O_2/100 mg ethofumesate. The calculated BOD of the control substances were 54. 8 and 55.3 mg O_2/100 mg D(+)Glucose and Sodium-L-glutaminate, respectively.

Based on the calculated average BOD-value of ethofumesate the BOD/COD ratio is:

$$\frac{BOD}{COD} = \frac{1.6}{157.3} = 0.010$$

Comments RMS

The study is acceptable. The low BOD:COD ratio of 0.01 indicates that ethofumesate is not biologically oxidised when related to its chemical oxygen demand.

Reference:	**ASSESSMENT OF READY BIODEGRADABILITY OF ETHOFUMESATE**
Notifier:	Taskforce
Author(s), year:	Douglas, M. T.; Sewell, I. G.;1989
Report/Doc. number:	A83351 / M-155620-01-1
Guideline(s):	OECD 301D
GLP:	yes
Deviations:	
Validity:	Valid
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods

The test was carried out according to OECD Guidelines 301D, the ready biodegradability closed bottle test. The chemical purity of ethofumesate was 99.9%. Sodium benzoate ($C_6H_5.COOH$) and aniline ($C_6H_5.NH_2$) were used as reference substances. Activated sludge for the test was taken from a sewage plant treating predominantly domestic sewage. The following solutions were tested: non-inoculated dilution water, inoculated dilution water, inoculated dilution water and filter paper, and three different inoculated samples: 3 mg/L ethofumesate, 3 mg/L sodium benzoate and 2 mg/L aniline. The solutions were filled on dark BOD bottles and kept in a water bath at 20±1°C. Duplicate bottles were withdrawn after 0, 5, 15 and 28 days. The free oxygen content was determined and degradation expressed as % of ThOD(NO_3) was calculated for ethofumesate and the reference substances.

Results

Table 5-11: O_2 depletion (mg O_2/l) and degradation (% of ThOD) after 28 days

Substance	O_2 depletion (mg O_2/l)	Degradation (% of ThOD)
Ethofumesate (3 mg/l)	0.575	10
Sodium benzoate (3 mg/l)	4.40	88
Aniline (2 mg/l)	4.17	68

Comments RMS

The study is acceptable.
The results indicate that ethofumesate is not readily biodegradable.

5.1.2.3 Simulation tests

Aerobic mineralisation in surface water

Reference:	**[^{14}C]Ethofumesate – Aerobic Mineralisation in Surface Water**
Notifier:	UPL/Agrichem
Author(s), year:	Caviezel, A., 2013
Report/Doc. number:	20130080
Guideline(s):	OECD 309
GLP:	Yes
Deviations:	
Validity:	Valid
Status:	New study
Justification:	New data requirement

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** [^{14}C]Ethofumesate
   Batch No.: 7350CDB001-1
   Radiochemical Purity: 98.7%
   Specific Radioactivity: 2.80 MBq/mg

2. **Test Material:** Ethofumesate
   CAS No.: 26225-79-6
   Batch No.: SZBB091XV
   Purity: 99.6%

3. **Reference Material:** [^{14}C(U)]Benzoic Acid
   Batch No.: 121214
   Radiochemical Purity: >99%
   Specific Radioactivity: 125 mCi/mmol (37.87 MBq/mg)

4. **Test system:** The test was performed using surface water without sediment (pelagic test). Water was freshly sampled from the pond from Fröschweiher on July 16, 2013. After one day of acclimation, the water was passed through a 0.2 mm sieve.

Table 5-12: Physiochemical parameters of the water system

System	Fröschweiher
Water parameters measured at field sampling:	
Temperature [°C]	22.0
pH (water)	7.68
Oxygen concentration [mg/L]	4.5
Redox potential (E_h) * [mV]	329
Sampling depth [cm]	0 – 20
Colour	Brown
Turbidity/Visibility	Approx. 15 cm
Water parameters measured post-handling:	
TOC [mg/L]	6.60
DOC [mg/L]	5.53
BOD	<4.00
Nitrate [mg/L]	0.97
Nitrite [mg/L]	<0.82
Ammonium [mg/L]	0.16
Orthophosphate [mg/L]	1.30
N total [mg/L]	1.15
P total [mg/L]	0.42

* The measured potential was corrected to Eh of a standard hydrogen electrode by adding 211 mV

B. STUDY DESIGN

1. Experimental conditions

The study was performed in an open gas-flow-through-system consisting of 300 mL Erlenmeyer flasks each containing 100 mL of surface water. The flasks with surface water were equilibrated for less than one week.

Samples were incubated in the dark at a temperature of 21.1 ± 0.1ºC under aerobic conditions. Each flask was aerated with moistened air. The samples were continuously and gently stirred to maintain particles and micro-organisms in suspension. After treatment, samples (except for those taken immediately after treatment, i.e. day 0) were connected to a volatile trapping system equipped with two absorption traps, one containing ethylene glycol and the other 2N NaOH (in this sequence) to trap organic volatiles and $^{14}CO_2$, respectively. Another set of samples (high dose) were maintained under sterilised conditions.

Two untreated control samples were used to measure physico-chemical parameters during the test. In addition, two samples were treated with [^{14}C-U]benzoic acid in order to test the microbial activity of the test water.

2. Sampling

Samples (duplicates for the high and low dose and single samples for the sterilised systems) were taken immediately after treatment (day 0) and after 7, 14, 21, 28, 42 and 62 days of incubation. Single samples treated with the reference test item [^{14}C(U)]benzoic acid were taken for analysis after 7 and 14 days.

Trapping solutions of samples were taken on the corresponding sampling day. Trapping solutions were in addition exchanged after 34 days of incubation.

The oxygen concentration and pH of the treated samples and the two untreated samples were measured at each sampling interval.

3. Description of analytical procedures

At each sampling interval, the volume of the water phase was recorded and the radioactivity present was determined by LSC using at least two replicates. Aliquots of the water phases were then submitted for HPLC analysis. At two sampling intervals, aliquots were removed from the water phase in order to obtain the remaining concentration of benzoic acid in the test system.

The volumes of the trapping solutions were recorded. Thereafter, radioactivity present in the trapping solutions was determined by LSC. In case when multiple trapping solutions are collected for a given sample during the incubation period, the total amount of volatile radioactivity is calculated as the sum of radioactivity in the corresponding trapping solutions.

The limit of detection (LOD) in samples was set to twice the background radioactivity or 42 dpm and the respective limit of quantification (LOQ) was set to three times the background radioactivity or 63 dpm per measurement sample aliquot.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mean radioactivity in the aqueous phases was between 96.7 and 100.3% of applied radioactivity for the high dose samples and between 95.3 and 100.8% of applied radioactivity for the low dose samples. For the sterilised samples (high dose) the mean recovery in the aqueous phase was between 96.3 and 101.6%AR.

The total radioactivity recovery including volatiles was between 95.3% and 101.8% AR throughout the study for all doses.

B. FINDINGS

HPLC analysis of the radioactivity in the water phase showed only [^{14}C]Ethofumesate and, thus, stability of the test item for 62 days of incubation in natural surface water. The mineralisation was marginal with maximum 1.1% (high dose) and 0.8% (low dose) at the end of the incubation period.

Table 5-13: Radioactivity in surface water following application of [^{14}C]Ethofumesate in % of applied radioactivity

Fröschweiher [%AR]	Replicate	Incubation time in days						
		0	7	14	21	28	42	62
High dose								
Aqueous phase	A	98.2	97.2	100.7	100.6	98.1	97.3	98.2
	B	96.8	96.3	99.9	98.2	100.7	96.5	98.2
	Mean	97.5	96.7	100.3	99.4	99.4	96.9	98.2
14CO2	A	n.p.	0.1	0.2	0.3	0.9	0.7	1.1
	B	n.p.	0.1	0.1	0.3	0.4	0.6	1.1
	Mean	n.p.	0.1	0.1	0.2	0.7	0.6	1.1
Other volatiles in EG	A	n.p.	<0.1	<0.1	0.1	0.2	<0.1	<0.1
	B	n.p.	<0.1	<0.1	0.1	0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	0.1	0.1	<0.1	<0.1
Total	A	98.2	97.2	100.9	101.0	99.2	98.0	99.2
	B	96.8	96.4	100.0	98.6	101.2	97.1	99.3
	Mean	97.5	96.8	100.4	99.8	100.2	97.6	99.2
Mean ± SD		98.8 ± 1.6						
High dose - Sterile								
Aqueous phase	-	96.8	96.7	99.6	99.1	101.6	96.3	98.8
14CO2	-	n.p.	0.1	0.1	<0.1	0.1	0.1	0.1
Other volatiles in EG	-	n.p.	<0.1	<0.1	<0.1	0.1	<0.1	<0.1
Total	-	96.8	96.8	99.7	99.2	101.8	96.3	98.9
Mean SD		98.5 ± 2.0						
Low dose								
Aqueous phase	A	96.5	97.5	100.1	99.7	100.7	97.2	97.5
	B	94.1	98.3	100.0	96.5	100.9	96.4	100.1
	Mean	95.3	97.9	100.0	98.1	100.8	96.8	98.8
14CO2	A	n.p.	0.1	0.1	0.4	0.4	0.4	0.8
	B	n.p.	0.1	0.2	0.1	0.6	0.4	0.8
	Mean	n.p.	0.1	0.2	0.2	0.5	0.4	0.8
Other volatiles in EG	A	n.p.	0.1	<0.1	0.4	0.4	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	0.2	0.2	<0.1	<0.1
Total	A	96.5	97.6	100.2	100.5	101.5	97.6	98.4
	B	94.1	98.4	100.2	96.6	101.5	96.9	100.9
	Mean	95.3	98.0	100.2	98.6	101.5	97.2	99.6
Mean SD		98.5 ± 2.0						

n.p. Not performed

As [^{14}C]Ethofumesate was stable, a degradation rate was not calculated.

III. CONCLUSIONS

The rate of biodegradation of Ethofumesate was investigated in natural pond surface water using the [^{14}C]labelled test item incubated at 21.1 ± 0.1 °C under aerobic conditions.

Ethofumesate was stable for 62 days of incubation. The mineralisation was marginal with maximum 1.1% (high dose) and 0.8% (low dose) at the end of the incubation period.

Comment RMS

In contrast to the study submitted by the notifier Taskforce, ethofumesate was stable for 62 days and mineralisation was low (1.8% in the high dose test, 0.8% in the low dose test).

The study is valid.

Reference:	**[14C]Ethofumesate: Aerobic mineralization in surface water**
Notifier:	Taskforce
Author(s), year:	Fahrbach, M. (2012)
Report/Doc. number:	M-439697-01-1
Guideline(s):	OECD 309
GLP:	Yes
Deviations:	
Validity:	Valid
Status:	New study
Justification:	New data requirement

MATERIALS AND METHODS

Materials :
Test Material : [Phenyl-UL-^{14}C]Ethofumesate
Specific Radioactivity: 3.78 MBq/mg

Test Water :
The test water was freshly sampled from a pond (Möhlin, AG, Switzerland) and consisted of natural water sampled at a depth of about 30 cm and filtered through a 0.2 mm sieve. The test water was acclimated under aerobic conditions and continuous agitation in the dark prior to treatment. At the day of application and at each sampling interval, the pH, redox potential and oxygen concentration of the water was measured in blank control duplicates (FB) and in the treated flasks

Table 5-14: Water characteristics

Pond		Test Water	
		Field sampling	Determined before treatment
Origin/Source		Fröschweiher pond, Möhlin AG/Switzerland	
Temperature	[°C]	13.8	-
Colour		yellow-brown	-
pH		6.95	-
Redox Potential*	[mV]	512.0	-
Oxygen content	[mg/L]	9.93	-
BOD	[mg/L]	-	1552.0
TOC	[mg C/L]	-	8.76
DOC	[mg C/L]	-	7.76
N_{tot}	[mg/L]	-	155.8
P_{tot}	[mg/L]	-	0.13
NO_3^-	[mg/L]	-	1.61
NO_2^-	[mg/L]	-	<0.83
NH_4^+	[mg/L]	-	0.27

Pond	Test Water	
	Field sampling	Determined before treatment
Origin/Source	Fröschweiher pond, Möhlin AG/Switzerland	
Dissolved orthophosphate (PO_4^{3-}) [mg/L]	-	0.002

BOD: Biological oxygen demand
TOC: Total Organic Carbon
DOC: Dissolved Organic Carbon
*: The measured redox potential value was converted to the standard hydrogen electrode by the addition of + 211 mV (Ag/AgCl electrode, WTW SenTix® ORP, 20 °C).
-: Not determined

Methods :
Study design
A volume of 300 mL pelagic was filled in all-glass metabolism flasks (inner diameter: about 5.3 cm, volume: ca. 500 mL). Each flask was equipped with a gas inlet and outlet and one absorption trap containing 60 mL of 2N sodium hydroxide to trap CO_2 and one absorption trap containing 50 mL ethylene glycol to trap organic volatiles, respectively.

The ^{14}C-labelled test item was applied to the water surface of each sample at two concentrations: 9.9 µg/L (low concentration, FTL) and 101.4 µg/L (high concentration, FTH), respectively. Several samples were treated with a higher test item amount (extended concentration, FTH, 1524 µg/L) in order to facilitate the production and isolation of metabolites. In addition, reference control samples (FC) were treated with [ring-^{14}C(UL)]Benzoic acid at a concentration of 11.0 µg/L in order to confirm the microbial activity. Furthermore, sterile controls (FS) and solvent controls were established.
During the incubation period, the samples were incubated in the dark and continuously agitated using magnetic stirrers. A stream of air was allowed to pass through the samples. Organic volatiles and ^{14}C-carbon dioxide were collected in ethylene glycol and sodium hydroxide traps, respectively.

Experimental Conditions
The time course and concentration dependency of the biodegradation of [^{14}C]Ethofumesate in aerobic surface water ("pelagic test") was investigated at 20 ± 3 °C in the dark

Sampling :
Duplicate samples of each Ethofumesate concentration were taken for analysis after 0, 7, 14, 21, 28, 58 and 88 days of incubation.

Analytical Procedures :
The test water was removed from the metabolism flasks. The glass material and the magnetic stirrer bars were rinsed with methanol. The test water was first submitted to LSC measurement for determination of its radioactivity content before being analyzed by HPLC and/or 1D-TLC and radiodetection either directly or after the water was concentrated under reduced pressure at 38 °C using a rotary evaporator (e.g. 75 mL were concentrated to 5.6 mL).

RESULT AND DISCUSSION

Mass balance and Distribution of Radioactivity :

Table 5-15: Material Balance in the Pond Test Water (Low Concentration, FTL) after Treatment with [^{14}C]Ethofumesate. Values Are Given in Percent of the Applied Radioactivity

Pond System Pelagic Low Conc. (% of applied)	Sample	Incubation Time in Days							
		0	7	14	21	28	58	88	Sterile*
Radioactivity in water	A	99.0	95.7	97.1	100.1	97.5	96.1	96.3	98.6
	B	98.9	97.8	98.9	98.1	99.3	98.6	95.2	98.6
	Mean	**99.0**	**96.7**	**98.0**	**99.1**	**98.4**	**97.3**	**95.7**	**98.6**
$^{14}CO_2$	A	n.p.	<0.1	0.2	0.1	0.2	0.9	0.3	0.2

Pond System Pelagic Low Conc. (% of applied)	**Sample**	**Incubation Time in Days**							
		0	**7**	**14**	**21**	**28**	**58**	**88**	**Sterile***
	B	n.p.	<0.1	<0.1	0.9	0.1	0.9	1.3	0.2
	Mean	**n.p.**	**<0.1**	**0.1**	**0.5**	**0.2**	**0.9**	**0.8**	**0.2**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
	Mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.1**
Total	A	99.0	95.7	97.3	100.2	97.8	96.9	96.7	98.9
	B	98.9	97.8	98.9	99.0	99.4	99.5	96.5	98.9
	Mean	**99.0**	**96.8**	**98.1**	**99.6**	**98.6**	**98.2**	**96.6**	**98.9**
MEAN +/- SD				**98.1**	±		**1.3**		

* Only replicate B (sterile control) was used for evaluation due to technical problems.
n.p.: Not performed.
SD: Standard Deviation.

Table 5-16: Pattern of [^{14}C]Ethofumesate and its Metabolites in the Pond Test Water (Low Concentration, FTL). Values Are Given in Percent of the Applied Radioactivity

Pond System Pelagic Low Conc. (% of applied)	**Sample**	**Incubation Time in Days**							
		0	**7**	**14**	**21**	**28**	**58**	**88**	**Sterile****
Parent	A	99.0	95.7	94.8	95.0	94.8	86.9	61.7	93.9
	B	98.9	97.8	96.1	94.8	90.8	86.6	55.1	93.9
	mean	**99.0**	**96.7**	**95.5**	**94.9**	**92.8**	**86.7**	**58.4**	**93.9**
M1 (BCS-CU88901)	A	*	*	2.2	5.1	2.7	9.2	8.7	<LOQ
	B	*	*	2.7	<LOQ	3.8	10.4	27.9	<LOQ
	mean	*****	*****	**2.5**	**4.2**	**3.2**	**9.8**	**18.3**	**<LOQ**
M2 (BCS-CW35117)	A	*	*	*	*	*	*	19.1	<LOQ
	B	*	*	*	*	4.7	*	7.7	<LOQ
	mean	*****	*****	*****	*****	**2.4**	*****	**13.4**	**<LOQ**
M3	A	*	*	*	*	*	*	5.0	*
	B	*	*	*	*	*	*	<LOQ	*
	mean	*****	*****	*****	*****	*****	*****	**4.0**	*****
non-resolved***	A	---	---	---	---	---	---	1.8	0.3
	B	---	---	---	---	---	1.6	1.3	0.3
	mean	**---**	**---**	**---**	**---**	**---**	**0.8**	**1.6**	**0.3**

*: Not detected
**: Only replicate B was used for evaluation due to technical problems. The sterile sample was used to examine abiotic degradation or other non-biological removal of the test item. The sample was worked-up on the last sampling interval (day 88). The sterile samples were autoclaved (121°C; 20 min) to stop the biological activity.
***: Adsorbed radioactivity which remained as origin on the TLC plate
<LOQ: Below Limit of Quantification

Table 5-17: Material Balance in the Pond Test Water (High Concentration, FTH) after Treatment with [^{14}C]Ethofumesate. Values Are Given in Percent of the Applied Radioactivity

Pond System Pelagic High Conc. (% applied)	Sample	Incubation Time in Days						
		0	**7**	**14**	**21**	**28**	**58**	**88**
Radioactivity in water	A	93.5	90.8	95.4	95.1	95.6	94.2	92.8
	B	97.0	95.4	96.7	96.8	96.7	93.7	93.5
	Mean	**95.2**	**93.1**	**96.0**	**95.9**	**96.1**	**94.0**	**93.1**
$^{14}CO_2$	A	n.p.	<0.1	<0.1	<0.1	0.1	0.3	0.9
	B	n.p.	<0.1	<0.1	<0.1	<0.1	0.3	0.8
	Mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**0.1**	**0.3**	**0.8**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	0.2	<0.1
	Mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.2**	**<0.1**
Total	**A**	**93.5**	**90.9**	**95.5**	**95.2**	**95.7**	**94.6**	**93.7**
	B	**97.0**	**95.4**	**96.7**	**96.9**	**96.8**	**94.2**	**94.3**
	Mean	**95.2**	**93.1**	**96.1**	**96.0**	**96.2**	**94.4**	**94.0**
MEAN +/- SD				**95.0**	±		**1.7**	

n.p.: Not performed.
SD: Standard Deviation.

Table 5-18: Pattern of [^{14}C]Ethofumesate and its Metabolites in the Pond Test Water (High Concentration, FTH). Values Are Given in Percent of the Applied Radioactivity

Pond System Pelagic High Conc. (% of applied)	Sample	Incubation Time in Days						
		0	**7**	**14**	**21**	**28**	**58**	**88**
Parent	A	93.5	90.8	93.3	91.2	92.4	81.7	79.3
	B	97.0	94.1	94.9	90.5	94.6	85.8	79.4
	mean	**95.2**	**92.5**	**94.1**	**90.9**	**93.5**	**83.8**	**79.3**
M1 (BCS-CU88901)	A	*	*	2.1	3.0	3.2	4.6	8.8
	B	*	1.2	1.7	6.3	*	4.9	14.1
	mean	*****	**<LOQ**	**1.9**	**4.6**	**1.6**	**4.7**	**11.4**
M2 (BCS-CW35117)	A	*	*	*	0.9	*	7.8	4.7
	B	*	*	*	*	2.1	3.0	*
	mean	*****	*****	*****	**<LOQ**	**1.0**	**5.4**	**2.4**

Not detected
<LOQ: Below Limit of Quantification

Non-extractable and Extractable Residues:
All residues were extractable.

Mineralization:
The maximum of formed ^{14}-CO_2 was 0.9% of AR and 0.8% for the low and high concentration, respectively.

Transformation of Test material and Transformation Products :
^{14}C]Ethofumesate was degraded slowly in the test water. In the low concentration samples (FTL) a significant degradation of the test item was observed after a lag phase of approximately 60 days. Immediately after application, its concentration in the water phase represented on average 99.0% (low concentration, FTL) and 95.2% (high concentration, FTH) of the

applied radioactivity decreasing to 58.4% and 79.3% after 88 days, respectively. The parent remained almost stable in the sterile control (FS) after an incubation period of 88 days.

Two major metabolites, designated M1 (R1 = BCS-CU88901; NC 20645, ethofumesate-carboxylic acid NC20645) and M2 (BCS-CW35117), were formed. Metabolite M1 reached maximum values of 18.3% (low concentration, FTL) and 11.4% (high concentration, FTH) of applied at the end the incubation period. Metabolite M2 reached maximum levels of 13.4% on day 88 at the low concentration (FTL). Metabolite M2 was identified as ethofumesate-acetic acid by LC-MS analysis and chromatographic behavior. At the high concentration (FTH) metabolite M2 reached its maximum on day 58 with 5.4% of applied radioactivity. In the low concentration samples (FTL) one additional minor radioactive fraction, designated M3 was detected. Metabolite M3 did not exceed 4.0% of the applied radioactivity in the low concentration samples and was not identified

Conclusion:
In aerobic surface water ethofumesate was degraded slowly. At the lower test item concentration the test item was degraded faster after a lag-phase of about 60 days. This indicates that the rate of Ethofumesate degradation in water is concentration dependent. Two predominant metabolites and one minor metabolite were formed, NC 20645 (ethofumesate carboxylic acid, reference substance BCS-CU88901) and BCS-CW35117 (ethofumesate acetic acid). The formation of carbon dioxide due to mineralization was low.

Comments RMS

The study is fully acceptable.

In contrast to the study presented by the notifier UPL, two major metabolites (NC 20645 and CW35117) were formed. Metabolite NC20645 reached maximum values of 18.3% (low concentration test, FTL) and 11.4% (high concentration test, FTH) of applied radioactivity at the end the incubation period. Metabolite CW35117 reached maximum levels of 13.4% on day 88 at the low concentration test (FTL).
The water was sampled at 13.8 °C, whereas the study was performed at 20 °C.

Biodegradation in water/sediment systems:

Reference:	**DEGRADATION AND METABOLISM OF 14C ETHOFUMESATE IN AQUATIC SYSTEMS**
Notifier:	Taskforce
Author(s), year:	Kellner, G.;1995
Report/Doc. number:	A87625/ W 526-1 / M-161568-01-1
Guideline(s):	BBA: IV, 5-1
GLP:	yes
Deviations:	
Validity:	Valid except for the kinetic evaluation
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods

The aerobic transformation of U-phenyl-labelled ^{14}C-ethofumesate (radiochemical purity 98.8%) was investigated in two sediment/water systems, according to BBA Guideline Part IV, 5-1, 1990. For sediment and water characteristics, see tables below.

Table 5-19. Sediment characteristics

Sediment	OC (%)	pH	clay (%)	silt (%)	sand (%)	water content (%)	CEC (mequ/100 g)
Rhine river	1.2	6.9	5.6	9.3	85	39 - 44	8.3
Anwiler Teich	1.4	6.9	29	30	42	48 - 49	23

Table 5-20: Water characteristics during the test period

Water	O_2 (% of saturation)	pH	redox-potential (mV)	hardness (°dH)	alkalinity (mg $CaCO_3$/l)
Rhine river	41 - 87	7.6 - 8.3	93 - 213	18	n.r.
Anwiler Teich	54 - 98	7.4 - 8.3	107 - 223	20	n.r.

n.r. = not reported

Sediment was introduced to a depth of ca. 2 cm (190 and 180 g w/w from river and pond, respectively) in 500 mL glass flasks. Surface water was added to achieve a water column of 6 cm height (290 ml). Each test system was treated with 0.14 mg ^{14}C-ethofumesate (corresponding to a field rate of 1.5 kg as/ha). The systems were ventilated with moistened air and incubated at 20±1°C in the dark after two weeks of equilibration. Volatile transformation products were trapped in NaOH and 2-methoxyethanol. Duplicate samples were taken for analysis 0, 0.25, 1, 2, 7, 14, 30, 61 and 103 days after treatment. The sediment was extracted with acetone/water (2:1). Radioactivity in the water phase and in the sediment extracts were quantified by LSC and identified by TLC. Non-extractable residues were combusted and measured by LSC.

Results

After 103 days of incubation, 32 and 27% (13 and 18% parent compound) of applied radioactivity was recovered in the river and pond water phase, respectively, while 57 and 64% (37 and 41% parent compound) was associated to the sediments. The material balance of applied ^{14}C-radioactivity is given in the table below.

Table 5-21: Balance of the radioactivity in the river water and sediment after various time intervals. Values are given in %AR.

		INCUBATION TIME IN								
		HOURS		DAYS						
		0	6	1	2	7	14	30	61	103
WATER	A	96.1	90.1	81.4	76.7	63.1	47.7	36.3	30.7	32.5
	B	95.7	89.0	83.7	72.9	55.6	51.9	39.0	30.6	31.1
	Mean	95.9	89.6	82.5	74.8	59.4	49.8	37.6	30.6	31.8
SEDIMENT Extractables	A	1.4	7.5	16.2	19.6	32.5	44.3	53.6	49.9	42.4
	B	1.8	10.5	13.7	24.4	37.9	41.0	50.7	49.5	42.4
	Mean	1.6	9.0	14.9	22.0	35.2	42.7	52.2	49.7	42.4
Non-extractables	A	0.1	0.2	0.2	0.1	0.3	0.6	2.7	11.4	13.7
	B	0.1	0.3	0.3	0.3	0.2	0.6	4.3	8.6	14.8
	Mean	0.1	0.2	0.3	0.2	0.3	0.6	3.5	10.0	14.2
TOTAL SEDIMENT	A	1.6	7.6	16.4	19.7	32.7	45.0	56.3	61.3	56.1
	B	1.9	10.8	14.0	24.7	38.1	41.5	55.0	58.1	57.2
	Mean	1.7	9.2	15.2	22.2	35.4	43.3	55.6	59.7	56.6
Volatile Compounds	A	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
14C-CO2	A	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.5	1.5
	B	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.5	1.5
	Mean	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.5	1.5
TOTAL RECOVERY	A	97.7	97.8	97.8	96.5	95.9	92.7	92.8	92.6	90.1
	B	97.6	99.8	97.7	97.7	93.7	93.5	94.1	89.2	89.9
	Mean	97.7	98.8	97.8	97.1	94.8	93.1	93.5	90.9	90.0
MEAN RECOVERY	±	Std. Dev.		94.9 ±		3.2				

Notes: n.d.: not determined

Table 5-22: Balance of the radioactivity in the pond water and sediment after various time intervals. Values are given in %AR.

		INCUBATION TIME IN								
		HOURS		DAYS						
		0	6	1	2	7	14	30	61	103
WATER	A	92.4	88.5	81.4	73.6	61.0	55.7	39.5	34.4	29.8
	B	93.3	89.0	82.2	74.1	63.1	52.0	42.6	36.8	24.1
	Mean	92.8	88.7	81.8	73.8	62.0	53.9	41.0	35.6	26.9
SEDIMENT Extractables	A	1.4	7.0	13.3	21.4	33.9	37.8	51.1	50.4	36.7
	B	0.6	8.1	13.5	22.3	33.3	41.4	49.1	47.9	50.4
	Mean	1.0	7.6	13.4	21.8	33.6	39.6	50.1	49.1	43.5
Non-extractables	A	0.1	0.3	0.3	0.7	0.9	1.2	3.3	10.2	25.1
	B	0.1	0.4	0.2	0.5	0.9	1.1	3.5	9.1	15.9
	Mean	0.1	0.3	0.3	0.6	0.9	1.2	3.4	9.6	20.5
TOTAL SEDIMENT	A	1.5	7.3	13.6	22.0	34.8	39.0	54.4	60.6	61.8
	B	0.7	8.5	13.7	22.8	34.2	42.5	52.7	57.0	66.4
	Mean	1.1	7.9	13.7	22.4	34.5	40.8	53.5	58.8	64.1
Volatile Compounds	A	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
14C-CO2	A	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.3	1.2
	B	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.3	1.2
	Mean	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.3	1.2
TOTAL RECOVERY	A	93.9	95.8	95.0	95.6	95.8	94.8	94.1	95.3	92.8
	B	93.9	97.5	96.0	96.9	97.4	94.6	95.4	94.1	91.7
	Mean	93.9	96.7	95.5	96.3	96.7	94.8	94.8	94.8	92.3
MEAN RECOVERY	±	Std. Dev.		95.1 ±		1.4				

Notes: n.d.: not determined

Table 5-23: Measured amounts of ethofumesate in percentage of applied radioactivity for the test system Rhine River (from Schmitt, 2008)

Days after application	Ethofumesate water	Ethofumesate sediment	NIR	NER	$^{14}C0_2$	NIR + NER+ $^{14}C0_2$
0*	95.9	n.d.	n.d.	0.1	n.d.	0.1
0.25	89.6	8.6	0.4	0.2	<0.1	0.6
1	81.7	14.5	1.4	0.3	<0.1	1.7
2	73.6	21.6	1.6	0.2	<0.1	1.8
7	57.6	34.6	0.6	0.3	<0.1	0.9
14	48.4	41	1.7	0.6	<0.1	2.3
30	27.2	49.3	13.3	3.5	0.1	16.9
61	18.7	45.5	16.1	10	0.5	26.6
103	12.6	36.7	25	14.2	1.5	40.7

n.d. = not detected

Table 5-24: Measured amounts of ethofumesate in percentage of applied radioactivity for the test system Anwiler Teich (from Schmitt, 2008)

Days after application	Ethofumesate water	Ethofumesate sediment	NIR	NER	$^{14}C0_2$	NIR + NER+ $^{14}C0_2$
0*	92.8	n.d.	n.d.	0.1	n.d.	0.1
0.25	88.7	7.3	0.3	0.3	<0.1	0.6
1	81.8	12.8	0.6	0.3	<0.1	0.9
2	73.8	21.3	0.5	0.6	<0.1	1.1
7	60.8	32.6	1	0.9	<0.1	1.9
14	52.2	38.3	1.3	1.2	<0.1	2.5
30	38.5	48.7	3.9	3.4	0.1	7.4
61	30.5	46.7	7.6	9.6	0.3	17.5
103	17.8	40.6	12.1	20.5	1.2	33.8

n.d. = not detected

The radioactivity referred to as “other” includes up to four unknown fractions, of which one amounted to a maximum of 15 and 6.6% (TLC) of applied radioactivity in the river- and pond waters, respectively. No other fraction amounted to more than 3% of applied.

RMS Comments

The study was conducted in accordance with the referred guidelines, except for the lack of identification of residues.The water:sediment ratio is narrower than required by guideline (3:2 instead of 3:1 to 4:1), but still acceptable. CO_2-free air was used for aeration, however, no alkalization occurred.

The study is acceptable with regard to the degradation of ethofumesate. The kinetic evaluation is reported in Schmitt (2008).

Reference:	**Ethofumesate - Fate and behaviour in water/sediment**
Notifier:	Taskforce
Author(s), year:	Blech, S.;1996
Report/Doc. number:	OFC00004877 / M-352106-01-1
Guideline(s):	Richtlinen für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren' part IV, 5-1
GLP:	Yes
Deviations:	
Validity:	Valid
Status:	Previous evaluation in DAR for original approval / baseline dossier.

Study summary based on previous draft assessment report

Materials and Methods

The aerobic transformation of U-phenyl-labelled ^{14}C-ethofumesate (radiochemical purity >98.5%) was investigated in two sediment/water systems, according to BBA Guideline Part IV, 5-1, 1990. For sediment and water characteristics, see tables below.

Table 5-25: Sediment characteristics at the beginning of the experiment

Sediment	Org. C (%)	pH	clay (%)	silt (%)	sand (%)	water content (%)	CEC (mval)
Waldwinkel	10	7.1	24	62	14	n.r.	33
Ruckhaltebecken	0.75	7.2	12	79	9.3	n.r.	7.9

Table 5-26: Water characteristics at the beginning of the experiment

Water	O_2 (% of saturation)	pH	redox-potential (mV)	hardness (mmol/l)	alkalinity (mg $CaCO_3$/l)
Waldwinkel	91	7.7	177	3.2	n.r.
Ruckhaltebecken	93	8.1	117	1.8	n.r.

n.r. = not reported

Sediment was introduced to a depth of ca. 2.5 cm (140 and 110 g w/w fromWaldwinkel and Ruckhaltebecken, respectively) in 500 mL flasks. Surface water was added to achieve a water column of 5.5 - 6.5 cm height**.** The test systems were treated with 0.4 mg ^{14}C-ethofumesate/L water (corresponding to a field rate of 1.2 kg as/ha, assuming 0.3 m water depth), pipetted on the watersurface of the systems. After one week of equilibration the systems were closed with absorption/ventilation devices and gently shaken (without whirling up the sediment) during incubation at 20±0.5°C in the dark. Volatile transformation products were trapped in NaOH and ethylacetate. Duplicate samples were taken for analysis 0, 1, 2, 7, 14, 30, 60±1, 100±4 and 230±5 days after treatment. The sediment was extracted with acetone and the water with trichloromethane. The radioactivity was quantified by LSC and identified by TLC. Non-extractable residues were combusted and measured by LSC.

Results

After 234 days of incubation in Waldwinkel and 225 days in Ruckhaltebecken, 5.1 and 26% (1.5 and 21% parent compound) of applied radioactivity was recovered in the water phase, while 81 and 58% (53 and 30% parent compound) was associated to the sediments. The material balance of applied ^{14}C-radioactivity at termination of the study is given in the table below.

Table 5-27: Material balance of applied ^{14}C-ethofumesate after 234 or 225 days of incubation in water/sediment systems maintained at 20±1°C.

System	NC8438 (%)	CO_2 (%)	unextractable (%)	other (%)	total (%)
Waldwinkel (234 d)	54	9.4	27	3.3	95
Ruckhaltebecken (225 d)	51	5.7	27	4.1	91

The radioactivity referred to as “other” includes a non extractable fraction assigned to metabolites, which amounted to a maximum of 3.6 and 5.9% (TLC) of applied radioactivity in the Waldwinkel and Ruckhaltebecken waters, respectively.

Table 5-28: Water/sediment system 'Waldwinkel' Distribution of radioactivity, summary (mean values) (%):

days	0	1	2	7	14	30	59	104	234
water (sum)	87.9	83.1	76.7	47.5	33.2	24.2	17	11.7	5.1
extractable part	87.2	81.9	75.3	46	31.7	21.3	13.4	9.4	1.8
radioactivity assigned to ethofumesate	87	81.4	73.2	45.8	31.2	20.8	13.4	9.2	1.5
non extractable part. radioactivity assigned to metabolites (sum)	0.7	1.2	1.4	1.5	1.4	2.8	3.6	2.3	3.3
sediment (sum)	12	16.3	27.4	50.8	63.7	71.7	77.7	81.1	80.7
extrectable part	12	16.2	27.1	50.2	62.8	70.3	74.6	74.4	53.8
radioactivity assigned to ethofumesate	11.8	15.1	26.6	49.4	61	66.8	71.3	72.2	52.5
bound residue	0.1	0.1	0.2	0.2	0.2	1.4	3.4	6.7	26.9
carbon dioxide	0	0	0	0	0	0.2	0.4	1.5	9.4
volatile substances	0	0	0	0	0	0	0	0	0
recovery	99.9	99.5	104.1	98.3	96.9	96.1	95.1	94.4	95.3
test substance (sum)	98.8	96.5	99.8	95.2	92.2	87.6	84.7	81.4	54
not classified radioactivity	0.4	1.6	2.6	1.1	2.3	4.1	3	2.4	1.7

Table 5-29: Water/sediment system 'Rückhaltebecken' Distribution of radioactivity, summary (mean values) (%):

days	0	1	2	7	14	30	61	98	225
water (sum)	91.0	88.9	90.4	76.5	66	56.3	49.6	42.5	26.4
extractable part	90.3	87.9	89.2	74.9	64.5	53.4	43.8	36.6	22.3
radioactivity assigned to ethofumesate	88.2	85.5	88.8	74.4	63	52.5	43.5	36.1	21.4
non extractable part, radioactivity assigned to metabolites (sum)	0.7	1.1	1.2	1.6	1.5	2.9	5.7	5.9	4.1
sediment (sum)	6	11.4	10.6	22.5	31.5	40.4	44.5	48.4	58.4
extrectable part	5.9	11.2	10.4	22	30.8	38.8	39.5	36.4	31.6
radioactivity assigned to ethofumesate	5.6	10.7	10.2	21.8	30.2	37.7	38.6	35.2	29.9
bound residue	0	0.2	0.2	0.1	0.2	1.6	5	12.1	26.9
carbon dioxide	0	0	0	0	0	0.1	0.6	1.5	5.7
volatile substances	0	0	0	0	0	0	0	0	0.1
recovery	97	100.3	101	99	97.6	96.8	94.7	92.5	90.6
test substance (sum)	93.8	96.2	98.9	96.2	93.2	90.2	82	71.3	51.3
not classified radioactivity	2.5	2.9	0.6	0.7	2.1	2	1.2	1.7	2.6

The dissipation half-lives of ethofumesate from the water phases in Waldwinkel and Ruckhaltebecken were 7 (6 - 9) and 50 (42 - 58) days, respectively. For the whole systems, the half-lives were extrapolated to 285 (CL 241 - 329) and 242 (CL 223 - 262) days, according to a Timme model based on the root function of first order.
The microbial biomass of the sediments was 23 and 15 mg/100 g dw in Waldwinkel and Ruckhaltebecken, respectively, at start of the incubation. At termination of the incubation, the corresponding values were 20 and 11 mg/100 g dw.

Comments RMS

The experiment was set up in duplicates but only the mean values reported. The study is valid. Degradation kinetics for the water and the sediment compartment were re-evaluated by the RMS according to level-I recommendations in FOCUS (2006). For the Waldwinkel study, for the sediment compartment no reliable kinetics could be determined.

System Rückhaltebecken

	Ethofumesate				
Compartment	kinetics		DT50 [d]	DT90 [d]	Chi^2
Total system degradation	SFO		250	830	1.4
Water dissipation	DFOP		52	457	2.4
Sediment dissipation	SFO	1000	1000	0.5	

System Waldwinkel

	Ethofumesate			
Compartment	kinetics	DT50 [d]	DT90 [d]	chi^2
Total system degradation	SFO	294	976	2.3
Water dissipation	DFOP	7.8	101	2.2
Sediment dissipation	n.a.	1000	1000	n.a.

Reference:	**[Phenyl-UL-14C]Ethofumesate:Aerobic aquatic metabolism**
Notifier:	Taskforce
Author(s), year:	Stupp, H. P. Weuthen, M.;2012
Report/Doc. number:	A87605 / W 505-1 / M-443554-01-1
Guideline(s):	OECD 308 US EPA OCSPP Test Guidelines No. 835.4300 and 835.4400
GLP:	Yes
Deviations:	
Validity:	Valid
Status:	New study

MATERIALS AND METHODS

Materials :

Test Material : [Phenyl-UL-^{14}C]Ethofumesate

Spec. Radioactivity 3.78 MBq/mg

Water / Sediment Test Systems :

The study was carried out with natural water/sediment systems from two locations:

Anglersee (Leverkusen, Germany): This small lake is a reclaimed gravel-pit, which is used for fishing only. The lake is entirely enclosed by a fence.

Hoenniger Weiher (close to Wipperfuerth, Germany): This is an artificially dammed pond in the course of the "Hoenniger Creek" forming "Hoenniger Weiher". On account of it's in- and outlet the pond (about 1000 m2 in surface area) has strong water current.

Table 5-30: Water characteristic

Properties of Waters

Parameter	**Anglersee**	**Hoenniger Weiher**
Temperature [°C][1]	1.0	1.0
pH[1]	8.6	7.2
Total Organic Carbon (TOC) [mg/L][2,3]	< 2 / 3 / 6	< 2 / 12 / 25
Redox Potential E_h [mV][1,5]	+ 435	+ 523
Oxygen Content [%][1]	95	96

Table 5-31: Sediment characteristics

Properties of Sediments		
Parameter	**Anglersee**	**Hoenniger Weiher**
Geographic Location	Leverkusen North Rhine-Westphalia, Germany	Wasserfuhr, close to Wipperfuerth North Rhine-Westphalia, Germany
Soil Taxonomic Classification (USDA)	Sand	Loamy Sand
Sand (2000 – 50 µm) [%]	95	77
Silt (< 50 – 2 µm) [%]	5	23
Clay (< 2 µm) [%]	0	0
pH[1]	7.6	7.4
pH	6.8 ($CaCl_2$); 7.0 (H_2O)	6.3 ($CaCl_2$); 6.5 (H_2O)
Organic Matter [%][2, 3, 4]	0.48 / 0.38 / 7.26	2.6 / 2.69 / 2.57
Organic Carbon [%][2, 3]	0.28 / 0.22 / 4.21	1.51 / 1.56 / 1.49
Soil Microbial Activity [mg CO_2 /h/kg sediment (dry weight)][2,3]	3.75 / 2.08 / 0.83	16.25 / 12.50 / 5.42
Cation Exchange Capacity [meq/100 g] [2]	3.5	6.3
Redox Potential E_h [mV][1,5]	+ 355	+ 469
Moisture [g H_2O ad 100 g dry weight]	23.9	50.1

[1] day of sampling
[2] start of acclimation
[3] DAT-0 / DAT-125
[4] %organic matter =%organic carbon x 1.724
[5] Potential difference between used electrode* and H_2-electrode at 20°C: 210 mV
Theoretical potential of used buffer solution for Pt-Ag/AgCl electrode at 25°C: 220 mV

Methods :
Study design
The test system consisted of special cylindrical glass container (volume about 1000 mL, inner diameter about 10.5 cm, surface area about 86.6 cm2, see Figure 4). The vessels were fitted with solid trap attachments permeable for oxygen but absorbing volatile compounds formed in the test systems to soda lime (CO_2) and polyurethane foam (organic volatiles).
For preparation of the test systems, wet sediment with a mass equivalent to a volume of 175 mL was weighed into each flask and 520 mL of the corresponding water were added. The volume ratio of water to sediment used was approximately 3:1 with a sediment layer of about 2 cm. The flasks were then fitted with trap attachments, stoppers and stirrers.
For acclimation of the test systems and for establishment of phase separation, the test systems were stored under the intended study incubation conditions for 19 days prior to application.

Experimental Conditions:
The test vessels were incubated in a climatic cabinet at about 20.0°C in the dark. Maintenance of aerobic conditions was achieved by a slight continuous movement of the water surface and the use of "open" test systems (so-called bio-meter flasks) with solid trap attachments permeable for air.

Sampling:
Duplicate samples of both test systems were taken and analyzed after 0, 3, 7, 22, 30, 65, 93 and 125 days of incubation

Analytical Procedures :
The water layers were decanted and centrifuged. The volumes of the water layers were determined and aliquots thereof were analyzed by liquid scintillation counting (LSC) to measure the radioactivity content. From day 3 onwards, aliquots of the water phases were taken before to determine the dissolved amount of CO_2. The sediment samples were extracted three times with 80 mL acetonitrile/water (80:20, v:v) at ambient temperature and once under reflux conditions with 80 mL acetonitrile/water (80:20, v:v), too. All extracts were combined and analyzed by LSC. Concentrates of the water

layers and the organic extracts were analyzed by high performance liquid chromatography with radiodetection (HPLC/radiodetection) to quantify the test item as well as possible transformation products.
The exhaustive extracted sediment phases were air-dried, homogenized and combusted in an oxidizer. The evolved CO_2 was trapped in a scintillation cocktail and measured by LSC to determine the amounts of non-extractable residues (NERs).
At the last sampling date, sediment aliquots were used to determine the amount of CO_2 trapped in the sediment as well as for a further characterization of the non-extractable residues.

RESULT AND DISCUSSION

Mass balance and Distribution of Radioactivity :

Table 5-32: Material balance and Biotransformation Angler See in % of AR

Compound	Source	Mean	DAT							
			0	**3**	**7**	**22**	**30**	**65**	**93**	**125**
Ethofumesate	Water Layer	Mean	98.1	76.6	72.8	61.6	58.7	36.8	26.2	22.0
	Sediment	Mean	1.1	15.8	18.9	25.5	28.3	23.9	17.9	14.2
	Entire System	Mean	**99.3**	**92.4**	**91.7**	**87.2**	**87.0**	**60.7**	**44.1**	**36.2**
NC 20645 (AE C639175)	Water Layer	Mean	n.d.	1.4	1.7	2.5	1.1	4.8	2.8	3.7
	Sediment	Mean	n.d.	0.1	0.4	n.d.	2.6	1.4	0.7	n.d.
	Entire System	Mean	**n.d.**	**1.5**	**2.1**	**2.5**	**3.7**	**6.2**	**3.5**	**3.7**
u2	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	n.d.
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	n.d.
u3	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	0.7	n.d.
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	0.7	n.d.
u4	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	3.8	2.6
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	3.8	2.6
u5	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	1.8
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	1.8
u6	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	3.0
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	3.0
u7	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	0.7
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	0.7
u8	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8
Total Extractable Residues *	Water Layer	Mean	98.1	78.0	74.5	64.2	60.5	45.1	35.1	36.6
	Sediment	Mean	1.1	15.8	19.3	25.5	30.9	25.3	18.6	14.2
	Entire System	Mean	99.3	93.7	93.7	89.7	91.5	70.3	53.7	50.8
$^{14}CO_2$ #		Mean	n.a.	0.1	0.1	0.1	0.3	2.8	8.5	15.3
Organic Volatiles #		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1
Non-Extractable Residues #		Mean	0.2	1.6	2.1	5.3	4.9	21.4	32.8	43.2
Total Recovery *		Mean	99.4	95.4	95.9	95.1	96.7	94.7	94.9	109.3

n.d.: not detected, n.a.: not analyzed, DAT: days after treatment, SD: standard deviation

* Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

Values taken from Material Balance Tables

Table 5-33: Material balance and Biotransformation Hönniger Weiher in % of AR

Compound	Source	Mean SD	DAT 0	3	7	22	30	65	93	125
Ethofumesate	Water Layer	Mean	96.4	66.3	56.7	37.7	34.5	19.5	18.1	10.5
	Sediment	Mean	2.4	27.3	35.4	50.1	50.3	50.2	50.0	35.9
	Entire System	**Mean**	**98.8**	**93.6**	**92.1**	**87.8**	**84.8**	**69.8**	**68.1**	**46.4**
NC 20645 (AE C639175)	Water Layer	Mean	n.d.	1.1	1.4	1.9	0.5	8.6	10.3	13.8
	Sediment	Mean	n.d.	n.d.	0.5	0.3	3.8	3.3	4.4	5.1
	Entire System	**Mean**	**n.d.**	**1.1**	**1.9**	**2.2**	**4.3**	**11.9**	**14.7**	**18.8**
u3	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.6	0.5
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.6	0.5
u4	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
u5	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
u6	Water Layer	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
	Sediment	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
Total Extractable Residues *	Water Layer	Mean	96.4	67.4	58.1	39.6	35.0	28.7	29.0	26.3
	Sediment	Mean	2.4	27.3	35.9	50.4	54.1	53.5	54.4	40.9
	Entire System	Mean	98.8	94.7	94.0	90.0	89.1	82.2	83.4	67.2
$^{14}CO_2$ #		Mean	n.a.	< 0.1	< 0.1	0.1	0.2	0.9	1.9	5.3
Organic Volatiles #		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1
Non-Extractable Residues #		Mean	0.2	2.4	3.6	6.0	7.9	11.3	13.1	25.7
Total Recovery *		Mean	99.0	97.1	97.6	96.1	97.2	94.5	98.3	98.1

n.d.: not detected, n.a.: not analyzed, DAT: days after treatment, SD: standard deviation

* Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

Values taken from Material Balance Table

Non-extractable and Extractable Residues:
The amount of extractable residues decreased from 99.3% / 99.8% of AR at DAT-0 to 50.8% / 67.2% of AR at the end of incubation for test systems Angler See and Hönniger Weiher, respectively. The amount of non-extractable residues increased from 0.2% / 0.2% of AR to 43.2% / 25.7% under the same conditions.
Due to the high formation rate of non-extractable residues a supplementary test with sterilized water/sediment systems was performed. Under sterile conditions significantly less NERs were formed (3.2% and 6.9% for Anglersee and Hoenniger Weiher, respectively) indicated that the formation of bound residues was primarily not a matter of strong adsorption or binding of ethofumesate. The binding of ethofumesate and its transformation products is due to metabolism and irreversible enzyme-mediated binding to sediment or incorporation into microbial processes.

Mineralization :
The maximum amount of ^{14}-CO_2 was 15.3% and 5.3% in the water sediment system Anger Weiher and Hönniger Weiher, respectively.

Transformation of Test Material and Transformation Products :

The dissipation time (DT_{50}) of ethofumesate from the water layer (sum of degradation and translocation processes into the sediment) was calculated to be 42.9 days for the Anglersee test systems and 10.0 days for the Hoenniger Weiher test systems.
The degradation half-lives (DT_{50}) of ethofumesate in the entire water/sediment systems were calculated to be 89.1 days for the Anglersee test systems and 139.3 days for the Hoenniger Weiher test systems, respectively.

The major metabolite appearing in the test systems was NC20645 (AE C639175, K+ salt of ethofumesate-carboxylic acid). It was identified by HPLC co-chromatography with the primary chromatographic method and a confirmation method using a non-radiolabeled reference item. This metabolite amounted to a maximum of 6.2% AR (DAT-65) in the entire Anglersee water/sediment systems and to a maximum of 18.8% AR (DAT-125) in the entire Hoenniger Weiher water/sediment systems.

The maximum amounts of a single minor radioactivity zone in the entire Anglersee and Hoenniger Weiher water/sediment systems were 3.8% and 0.7% AR, respectively. Due to the low amounts of the minor metabolites, identification procedures were not performed.

Ethofumesate is a racemate of two enantiomers. In order to demonstrate the same behavior of both enantiomers, water and sediment fractions from both test systems (DAT-125) were isolated by the standard non-chiral HPLC. These fractions were analyzed with a chiral HPLC method. The chromatograms obtained for Ethofumesate (DAT-125) and for pure solutions of the enantiomers demonstrated that the ratio of the two enantiomers in water and in sediment did not change during the time of incubation.

Conclusion:
Ethofumesate was moderately fast degraded in 2 different water / sediment systems. The main metabolite was NC 20645 (ethofumesate carboxylic acid). In addition some minor metabolites were detected in low amounts (max. 3.8%). The behavior of the enantiomers is the same in water and in sediment.

Comments RMS

The study is valid. The kinetic evaluation is carried out in Chapple (2013).

Reference:	**Kinetic evaluation of the degradation of ethofumesate in an aerobic water-sediment system**
Notifier:	Taskforce
Author(s), year:	Chapple, A.C.;2013
Report/Doc. number:	EnSa-13-0250 / M-459125-01-1
Guideline(s):	Not applicable
GLP:	No
Deviations:	
Validity:	Valid
Status:	New study

The degradation and dissipation behavior of ethofumesate in water-sediment systems was investigated by kinetic evaluation of an aerobic laboratory water-sediment study conducted with ^{14}C-labelled ethofumesate ([phenyl-UL-^{14}C]-AE B049913) in two different test systems: a sand from Anglersee, Germany, and a loamy-sand from Hoenniger Weiher, Germany (Stupp and Weuthen, 2013).
According to the recommendations of FOCUS (2006), (Level I) dissipation half-lives of ethofumesate in water and sediment were determined as well as the degradation DT_{50} for the total systems. An overview over the arithmetic DT_{50} values for use as inputs in environmental fate models is given in as well as for use in assessing persistence endpoints. A Level II degradation assessment was attempted but the high correlations between the various transformation factors so derived were sufficiently high as to render the analysis invalid. (It is, however, reported in detail.)
Generally, where the evaluations were done using SFO kinetics, the persistence endpoints are equal to those for modeling purposes. Only in the case of the dissipation from the water phase did ethofumesate show a non-SFO behavior and consequently both modeling and persistence endpoints were obtained using different kinetic models, according to FOCUS (2006).

Total system Angler See:

Table 5-34: DT_{50} values for ethofumesate and its metabolite and results of statistical evaluation of the model fits using SFO kinetic for total system Anglersee

Substance	DT_{50}	DT_{90}	chi^2 test	t-test probability	Visual acceptability	
					Curve	Residues
Ethofumesate	89.0	295.5	4.2	<0.001	++	+
Ethofumesate-carboxylic acid NC 20645	18.7	62.2	18.1	<0.001	+	-

Other details: M_0 99.49; Formation fraction 0.385

Total system Hönniger Weiher:

Table 5-35: DT50 values for ethofumesate and its metabolite and results of statistical evaluation of the model fits using SFO kinetic for total system Hoenniger Weiher [NR: not reliable]

Substance	DT_{50}	DT_{90}	chi^2 test	t-test probability	Visual acceptability	
					Curve	Residues
Ethofumesate	141.2	468.9	3.4	<0.001	+	++
Ethofumesate-carboxylic acid NC 20645	>1000[NR]	>1000[NR]	9.9	0.5	++	+

Other details: M_0 97.06; Formation fraction 0.416 [NR] Not Reliable

RESULT AND DISCUSSION

DT_{50} values for ethofumesate in the entire system ranged from 89.0 to 141.2 days, with an arithmetic mean of 115.1 days.

Comments RMS

The study is valid. Ethofumesate and NC 20645 were assessed for the entire system, for both water and sediment systems by the notifier and accepted by the RMS.
In the Hoenniger Weiher water phase, concentrations of metabolite NC20645 were increasing at study end; therefore, no reliable DissT50 could be calculated.

The relevant endpoints for **ethofumesate** are:

	water phase			**sediment**		
	DissT50 /DT90 (d)	Chi^2 (%)	Kinetic model	DissT50 /DT90 (d)	Chi^2 (%)	Kinetic model
Anglersee	43 / 187	2.3	DFOP	96 / 320	3.2	SFO
Hönniger W.	9.9 / 130	4.4	DFOP	1000	1000	SFO

	Total system		
	DegT50 /DT90 (d)	Chi^2 (%)	Kinetic model
Anglersee	89 / 296	4.2	SFO
Hönniger W.	141 / 469	3.4	SFO

The relevant endpoints for **NC20645** are:

	water phase			**sediment**		
	DissT50 /DT90	Chi^2	Kinetic model	DissT50 /DT90	Chi^2	Kinetic model

	(d)	(%)		(d)	(%)	
Anglersee	1000	-		35.6 / 118	3.2	SFO
Hönniger W.	1000	-		1000	-	

	Total system		
	DegT50 /DT90 (d)	Chi² (%)	Kinetic model
Anglersee	18.7 / 62	18.1	SFO
Hönniger W.	1000	-	SFO

Reference:	**Kinetic evaluation of the degradation of ethofumesate in an aerobic water/sediment system**
Notifier:	Taskforce
Author(s), year:	Schmitt, W.;2008
Report/Doc. number:	MEF-08/247 / M-301623-01-1
Guideline(s):	Not applicable
GLP:	No
Deviations:	
Validity:	Valid
Status:	New study

EXECUTIVE SUMMARY

The degradation and dissipation kinetics of [^{14}C]-ethofumesate in aquatic systems was investigated by evaluating respective experimental data from three different water/sediment systems. These include a river water and loamy sand sediment (Rhine River) system and a pond water and clay loam sediment (Anwiler Teich) system, [Kellner G., 1995] as well as a pond system with sandy sediment (Hubertus-see) [Celorio, J., 1984].

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics [FOCUS, 2006] and considered modeling endpoints. It includes an analysis of the total system degradation and single phase dissipation by analyzing the decline curves.

Table 5-36: SFO degradation parameters for the total system.

System	DT_{50} [days]	DT_{90} [days]
Hubertussee (Celorio, J., 1984, M-155553-01-1)	162	538
Rhine River (Kellner, G., 1995, M-161568-01-1)	103	342
Anwiler Teich (Kellner, G., 1995, M-161568-01-1)	164	543

Table 5-37: SFO dissipation parameters for ethofumesate in the water phase

System	DT_{50} [days]	DT_{90} [days]
Hubertussee	31.2	104
Rhine River	34.8*	116
Anwiler Teich	56.8*	189

*derived from biphasic model

Table 5-38: SFO Dissipation parameters for ethofumesate in the sediment phase

System	DT_{50} [days]	DT_{90} [days]
Rhine River	174	578
Anwiler Teich	279	928

RESULT AND DISCUSSION

DT_{50} values for ethofumesate in the entire system ranged from 103 to 164 days, with a geometric mean of 140 days.

The following figures and tables show the input parameters and the results of the kinetic evaluation carried out by the notifier.

Table 5-39: Measured amounts of ethofumesate in percentage of applied radioactivity for the test systems Rhine River [Kellner G., 1995].

Days after application	Ethofumesate water	Ethofumesate sediment	NIR	NER	$^{14}C0_2$	NIR + NER+ $^{14}C0_2$
0*	95.9	n.d.	n.d.	0.1	n.d.	0.1
0.25	89.6	8.6	0.4	0.2	<0.1	0.6
1	81.7	14.5	1.4	0.3	<0.1	1.7
2	73.6	21.6	1.6	0.2	<0.1	1.8
7	57.6	34.6	0.6	0.3	<0.1	0.9
14	48.4	41	1.7	0.6	<0.1	2.3
30	27.2	49.3	13.3	3.5	0.1	16.9
61	18.7	45.5	16.1	10	0.5	26.6
103	12.6	36.7	25	14.2	1.5	40.7

n.d. = not detected

Table 5-40: Measured amounts of ethofumesate in percentage of applied radioactivity for the test systems Anwiler Teich [Kellner G., 1995].

Days after application	Ethofumesate water	Ethofumesate sediment	NIR	NER	$^{14}C0_2$	NIR + NER+ $^{14}C0_2$
0*	92.8	n.d.	n.d.	0.1	n.d.	0.1
0.25	88.7	7.3	0.3	0.3	<0.1	0.6
1	81.8	12.8	0.6	0.3	<0.1	0.9
2	73.8	21.3	0.5	0.6	<0.1	1.1
7	60.8	32.6	1	0.9	<0.1	1.9
14	52.2	38.3	1.3	1.2	<0.1	2.5
30	38.5	48.7	3.9	3.4	0.1	7.4
61	30.5	46.7	7.6	9.6	0.3	17.5
103	17.8	40.6	12.1	20.5	1.2	33.8

n.d. = not detected

Comments RMS

The study is valid. Results from the experiment Hubertussee were not further considered since the respective study (Celorio, 1984) was considered not valid.

For both the Rhine River and the Anwiler Teich system, the SFO model was the most appropriate for the total system. In turn, DFOP clearly gave the best fits for the water phase of the two systems. For the sediment phase, in both studies only three data points were available and therefore, no reliable endpoints could be determined.

The relevant endpoints are:

	Water phase. DissT50 /DT90 (d)	Chi² (%)	Kinetic model	Sediment phase DissT50 /DT90 (d)	Chi² (%)	Kinetic model
Rhine River	13.3 / 94.0	4.7	DFOP	1000	-	SFO

Anwiler Teich	23.1 / 155	2.5	DFOP	1000	-	SFO
	Total system DegT50 /DT90 (d)	Chi² (%)	Kinetic model			
Rhine River	103 / 342	1.1	SFO			
Anwiler Teich	164 / 543	2.0	SFO			

Reference:	**Degradation and Metabolism of Ethofumesate in two Water/Sediment Systems under Aerobic Conditions – Laboratory Test**
Notifier:	UPL
Author(s), year:	Heintze, A. (2003)
Report/Doc. number:	20011407/01-CUWS
Guideline(s):	BBA Guideline, part IV, 5-1 (1990) and SETAC recommendations (1995).
GLP:	Yes
Deviations:	
Validity:	Valid
Status:	New study

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** [^{14}C U-ring]Ethofumesate
   Batch No.: 00BDR495/2/1
   Radiochemical Purity: 99.3%
   Specific activity: 1.72 GBq/mmol (46.4 mCi/mmol)
   CAS No.: 26225-79-6

2. **Test material:** Ethofumesate
   Batch No.: #1997/1
   CAS No.: 26225-79-6
   Purity: 98.59%

3. **Test material (reference):**
   EDB (2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-ol)
   HDBM (2,3-dihydro-2-hydroxy-3.3-dimethyl-benzofuran-5-yl methanesulphonate, =NC 8493)
   HDS (2-(2-hydroxy-5-methanesulphonyloxyphenyl)-2-methylpropionic acid, = NC 20645)
   Ethofumesate-2-keto ([2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulphonate)

4. **Water/Sediment**: Two test systems were used, pond and creek sampled from different locations. The test system was characterised and acclimated at 20°C under aerobic conditions in the dark over a period of 44 days. The water sediment systems were sieved through a 2 mm mesh and water was filtered through a 0.2 mm sieve, the sediment was sieved through a 2.00 mm sieve.

Table 5-41: Characterization of water and sediment samples

Parameter	Pond (silty loam)			Creek (sand)		
	Before start / Beginning of the study	After 140 days	After 232 days	Before start / Beginning of the study	After 140 days	After 232 days
Water						
Total P [mg/L]	0.06 / < 0.02	0.38	0.075	< 0.02	0.58	0.18
Ca/Mg/Na/K [mg/L]	68/30/9.3/2.3 [a]			44/21/10/3.3 [a]		
Total N [mg/L]	< 1 / < 1	< 1	< 1	3.6 / < 1	< 1	< 1
Total organic carbon [mg/L]	11.7 / 103	6.7	8.8	2.3 / 97	4.2	10
Temperature [°C]	16.8 [a]	n.d.	n.d.	6.0 [a]	n.d.	n.d.
pH	7.92 [a]	n.d.	n.d.	8.24 [a]	n.d.	n.d.
Oxygen [mg/L]	10.4 [a]	n.d.	n.d.	10.5 [a]	n.d.	n.d.
Redox potential [mV]	+ 135 [a]	n.d.	n.d.	+ 141 [a]	n.d.	n.d.
Sediment						
Total P [mg/L]	470 [a]	n.d.	n.d.	69.5 [a]	n.d.	n.d.
Total N [mg/L]	715 [a]	n.d.	n.d.	112 [a]	n.d.	n.d.
pH	7.8 [a]	n.d.	n.d.	7.5 [a]	n.d.	n.d.
Total organic carbon [%]	1.14 [a]	n.d.	n.d.	0.14 [a]	n.d.	n.d.
Sand/slit/clay [%]	21.9/60.9/17.2 [a]	n.d.	n.d.	88.9/6.9/4.1 [a]	n.d.	n.d.
Cation exchange capacity [mval/100 g]	22.9 [a]	n.d.	n.d.	4.27 [a]	n.d.	n.d.
Redox potential [mV]	- 150 [a]	n.d.	n.d.	+ 566 [a]	n.d.	n.d.
Microbial biomass [µg C/g dry matter]	832 ± 345	1819	2373	< 10 ± 0	454	433

a determined at the time of sampling
n.d. not determined

B. STUDY DESIGN

1. Experimental conditions

The study was performed with a closed glass flow system using 1000 mL all-glass metabolism flasks (≈ 10.1 cm inner diameter) containing about 500 ± 100 mL water and 300 ± 100 g sediment. The height of the water column was about 6 cm and sediment was about 2.5 cm thick (bulk density of 1.5 g/cm^3). The system was aerated by shaking with CO_2-free, moistened air. The organic volatiles in the flask were trapped. 6 flasks were not treated and served as control and were used to determine the biomass. The samples were incubated at 20 ± 2°C protected from light for incubation periods up to 232 days.

Each test system was treated with 249 µg Ethofumesate/80 cm^2, equivalent to 1.556 kg/ha.

2. Sampling

The organic volatiles were trapped with Tenax volatile trap. The $^{14}CO_2$ was trapped by sodium hydroxide solution.

Duplicate samples were collected at sampling intervals 0, 6, 24, 48 hours and 7, 14, 29, 61, 103, 121 and 230 days after treatment.

2. Description of analytical procedures

The water was separated from the sediment by pour-out. The organic volatiles were extracted from Tenax trap with 15 ML acetone and radioactivity in the extracts was determined by LSC of an aliquot. The sodium hydroxide trapped CO_2 was determined by LSC.

After pour out of the water phase, the sediment was mixed by stirring and shaking and 100 mL acetonitrile/water (1/1, v/v) was added to the aliquots of about 100 g w.w. and amount of acetic acid sufficient to get an pH below 5.0 was added to the extracts. The incubation flasks were closed with a carbon dioxide trap and shaken overnight. The dispersed sediment was transferred to a 200 mL glass centrifuge tube and centrifuged for 10 minutes at 2600 rpm. The extraction was repeated twice and the radioactivity after each extraction step and in the combined extracts was determined by LSC of an aliquot. The sediment was afterwards extracted minimum two times with 80 mL pure acetone and the radioactivity after each extraction step and in the combined extracts was determined by LSC of an aliquot.

Partitioning of the extractables was characterised by TLC. The fractions were co-chromatographed with the reference compounds.

After the final extraction the sediment was dried and the total amount of non-extractable radioactive residues in sediment was determined by combustion and LSC.

After pour out of water from the incubation flasks the radioactivity in the water was determined by LSC. The water phase was added with 10% of its volume of acetonitrile and radioactivity was determined by LSC. An aliquot of around 100 mL was transferred to a 300 mL Erlenmeyer flask, acidified with acetic acid to reach a pH below 5.0 and closed with a carbon dioxide trap. The assemble was shaken overnight and the amount of radioactivity in the carbon dioxide trap was determined. Afterwards the remaining non-volatile radioactivity in the aqueous phase was determined by LSC on an aliquot.
Partitioning of the dissolved radioactivity was characterised by two TLC systems. The fractions were co-chromatographed with the reference compounds.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mean recovery from the pond water/sediment system during the whole study was 98.2% AR (91.4% - 113.1%) and the mean recovery from creek water/sediment system during the whole study was 100.6% AR (79.3% - 115.9)

B. FINDINGS

In the pond system the CO_2 trapped from air increased to 4.2% AR after 230 days. In the sediment radioactivity increased from 1.5% AR immediately after the treatment to 68.7% AR after 230 days. In the water phase the radioactivity decreased from 96.2% AR on day 0 to 28.1% AR after 230 days. The extractable residues increased from 1.4% AR (0 days) to approx. 43.9% AR after 61 days and decreased to 26.4% AR after 230 days. The un-extractables reached 41.9% AR at the end of the study. No organic volatiles could be found throughout the incubation period. Total mineralisation to carbon dioxide was 6.1% AR after 230 days.
The metabolite HDS increased to maximum rates of 10.6% AR after 103 days and 10.7% after 230 days. Other metabolites were below 5% AR at all sampling dates.
In the creek system the CO_2 trapped from air increased to 4.6% AR after 230 days. In the sediment radioactivity increased from 1.7% AR immediately after the treatment to 35.3% AR after 230 days. In the water phase the radioactivity decreased from 105.8% AR (0 days) to 44.7% AR after 230 days. The extractable residues increased from 1.6% AR (0 days) to approx. 26.6% AR after 29 days and decreased to 15.5% AR after 230 days. The un-extractables reached 19.4% AR at the end of the study. No organic volatiles could be found throughout the incubation period. Total mineralisation to carbon dioxide was 9.1% AR after 230 days.
The metabolite HDS increased to maximum rates of 7.5% AR after 61 days and was not detected in the system after 230 days. Other metabolites were below 5% AR at all sampling dates.

Table 5-42: Distribution of radioactivity in the pond and creek water/sediment system in % of AR

Time	CO_2 trapped directly	Water			Sediment				Sum
		Total after sampling	SNV [a]	CO_2	Total after sampling	Extract	CO_2	NER	
[d]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Pond System									
0	0.0	96.2	96.1	0.1	1.5	1.4	0.0	0.1	97.7
0.25	0.0	94.9	94.8	0.1	1.7	1.5	0.0	0.2	96.6
1	0.0	92.0	91.9	0.1	3.1	2.8	0.0	0.4	95.2
2	0.0	99.3	99.1	0.2	7.2	6.5	0.0	0.6	106.5
7	0.0	70.9	70.7	0.2	26.7	24.2	0.0	2.4	97.5
14	0.0	61.9	61.6	0.3	34.8	32.3	0.0	2.5	96.7
29	0.0	53.5	53.3	0.2	41.5	39.4	0.0	2.1	95.1
61	0.6	48.2	47.8	0.4	49.5	43.9	0.1	5.5	98.3
103	1.3	41.9	41.2	0.6	53.1	40.7	0.1	12.4	96.4
121	1.1	42.8	41.4	1.5	53.3	38.8	0.4	14.1	97.3
203	4.2	28.1	26.7	1.4	68.7	26.4	0.5	41.9	101.1
Creek System									
0	0.0	105.8	105.7	0.1	1.7	1.6	0.0	0.1	107.5
0.25	0.0	109.8	109.7	0.1	2.2	2.0	0.0	0.1	111.9
1	0.0	100.1	100.0	0.1	2.8	2.5	0.0	0.2	102.8
2	0.0	98.4	98.2	0.2	6.1	5.8	0.0	0.3	104.5
7	0.0	84.5	84.3	0.2	17.6	16.9	0.0	0.6	102.1
14	0.1	80.7	80.4	0.2	21.9	21.2	0.0	0.7	102.7

Time	CO_2 trapped directly	Water			Sediment				Sum
		Total after sampling	SNV [a]	CO_2	Total after sampling	Extract	CO_2	NER	
[d]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
29	0.3	72.1	72.0	0.1	27.3	26.6	0.0	0.7	99.7
61	1.0	67.4	66.7	0.6	29.5	24.6	0.0	4.9	97.9
103	1.1	63.8	62.6	1.2	30.8	22.3	0.2	8.3	95.7
121	1.0	64.8	60.9	3.8	31.2	21.7	0.4	9.0	96.9
203	4.6	44.7	40.6	4.2	35.3	15.5	0.4	19.4	84.6

a soluble but not volatile after acid treatment

Table 5-43: Characterisation of the radioacticity in the water phase of the creek system in % of applied radioactivity

Time (d)	Ethofumesate (%)	Keto-Ethofumesate (%)	EDB (%)	HDBM (%)	HDS (%)
0	105	0	4.7	0	0
0	97.7	0	4	0	0
0.25	100.6	0	4.8	0.8	0
0.25	108.7	0	3.9	0.6	0
1	94	0	3.8	0	0
1	97.7	0	3.1	1.6	0
2	95.6	0	2.8	1.2	0.8
2	91.4	0	2.3	1	1.2
7	78.9	0	5.1	0	0
7	80.6	0	0	0	4.1
14	75.4	0	0	0	3.7
14	77.9	0	0	0	3.8
29	66.7	0	1.8	0	3.1
29	66.8	0	1.5	0	3.9
61	54.3	0	0	0	9.7
61	64.2	0	0	0	5.2
103	62.3	0	0	0	4.2
103	52.4	0	0	0	6.3
121	60.3	0	0	0	8.8
121	50.2	0	0	0	2.6
230	44.7	0	0	0	0
230	36.5	0	0	0	0

Table 5-44: Characterisation of the radioacticity in the sediment of the creek system in % of applied radioactivity

Time (d)	Ethofumesate (%)	Keto-Ethofumesate (%)	EDB (%)	HDBM (%)	HDS (%)
0	1.2	0	0	0	0
0	2	0	0	0	0
0.25	1.7	0	0	0	0
0.25	2.4	0	0	0	0
1	3	0	0	0	0

Time (d)	**Ethofumesate (%)**	**Keto-Ethofumesate (%)**	**EDB (%)**	**HDBM (%)**	**HDS (%)**
1	2.1	0	0	0	0
2	5.6	0	0	0	0
2	6	0	0	0	0
7	16.9	0	0	0	0
7	16.9	0	0	0	0
14	22.6	0	0	0	0
14	19.7	0	0	0	0
29	25.7	0	0	0	0
29	27.5	0	0	0	0
61	26.3	0	0.8	0	0
61	21.6	0	0.5	0	0
103	23.2	0	0	0	0
103	21.4	0	0	0	0
121	23.2	0	0	0	0
121	20.3	0	0	0	0
230	15.7	0	0	0	0
230	15.4	0	0	0	0

Table 5-45: Characterisation of the radioacticity in the water phase of the pond system in % of applied radioactivity

Time (d)	Ethofumesate (%)	Keto-Ethofumesate (%)	EDB (%)	HDBM (%)	HDS (%)
0	96.9	0	2.2	0	0
0	88.5	0	4.6	0	0
0.25	88.6	0	2.5	0	0
0.25	93.3	0	4	1.2	0
1	91.7	0	2.5	1.1	0
1	83.3	0	3	2.1	0
2	89.9	0	2	0.7	0.3
2	101.3	0	2.3	1.1	0.6
7	68.8	0	0	0	2.4
7	65.9	0	0	0	4.2
14	58.1	0	0	0	3.2
14	57.3	0	0	0	4.6
29	50.3	0	0	0	3
29	48.1	0	0	4.6	0
61	43.3	0	0	0	5.6
61	41.5	0	0	0	5.2
103	30.8	0	0	0	9.3
103	33.7	0	0	0	8.7
121	35.5	0	0	1.1	5.2
121	34.9	0	0	0.4	5.6
230	15	0	1.1	2.1	8.6
230	11.6	0	0.8	1.3	12.8

Table 5-46: Characterisation of the radioacticity in the sediment of the pond system in % of applied radioactivity

Time (d)	Ethofumesate (%)	Keto-Ethofumesate (%)	EDB (%)	HDBM (%)	HDS (%)
0	1.5	0	0	0	0
0	1.3	0	0	0	0
0.25	1.4	0	0	0	0
0.25	1.6	0	0	0	0
1	3	0	0	0	0
1	2.5	0	0	0	0
2	6.2	0	0	0	0
2	6.9	0	0	0	0
7	22.8	0	0.7	0	0
7	24.4	0	0.6	0	0
14	31.8	0	0	0	0
14	32.7	0	0	0	0
29	39.4	0	0	0	0
29	-	-	-	-	-
61	40.9	0	1.4	0	0
61	43.6	0	2	0	0
103	38.6	0	0	1.2	1
103	38.3	0	0	0	2.2
121	35.3	0	0	1	0.8
121	38.9	0	1.6	0	0
230	25.2	0	2.4	0	0
230	22	0	3.2	0	0

Table 5-47: Sums of Ethofumesate and its metabolites in water and sediment of the pond and creek system in % AR

Time [d]	**0**	**0.25**	**1**	**2**	**7**	**14**	**29**	**61**	**103**	**121**	**230**
Pond System											
Ethofumesate	94.1	92.5	90.3	102.2	91.0	90.0	89.7	84.7	70.7	72.3	36.9
Keto-Ethofumesate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
EDB	3.4	3.3	2.8	2.2	0.7	0.0	0.0	1.7	0.0	0.8	3.8
HDBM	0.0	0.6	1.6	0.9	0.0	0.0	0.0	0.0	0.6	1.3	1.7
HDS	0.0	0.0	0.0	0.5	3.3	3.9	3.0	5.4	10.6	5.8	10.7
Creek System											
Ethofumesate	103.0	106.7	98.4	99.3	96.7	97.8	93.4	83.2	79.7	77.0	56.2
Keto-Ethofumesate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
EDB	4.4	4.4	3.5	2.6	2.6	0.2	1.7	0.7	0.0	0.0	0.0
HDBM	0.0	0.7	0.8	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HDS	0.0	0.0	0.0	1.0	2.1	3.8	3.5	7.5	5.3	5.7	0.0

The half-lives of Ethofumesate in water/sediment system were 188 days (1st order) in pond system and 275 days (1st order) in creek system. The DT_{90} values were not calculated.

Table 5-48: Degradation parameters of Ethofumesate in water/sediment systems

System	Phase	Kinetics	Confidence limits (95%) [days]	DT_{50} [days]
Pond	Water phase	1st order	76.0 - 97.9	86.9
		Sqrt 1st order	28.1 – 42.4	34.9
	Whole system	1st order	175 - 200	188
		Linear	191 - 210	201
Creek	Water phase	1st order	163 - 199	181
		Sqrt 1st order	121 - 153	136
	Whole system	1st order	264 - 286	275
		Linear	243 -264	253

III. CONCLUSION

The half-lives of Ethofumesate in water/sediment system were 188 days (1st order) in pond system and 275 days (1st order) in creek system. One metabolite, HDS (= NC 20645), was found in the water/sediment systems above 5% for two succeeding sampling point (creek system) or above 10% (pond system).

Comments RMS
Although the study was conducted according to the BBA Guideline, part IV, 5-1 (1990) and SETAC recommendations (1995), the study was in accordance with the relevant OECD 308 guideline. Significant details on the analytical method are missing, such as LOQ and LOD. This has also consequences for the subsequent kinetic evaluation. After first detect concentrations below LOD should be set to ½ LOD. Although the impact on the endpoints might be limited, clarification regarding analytical methods (LOD, LOQ) has to be provided.

Response notifier UPL/Agrichem:
The notifier was requested provide clarifications regarding LOD and LOQ of the method of analysis. The following statement was provided:
The LOD for the water phase is 0.75 µg/L (i.e. 1 kBq/L). This corresponds to 0.15% of applied radioactivity (AR), assuming a water volume of 500 mL per flask and an amount of 249.5 µg (i.e. 333 kBq) test item per flask which corresponds to 0.50 µg test item or 666 Bq radioactivity, resp., per mL water.
The LOD for the sediment extract is 2.62 µg/kg assuming a dilution factor of 3.5 during extraction. This corresponds to 0.32 % AR, assuming a mass of 300 g sediment (wet weight) per flask and an amount of 249.5 µg (i.e. 333 kBq) test item per flask which corresponds to 0.83 µg test item or 1110 Bq radioactivity, resp., per g sediment.

Conclusion RMS:
The study is acceptable, reliable endpoints can be derived.

Reference:	**Calculations of the environmental fate endpoints in water/sediment systems for Ethofumesate according to recommendations of the FOCUS working group on degradation kinetics**
Notifier:	UPL
Author(s), year:	Stangelj, A.; 2014
Report/Doc. number:	210790-CA-07020203-01
Guideline(s):	Not applicable
GLP:	No
Deviations:	
Validity:	Valid
Status:	New study

A kinetic analysis of the Heintze 2003 study was performed according to recommendations of the FOCUS workgroup on degradation kinetics (2006) and is reported in Stangelj (2014). The results of this analysis support the degradation as reported in the Heintze 2003 study. The analysis is submitted under KCA 7.2.2.3/02 and shortly summarised below.

Water/sediment degradation rates derived from experimental values obtained in a laboratory study with ^{14}C-Ethofumesate (Heintze, 2003) were calculated according to recommendations of the FOCUS workgroup on degradation kinetics (2006 & 2011).

Degradation data of two water-sediment systems were used in calculations performed with the model software KinGUI version 2.0. Modelling was done using all data, no weighting and M0 (total amount at time 0) were not fixed for the parent. M0 of the metabolites were fixed to 0. Flows from parent to metabolites as well as from parent or metabolite, resp., to sink were considered for the simultaneous fittings.

The data were optimized and integrated according to standard recommendations and assuming SFO (single first order) kinetics and FOMC (first order multi compartment) kinetics for Ethofumesate and assuming single first order kinetics for the metabolite. The calculated output data (consisting of daily percentages of the nominally applied concentration) and residuals (differences between calculated concentrations and actual measured concentrations) were graphically fitted and visually assessed. Following an acceptable visual assessment, the deviations between observed and calculated values relative to the uncertainty of the measurements were assessed using the chi-square (χ^2) statistical test where the FOCUS trigger level of < 15% was applied. A test of the confidence of the calculated data returned after optimisation was performed using a t-test and employing the FOCUS trigger value for probability of < 0.05.

For Ethofumesate, the obtained results indicate that SFO was the model that clearly fits best for both water-sediment systems. In all cases the kinetic evaluation using SFO resulted in better curve fittings and lower χ^2 values. For FOMC the probabilities of the t-test indicated that the parameters α and β are not significantly different from zero. FOMC did also not provide better Chi² (χ^2) values than the SFO model.

For parent/metabolite combination (pond and creek system) the simultaneous fittings led to acceptable results. The resulting curve fittings and residual plots for parent and metabolite were visually acceptable considering the inherent scatter of degradation data. However, the Chi² (χ^2) value for the metabolite significantly exceeds the trigger of 15 which was considered to be acceptable due to the scatter of degradation data. Furthermore, only the values of pond system can be used for the metabolite, due to inadequate simultaneous fitting for the creek system as the Chi² for the metabolite fitting is above 40.

Table 5-49: Whole system modelling and persistence endpoints for Ethofumesate and metabolite based on data obtained in two water/sediment system (pond and creek) and considering FOCUS kinetics

Substance	Kinetic model	degDT50 (d)	degDT90 (d)	Formation fraction	Plots visually acceptable	Chi2	t-test	EF
Pond								
Ethofumesate	SFO	217.7	723.1	n.a.	yes	5.151	<0.05	0.9888
NC 20645	SFO	99.1	329.2	0.443	yes	32.445	<0.05	
Creek								
Ethofumesate	SFO	204.8	680.2	n.a.	yes	3.711	<0.05	0.9903
NC 20645	SFO	13.4	44.6	1	yes	40.056	<0.05	

Table 5-50: Results of the kinetic evaluation for the active substance Ethofumesate (parent to sink)

Water-sediment system	Kinetic model	degDT50 (d)	degDT90 (d)	Plots visually acceptable	Chi2 (trigger:15)	t-test (trigger:0.05)	EF
Pond	SFO	217.3	722	yes	5.049	<0.05	0.8864
	FOMC	217.5	728.2	yes	5.162	a: 0.406 b: 0.406	0.886
Creek	SFO	208.6	692.9	yes	3.626	<0.05	0.8985
	FOMC	208.7	696.6	yes	3.709	a: 0.43 b: 0.43	0.8983

Comment RMS

The notifier has provided calculations for the whole system, however dissipation kinetics of ethofumesate in the water and sediment compartment were not calculated by the notifier. For both systems, the notifier carried out simultaneous fittings (parent/metabolite) as well as the fitting for the parent only.

The RMS has performed additional kinetic evaluations according to level-I (FOCUS, 2006).

The dissipation kinetics for metabolite NC20645 in the total system of the experiment "Pond" are acceptable. In the water phase, maximum occurrence was not reached at study end - no degradation kinetics could be derived. NC 20645 occurs only at two sampling dates in the sediment phase (<3% AR).

The dissipation kinetics for metabolite NC20645 in the total system of the experiment "Creek" showed a very large scatter and the fits are visually unacceptable. For the water compartment, acceptable fits were achieved. The metabolite was not detected in the sediment phase.

The endpoints for **ethofumesate** are:

	$DissT_{50}/DissT_{90}$ Water	Chi^2	Kinetic model	$DissT_{50}/DissT_{90}$ sediment	Chi^2	Kinetic model
Pond	37 / 343	5.7	DFOP	258 / 857	6.6	SFO
Creek	141 / 804	2.4	DFOP	273 / 907	1.7	SFO

	$DegT_{50}/DegT_{90}$ Total system	Chi^2	Kinetic model
Pond	217 / 722	5.0	SFO
Creek	209 / 693	3.6	SFO

The endpoints for **NC20645** were derived from the simultaneous fittings (parent/metabolite) and are:

	$DissT_{50}/DissT_{90}$ Water	Chi^2 model	Kinetic	$DissT_{50}/DissT_{90}$ sediment	Chi^2	Kinetic model
Pond	1000	-	SFO	1000	-	-
Creek	81 / 269	11.7	SFO	-	-	-

	$DegT_{50}/DegT_{90}$ Total system	Chi^2	Kinetic model
Pond	99 / 329	32.4	SFO
Creek	1000	-	SFO

5.1.3 Summary and discussion of degradation

Aquatic hydrolysis

Ethofumesate is stable to hydrolysis at pH 4, pH 7 and pH 9. No major degradation products were observed.

Hydrolytic degradation (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.1.1

Hydrolytic degradation of the active substance and metabolites > 10 %	pH 5: stable at 20 °C
	pH 7: stable at 20 °C
	pH 9: stable at 20 °C

Aquatic photolysis

In the first evaluation for approval, the photolytic degradation of ethofumesate was reported for a number of studies with variable results. Aqueous photolysis at pH 7 with filtered light from an Hg-arc lamp resulted in a DT_{50} of 28-31 hours (3-5 fold intensity of natural sunlight) in irradiated solutions. However, due to 41% of unidentified radioactivity in this study and experimental deficiencies in other aqueous photolysis studies, new studies were conducted by both notifiers. In both new aqueous photolysis studies, a multitude of transformation products was formed; none of them exceeding 10% AR. A similar degradation pattern is observed in a study investigating the photolysis of ethofumesate in natural water, which was performed for registration in Japan and is an optional data requirement. The results mirrored the findings of the study on aqueous photolysis in buffered solution. A large number of unidentified photodegradates were formed, two of them above 5% AR

Aqueous photochemical degradation (Regulation (EU) N° 283/2013, Annex Part A, points 7.2.1.2 / 7.2.1.3)

Photolytic degradation of active substance and metabolites above 10 %	DT_{50} : 15.6 d Natural light, 33°N; DT_{50} 53.2 days
Quantum yield of direct phototransformation in water at Σ > 290 nm	$1.92 \cdot 10^{-4}$ mol · Einstein $^{-1}$

Biological degradation

Three **readily biodegradability studies** were conducted, all three indicating that no rapid biodegradation occurs. Contrasting results were reported for the new **aerobic mineralization studies in water**. In the study by the notifier UPL, ethofumesate was found to be stable in natural surface water until day 62 of incubation and the mineralisation was marginal with a maximum of 1.1% (high-dose test) and 0.8% (low-dose test) at the end of the incubation period. The new study on aerobic mineralization in surface water submitted by the notifier Taskforce, however, showed that after a lag phase of 60 days a significant degradation of ethofumesate was observed: the remaining amounts of ethofumesate after 88 days were 58.3% AR and 79.3% AR in the low- (10 µg/L) and high-dose (100 µg/L) experiment, respectively. The main metabolite formed was NC 8493 (ethofumesate-2-hydroxy) with a maximum amount of 18.3% AR. The metabolite identified as BCS CW35117

(ethofumesate acetic acid) was formed at 13.4% AR and 2.4% AR in the low-dose and high-dose experiment, respectively.
In conclusion Ethofumesate was characterised not `rapidly degradable`.

Table 5-51: Aerobic mineralisation in surface water (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.2.2 and Regulation (EU) N° 284/2013, Annex Part A, point 9.2.1)

Parent										
System identifier (indicate fresh, estuarine or marine)	pH water phase	pH sed [a]	t. °C[b]	DT_{50} /DT_{90} whole sys. (suspended sediment test)		St. (χ^2)	DT_{50} /DT_{90} Water (pelagic test)		St. (χ^2)	Method of calculation
				At study temp	Normalised to *x* °C[c]		At study temp	Normalised to x °C[c]		
Fresh (Fröschweiher)	7.68	-	22°	-	-	-	1000	-	-	SFO
Fresh (Möhlin)	6.95	-	20°	.	-	-	331 /1000		1.4	SFO

[a] Measured in [medium to be stated, usually calcium chloride solution or water]
[b] Temperature of incubation=temperature that the environmental media was collected or std temperature of 20°C
[c] Normalised using a Q10 of 2.58 to the temperature of the environmental media at the point of sampling.

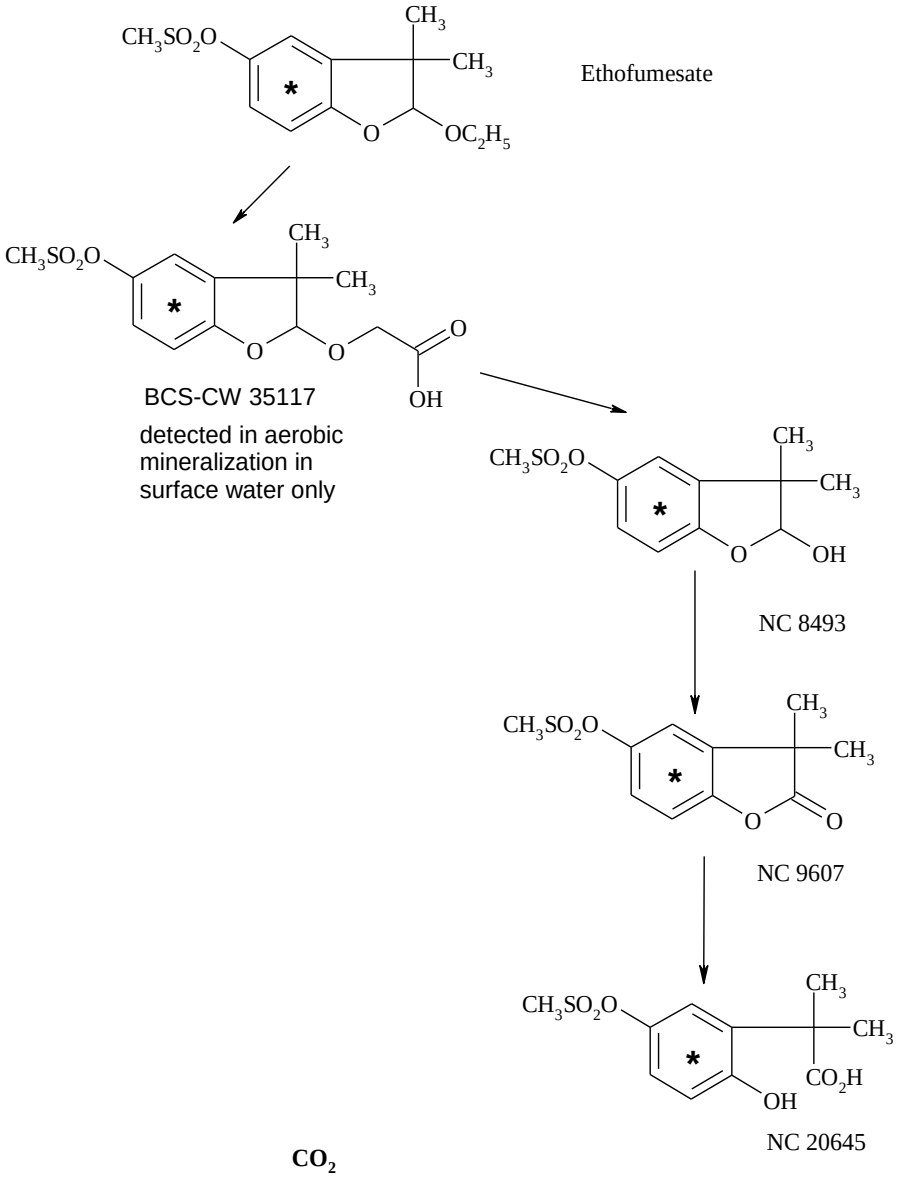
Three **dark water/sediment studies** submitted for the previous evaluation were found to be not valid anymore, mainly due to experimental insufficiencies. For instance, in two of these studies only the pH of the water phase was reported whereas in one study only the sediment pH was determined. In addition, metabolites above 10% AR were not identified within these studies. Therefore, new water sediment studies were submitted by both notifiers. Mineralisation of the active substance ranged between 1.2 % AR and 15.3% AR after 103 and 125 days, respectively. Non-extractable residues in the sediment compartment ranged between 14.2 % AR and 43.2% AR at study end. Whole system half-lives ranged between 89 and 294 days (geomean 170 d; n = 8).

Table 5-52: Water / sediment study (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.2.3 and Regulation (EU) N° 284/2013, Annex Part A, point 9.2.2)

Parent	Distribution Max. 72.2%AR in Sediment after 104 d									
Water / sediment system	pH water phase	pH sed [a]	t. °C	$DegT_{50}$ /DT_{90} whole sys.	St. (χ^2)	$DissT_{50}$ /DT_{90} water	St. (χ^2)	$DissT_{50}$ /DT_{90} sed	St. (χ^2)	Method of calculation
Rückhaltebecken	8.1	7.2[1]	20	250 / 830	1.4	52 / 457 [c]	2.4	1000	-	SFO
Waldwinkel	7.7	7.1[1]	20	294 / 976	2.3	7.8 / 101 [c]	2.2	1000	-	SFO
Anglersee	8.6	6.8[2]	20	89 / 296	4.2	43 / 187 [c]	2.3	96 / 320	3.2	SFO
Hönniger Weiher	7.2	6.3[2]	20	141 / 469	3.4	9.9 / 130 [c]	4.4	1000	-	SFO
Rhine River	7.9	6.9[2]	20	103 / 342	1.1	13.3 / 94 [c]	10.1	1000	-	SFO
Anwiler Teich	7.9	6.9[2]	20	164 / 543	2.0	23 / 155 [c]	2.5	1000	-	SFO
Pond	7.9	7.8[2]	20	217 / 722	5.0	37 / 343 [c]	5.7	258 / 857	6.6	SFO
Creek	8.2	7.5[2]	20	209 / 693	3.6	141 / 804 [c]	2.4	273 / 907	1.7	SFO
Geometric mean at 20°C[b]				170 / 564		-		536 / 840		

[a] Measured in water (1) or CaCl2 (2)
[b] Normalised using a Q10 of 2.58
[c] DFOP

Degradation pathway of ethofumesate in water/sediment systems



5.2 Environmental distribution

In **the aerobic soil degradation studies** evaluated in the course of the first approval, ethofumesate was slowly degraded (lab DT50 up to 211 days). The main degradation products were carbon dioxide and non-extractable residues. Ethofumesate was degraded in soil through the action of soil micro flora via either dealkylation (NC 8493, ethofumesate- 2- hydroxy) followed by oxidation (NC 9607, ethofumesate-lactone) and ring opening (NC 20645, ethofumesate-carboxylic acid). These studies, however, were often characterized by unappropriate handling of the experimental soils (storage of the soils outdoors or under ambient conditions for up to three months, low microbial biomass levels, no pre-incubation prior application of the spiking solutions). The newly submitted aerobic soil degradation studies confirmed the previously established degradation route, but degradation was faster due to the use of freshly sampled soils. Considering the valid studies from the previous evaluation and the new studies, ethofumesate was generally moderately fast degraded (DT50 lab: non

normalized 9.4 – 157 d; geomean nornalized to pF2 and 20°C = 21.6 d). The main degradation products were carbon dioxide and unextractable residues. Ethofumesate is degraded to NC 8493 (ethofumesate- 2- hydroxy) followed by NC 9607 (ethofumesate-lactone) and NC 20645 (ethofumesate-carboxylic acid) or the loss of the methanesulfonate moiety to transient degradates which are converted to non-extractable residues (21 - 64% AR; n = 17) and mineralized to CO_2 (4 - 60% AR; n = 17) at 100 days. Metabolites were detected in minor amounts only (< 5% AR).

Under **anaerobic** conditions, ethofumesate was not mineralized (CO_2-evolvment during anaerobiosis 2.5% AR after 152 days). It was regarded as stable under anaerobic soil conditions and therefore the anaerobic degradation is not considered to contribute significantly to the degradation route of ethofumesate.

In the studies submitted for the first approval, **soil photolysis studies** showed inconsistent results. The DT50 of the degradation of ethofumesate in soil under environmental conditions was 65 days and 13.8 days. In the first study one main phototransformation product was identified (NC 8493 with maximum amounts of about 30%). This metabolite was also observed as transient metabolite in the soil metabolism study. One minor product < 5% was formed. In the second study three radioactive fractions were detected but not identified (D2, D3 < 5%, D4 at 7.1% AR at day 30). The previous photolysis in soil studies were repeated due to experimental insufficiencies and the occurrence of considerable levels of unidentified radioactivity. In the new studies, the main transformation product was NC 8493 (max. 24.2 %,). A second minor transformation product was identified as NC 20645 (max. 4.8%). All other metabolites did not exceed 1%.

Metabolites NC 20645 and NC8493 and/or their respective glycoside conjugate were considered to represent the Peak A detected in Lysimeter studies carried out for the first approval of ethofumesate. The theoretically possible back reaction of NC 20645 to NC 9607 was investigated and was shown not to contribute significantly to the degradation of NC20645. Degradation rates of the soil metabolites NC 8493, NC 9607 and NC 20645 were determined in three separate studies. The DT50 were less than 1,5 hours for NC 8493, NC 9607 and 1-3 hours for NC 20645. This fast degradation is in line with the observed very low occurrence in the aerobic soil metabolism studies. The groundwater risk assessment was carried out for both NC20645 and NC8493 as aglycon.

For the first approval of ethofumesate, several **field dissipation studies** were submitted. In the previous list of end-points, values for 13 sites were included with DT50 values (not normalized) of 15 to 250 days with a mean of 77 days and a median of 56 days. Several of these studies were not considered acceptable after the current re-evaluation due to insufficient sampling depth. In addition to the existing field studies, the notifier UPL submitted new field studies. These field dissipation studies were evaluated to determine DT50 values, normalized to standard conditions of 20°C and field capacity for use in modeling (DT50 13.5 – 112 days; geomean 40.7 days). Legacy field studies were evaluated in accordance with the EFSA guidance (e.g. exclusion of data points before 10 mm rain). Therefore, it appears to be justified to consider them as equivalent with new studies specifically designed to minimize surface processes. Since DT50 from field studies were shown, not to be statistically different from the lab degradation studies' population (based on EFSA's excel sheet "EFSA DegT50 Endpoint Selector"), the combined geomean of lab and field studies (26.2 d) was used in the further groundwater and surface water assessment.

Table 5-53: Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1)

Parent	Aerobic conditions								
Soil type (indicate if bare or cropped soil was used).	Location (country or USA state).		pH[a)]	Depth (cm)	DT_{50} (d) actual	DT_{90} (d) actual	St. (χ^2)	DT_{50} (d) Norm[b)].	Method of calculation
MainzA Loamy silt	Germany	bare soil	7.5	0-30	116	384	13.3	69.5	SFO
MainzB Loamy silt	Germany	bare soil	7.5	0-30	114	379	11.3	47.4	SFO
Mainz A/B Loamy silt	Germany	bare soil	7.5	0-30	-	-	-	57.4 [d)]	SFO
SpeyerA Silty sand	Germany	bare soil	6.7	0-30	21 α = 0.004 β = 0.05	333	12.5	47.2 [e)]	FOMC
SpeyerA Silty sand	Germany	bare soil	6.7	0-30					DFOP
SpeyerB Silty sand	Germany	bare soil	6.7	0-30	13.6 k1 = 0.09528 k2 = 0.00772 g = 0.6392	166	3.9	46.5 [c)]	DFOP
Isleham Loamy sand bare	UK	bare soil	7.5	0-30	59	196	12.3	25.7	SFO
Willingham Sandy clay loam bare	UK	bare soil	7.5	0-30	44	147	22	18.0	SFO
Fresno Sandy loam	California	cropped with alfalfa and sugar beet	6.5	0-90	89	295	20.7	112	SFO
Northwood Clay loam	North Dakota	cropped with alfalfa and sugar beet	7.3	0-90	1000	-	-	-	SFO
Weeze sand	Germany	bare soil	5.8	0-30	**157**	522	15.0	75.7	SFO

Table 5-53: Rate of degradation field soil dissipation studies (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.2.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.2.1)

Parent	Aerobic conditions								
Soil type (indicate if bare or cropped soil was used).	Location (country or USA state).		pH[a)]	Depth (cm)	DT_{50} (d) actual	DT_{90}(d) actual	St. (χ^2)	DT_{50} (d) Norm[b)].	Method of calculation
Nierswalde Sandy loam	Germany	bare soil	3.5	0-30	1000	-	-	-	SFO
NZ11007/1 Clay loam	UK	bare soil	7.13	0-30	21.6	72	16	15.2	SFO
NZ11007/2 Silty clay loam	Germany	bare soil	7.57	0-30	10.2	74	4.1	13.5	SFO
NZ11007/3 Silty clay loam	France	bare soil	7.72	0-30	35.9 k1 = 0.03878 k2 = 0.003795 g = 0.5968	367	6.1	110 [c)]	DFOP
NZ11007/4 Loam	Spain	bare soil	7.7	0-30	12.3 k1 = 0.1805 k2 = 0.00662 g = 0.0518	237	12.0	60 [c)]	DFOP
Geometric mean (if not pH dependent)								37.8	
pH dependence					No				

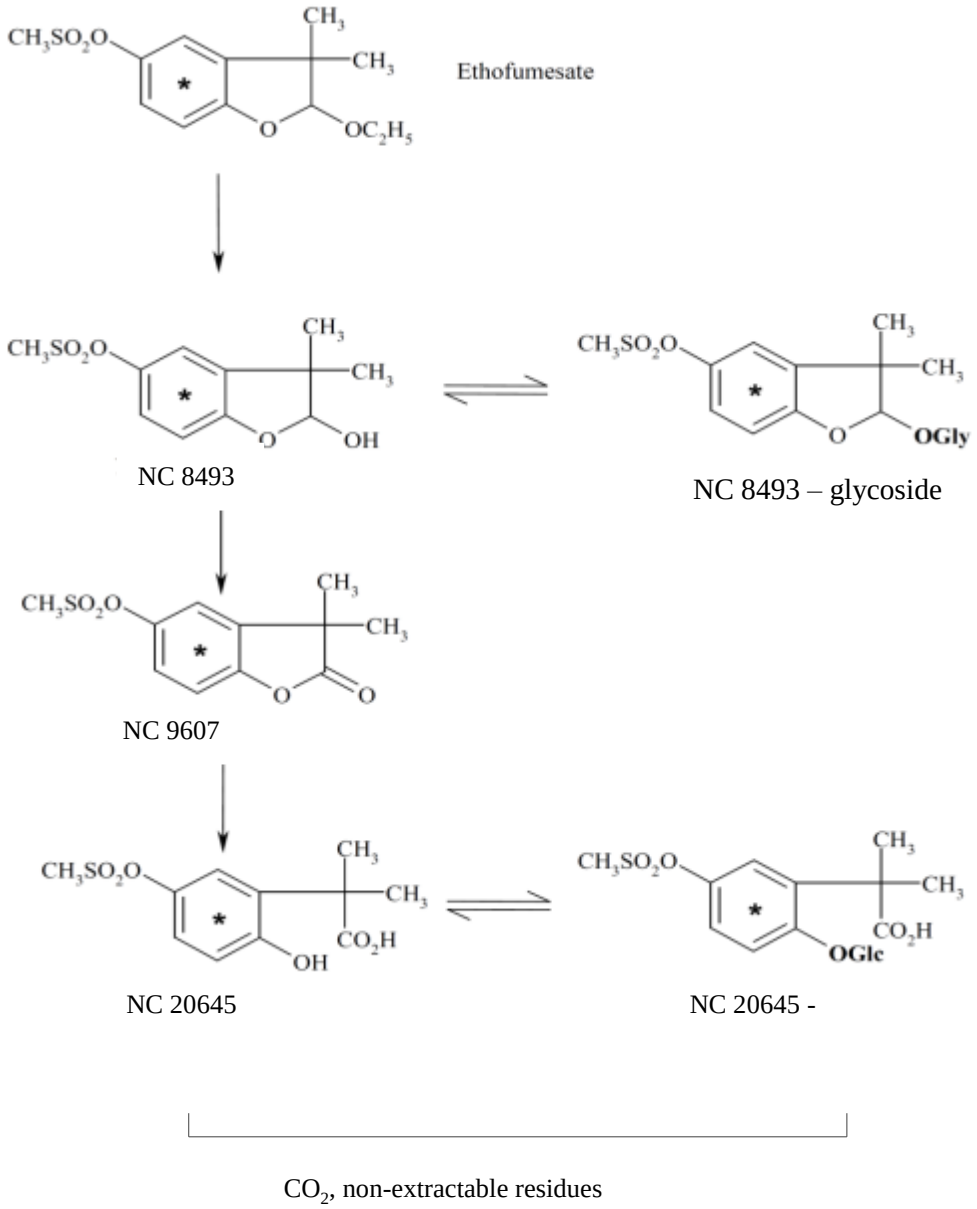
Table 5-54: Rate of degradation in soil (aerobic) laboratory studies active substance (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.2.1.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.1.1)

Parent	Dark aerobic conditions						
Soil type		pH ($CaCl_2$)	t. °C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20 °C pF2/10kPa [b)]	St. (χ^2)	Method of calculation
Sandy Loam Abington		7.0	25°C / 75 % of WHC at 33kPa	137 / 454	208	5.8	SFO
Loam/Silt Loam Terling		5.8	25°C / 75 % of WHC at 33kPa	68.7 / 228	80.5	3.0	SFO

Sandy Loam AX		6.1	20.7 °C / 55 %	28.5 / 94.7	30.4	5.1	SFO
Silt Loam HF		6.5	20.7 °C / 55 %	19.4 / 64.4	20.5	3.3	SFO
Sandy Loam WW		5.4	20.7 °C / 55 %	19.7 / 65.6	21.1	5.3	SFO
Clay Loam DD		7.2	20.7 °C / 55 %	19.1 /63.6	20.4	2.0	SFO
Sand Lufa 2.2		5.8	20°C / 40 % MWHC	69.9 / 232	69.9	15.4	SFO
Silt Loam Fislis		6.82	20°C / pF 2.5	16.0 / 53.0	14.1	2.2	SFO
Loam Horn		7.23	20°C / pF 2.5	9.4 / 31.2	8.5	6.2	SFO
Clay Montesqiuieu		7.37	20°C / pF 2.5	20.4 / 67.8	17.9	4.8	SFO
Sandy Loam Sevelen		7.51	20°C / pF 2.5	11.7 / 38.7	9.3	3.4	SFO
Loam Mussbach		7.21	20°C / 50 %	17.72 / 58.86	15.2	6.0	SFO
Sandy loam Lufa 5.2		7.3	20°C / 50 %	15.36 / 51.01	14.5	6.9	SFO
Loamy sand Lufa 2.2		5.5	20°C / 50 %	12.78 / 42.47	12.8	7.9	SFO
Clay loam UK1		6.80	20°C / 50 %	25.52 / 84.79	25.5	6.5	SFO
Sandy loam UK2		6.83	20°C / 50 %	23.29 / 77.37	23.3	3.5	SFO
Loam North France		7.41	20°C / 50 %	13.63 / 45.28	11.4	9.6	SFO
Silt loam Austria		7.14	20°C / 50 %	12.53 / 41.61	12.5	4.5	SFO
Silt loam Spain		7.38	20°C / 50 %	17.27 / 57.36	15.5	4.1	SFO
Geometric mean (if not pH dependent)					21.6		
pH dependence					No		

a) Measured in $CaCl_2$
b) Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7

The following metabolic pathway including formation of conjugates observed in outdoor studies is proposed (all metabolites < 5%):



The behaviour of both **enantiomers** of ethofumesate was exemplarily investigated under laboratory conditions in soil and water/sediment. Leachate in the tailor made lysimeter simulation study was investigated Stupp, H.-P., Junge, T. (2013). The rate of the enantiomers was stable. In addition, the ratio of enantiomers was investigated in a water/sediment study (Stupp, H.-P., Weuthen, M., 2012). A supplementary test in this study showed that the ratio of the enantiomers was stable. In one scientific paper, the potentially enantioselective degradation of ethofumesate was investigated in four Chinese soils under laboratory conditions. No significant difference was observed in three out of four soils. In one of the four soils, a minor difference (max. enantiomeric ratio: 1.65) was observed. In this soil, the half-life of the (+) enantiomer was in the typical range of the other soils, whereas the half-life of the (-) enantiomer was faster.–However, the study cannot be considered reliable for regulatory purposes and therefore, no conclusion can be made that enantioselective degradation occurs. Therefore, it is considered adequate that all studies on the active substance where performed using the racemic mixture.

5.2.1 Adsorption/Desorption

Ethofumesate was rapidly and strongly adsorbed to soil in laboratory tests with Kfoc ranging between 97 and 208 mL/g (geomean 118 mL/g; n = 12). An additional time-dependent sorption study was submitted by the notifier Taskforce. The increase of sorption over time was defined as the ratio of concentration of [Phenyl-UL-14C]Ethofumesate in soil to the concentration in aqueous 0.01 M $CaCl_2$ extracts (R_{TDS} value). At study end (91 days), the mean R_{TDS} value increased by a factor of 1.4-3.0 indicating effects of ageing on adsorption of thofumesate.

Table 5-55: Soil adsorption active substance (Regulation (EU) N° 283/2013, Annex Part A, point 7.1.3.1.1 and Regulation (EU) N° 284/2013, Annex Part A, point 9.1.2.1)

Parent							
Soil Type	OC %	Soil pH[a)]	K_d (mL/g)	K_{doc} (mL/g)	K_F (mL/g)	K_{Foc} (mL/g)	1/n
Mueller Podsol	1.5	6.1			3.7	247	0.96
Mueller Parabraunerde	1.1	7.6			1.1	100	0.91
Mueller light sand	1.5	6.7			3.0	200	0.94
Bruhl Sandy loam	1.16	6.0			1.13	97	0.84
Cameron Sand	1.12	4.6			0. 7	63	0.92
Cameron Acidic sandy loam	1.45	5.7			0.7	48	0.92
Cameron Alkaline Sandy loam	1.66	7.3			0.8	48	0.93
Icklingham, Sand	0.35	6.8			0.73	209	0.87
Abington, sandy loam	1.9	7.4			2.3	121	0.93
Terling, silt clay loam	3.2	6.6			5.3	166	0.89
Shelford clay	4.9	6.6			6.2	127	0.82
UPL loamy sand	1.41	7.3			2.6	187	0.93
Geometric mean (if not pH dependent)					1.74	118	
Arithmetic mean (if not pH dependent)							0.905
pH dependence			No				

[a)] Measured in [medium to be stated, usually calcium chloride solution or water]

5.2.2 Volatilisation

The vapour pressure of ethofumesate is 0.00065 Pa at 25°C indicating a moderate potential for volatilization from plant and soil. Since the compound is rapidly degraded in air (DT_{50} = 4.1 hours), no further investigation of its transport in air is required. It is unlikely that the compound is transported in air over long distances or accumulates in air.

Fate and behaviour in air (Regulation (EU) N° 283/2013, Annex Part A, point 7.3.1)

Direct photolysis in air	Not studied - no data requested
Photochemical oxidative degradation in air	DT_{50} of 4.1 hours derived by the Atkinson model (version not specified). OH (24 h) concentration assumed = 5×10^5
Volatilisation	No volatilisation expected
Metabolites	None

5.2.3 Distribution modelling

No information available

5.3 Aquatic Bioaccumulation

Table 5-56: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water EC A.8, OECD 117 (shake flask method)	Log P_{OW} = 2.7 (at 25 °C, pH 6.44)	Test substance: Ethofumesate pure Batch: C66/87 Purity: 99.9%	Bright, A.A.S., Stalker, A.M., 1990 Reference No. M-155196-01.1
Fish bioaccumulation test US EPA guideline 165-4	$BCF_{steady-state}$ = 144 CT_{50} < 3 days Depuration after 14 days greater than 99%	Test species: *Lepomis macrochirus* (Bluegill sunfish) Test substance: ^{14}C-Ethofumesate	Caley, C.Y., Cameron, B.D., Chapleo, S., Hall, B.E., Wright, J.G., 1992 Study No. A817617 Reference No. M-161555-01-1
Fish bioaccumulation test No test guideline	$BCF_{steady-state}$ = 67 $BCF_{kinetic}$ = 72 CT_{50} = 0.199 days Depuration after 14 days greater than 99%	Test species: *Lepomis macrochirus* (Bluegill sunfish) Test substance: ^{14}C-Ethofumesate	Barrett, K.L., Lattimore, A.E., 1991 Study No. A83371 Reference No. M-155639-01-1

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No estimations are available.

5.3.1.2 Measured bioaccumulation data

Reference:	**Bioaccumulation test in bluegill sunfish ^{14}C-Ethofumesate**
Author(s), year:	Caley, C.Y., Cameron, B.D., Chapleo, S., Hall, B.E. and Wright, J.G., 1992
Report/Doc. number:	Study no. A817617, Reference no. M-161555-01-1
Guideline(s):	US EPA guideline 165-4
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Materials and Methods:

Test material	[^{14}C]-ethofumesate
Lot/Batch #	CFQ-5802
Specific activity	225 µCi mg^{-1}
Radiochemical purity	> 97% (re-purified)
Positiv control	Charcoal-filtered dechlorinated tap water
Test organism:	*Lepomis macrochirus*
Size	25 – 46 mm
Body weight	0.345 – 1.655 g (mean 0.87 g)
Loading	Control: 0.19 g/L/day Treatment: 0.22 g/L/day

Source	Monkfield Aquatics, Cambridge, UK
Diet/Food	Fish were fed daily with Promin Tropical Fish Food, Barton Stacey, Winchester, UK, during holding and test periods. Basic constituents of this are Protein, 50%; Ash, 10%; Fibre, 3%; Moisture, 4%. During the test, any excess food was removed after 30 min.
Acclimatisation period:	min. 14 days prior to test initiation
Environmental conditions	
Temperature	20.4 – 23.0 °C
Photoperiod	16 hours light : 8 hours dark
pH	7.70 – 8.49
Dissolved oxygen	61 – 78%
Total hardness	52 – 70 mg $CaCO_3$/L
Alkalinity	50 – 66 mg $CaCO_3$/L
Conductivity	160 – 240 µS/cm

Study design :
Experimental conditions:
The bioaccumulation of [^{14}C]-ethofumesate (purity > 97%) in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 0.124 mg/L. Following pre-equilibration of the test system for 3 days, fish with a mean fresh weight of 0.87 g were exposed to ethofumesate for 28 days followed by a 14 days depuration period. The test was conducted in two aerated 50 L glass tanks with 68 fish receiving ethofumesate stock solution and charcoal-filtered dechlorinated tap water (264.646 L day^{-1}) and 58 control fish receiving dilution water alone. The fish were fed daily during the study.

Observations:
Fish were observed daily for signs of disease, stress, irritation and other effects.

The pH, temperature range, conductivity and dissolved oxygen concentration were measured daily in each tank. Total hardness was measured at weekly intervals.
Water samples were taken daily from each tank and analysed directly for total radioactivity. Additional water samples were also taken on days 0, 3, 7, 14, 21 and 28 of the uptake phase and on days 3, 7 and 14 of the depuration phase. These were filtered (Whatman No. 1 Filter Paper Discs) prior to analysis of total radioactivity. Duplicate aliquots of water were removed from each tank during the pre-equilibration phase (24 h before adding fish), on days 0, 2, 7, 8, 14, 19, 21, 24 and 28 of the uptake phase and on days 3, 7 and 14 of the depuration phase. Concentrations of ethofumesate were then determined by reversed-phase HPLC with u.v. detection.

Five test and 5 control fish were taken on days 0, 1 (test fish only), 3, 7, 14, 21 and 28 of the uptake phase and days 1, 3, 7 and 14 of the depuration phase. After killing, fish were blotted dry, weighed and the length (from the tip of the snout to the caudal peduncle) measured. Each fish was dissected into edible tissue (muscle) and viscera and carcass fractions representative of non-edible tissue. Total radioactivity in each tissue fraction was determined.

Calculations:
Bioconcentration factors, for test fish tissues and whole fish from the uptake phase, were calculated

from total radioactivity detected in fish and water as follows:

Bioconcentration factor (BCF) = µg equiv./g fresh weight test fish / µg equiv./mL test water

Statistical analysis was used to define the extent of the period of apparent steady-state for bioaccumulation.

Results and discussion:
Validity:
The study was conducted prior to adoption of the latest version of OECD 305 (2012). However, all validity criteria according to OECD 305 were fulfilled by meeting the following criteria:

- Temperature variation was less than ± 2°C (20.4 – 23.0 °C).
- The concentration of dissolved oxygen did not fall below 60% saturation (61 – 78%).
- The variation of the test substance concentration during exposure was maintained within ± 20% of the mean of the measured values during the uptake phase, i.e. concentrations of ethofumesate measured by HPLC ranged 86 – 124% of their mean (0.121 mg L^{-1}). The slight deviation in the upper limit is not considered to have an impact on the validity of the results.
- The concentration of the test substance was below its limit of solubility in water.
- The mortality in both control and treated fish was less than 10% at the end of the test (≤ 5%).

Exposure concentration:
A mean test concentration of 0.121 mg equiv./L was determined by analysis of total radioactive residues, uptake phase concentrations ranging from 0.094 to 0.149 mg equiv./L during the uptake phase. A mean test concentration of 0.121 mg/L was determined by reversed-phase HPLC analysis, measured concentrations ranging from 0.104 to 0.150 mg/L during the uptake phase. Test material was not detectable in water from the control experiment throughout the test or in water from the test tank throughout the depuration phase. Test material was not detected in extracts of the fish diet used and extracts from control fish did not contain detectable quantities of ethofumesate from any possible previous exposure.

Residue in fish:
During the uptake phase, most of the radioactivity was accumulated in the viscera (> 71%). Approximately 2% – 12% and 5% – 19% of the total radioactivity were accumulated in muscle and carcass, respectively. An apparent steady-state for the viscera and whole fish was achieved after 24 hours and for the muscle and carcass after three days. The mean bioconcentration factors (BCF) for the apparent steady-state period for viscera, muscle and carcass were 1280x, 36x and 43x, respectively. The BCF for the whole fish was 144x. These correspond to 146.45 (viscera), 4.23 (muscle), 5.01 (carcass) and 16.6 (whole fish) µg [^{14}C]-ethofumesate equivalents/g fresh weight. All results for control fish and tissues were below the limit of reliable determination.

Elimination of radioactivity from the fish was rapid. Over 99% of the radioactivity measured at the end of the uptake phase was eliminated within three days after transfer of the fish to fresh water.

Tissue extracts from test fish sampled after 1, 3 and 28 days of exposure were examined by thin layer chromatography (TLC). Principal radioactive components were ethofumesate, 2,3-dihydro-3,3-dimethyl-2-hydroxy-5-benzofuranyl methanesulfonate (NC 8493) together with 2,3-dihydro-3,3 dimethyl-2-oxo-5-benzofuranyl methanesulfonate (NC 9607) and also a further hydroxy acid derivative (NC 20645).

The results based on total radioactivity are summarised as follows:

Table 5-57: Results based on total radioactivity

Parameter	Description	Value
Cf_{SS}	Concentration in fish at steady state [µg equiv./g]	16.6
Cw	Concentration in water at steady state [mg equiv./L]	0.121
BCF_{SS}	Steady-state bioconcentration factor [L/kg]	144
CT_{50}	Clearance time [days]	< 3
Depuration after 14 days	Depuration after 14 days [%]	> 99%

Mortality was 3% and 5% in the treatment and the control, respectively. No other effects were observed throughout the study.

Conclusion:
The bioaccumulation of [^{14}C]-ethofumesate (purity > 97%) in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 0.124 mg/L. In whole fish, apparent steady-state was achieved after 24 h exposure to the test material. The steady-state bioconcentration factor for whole fish was 144 based on total radioactivity, corresponding to 16.6 µg [^{14}C]-ethofumesate equivalents/g fresh weight. After exposure, a rapid depuration was observed (> 99% within 3 days).

Comment RMS: The fish bioconcentration study was conducted according to the US EPA test guideline 165-4. According to the US EPA test guideline and the current valid OECD test guideline 305 (2012) the study is considered valid.
Even though some information is missing in the study report (lipid content of fish) the results of the study are acceptable to be used in the risk assessment.

Reference:	**Determination of the accumulation and elimination of [^{14}C]-Ethofumesate in bluegill sunfish (*Lepomis macrochirus* L.)**
Author(s), year:	Barrett, K.L. and Lattimore, A.E., 1991
Report/Doc. number:	Study no. A83371, Reference no. M-155639-01-1
Guideline(s):	None
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Materials and Methods:

Test material	[^{14}C]-ethofumesate
Lot/Batch #	CFQ-6191
Radiochemical purity	97.5% (by TLC)
Positive control	Acetone (100 µg/L)
Test organism	*Lepomis macrochirus*
Size	43.57 mm (SD ± 2.99)
Body weight	1.5330 g (SD ± 0.3046)
Loading	max. 0.11 g fish/L
Source	Monkfield, The Aquatic Centre, 35 Cherry Hinton Road, Cambridge, UK

Diet/Food	Throughout the study period the fish were fed daily with a proprietary brand of food (Promin® coarse) at a nominal rate of 2% of the average fish weight present per day.
Acclimatisation period	14 days
Environmental conditions	
Temperature	20.9 – 23.2 °C
Photoperiod	12 hours light : 12 hours dark
pH	6.52 – 7.40 (mean 6.93 ± 0.19)
Dissolved oxygen	90 – 102%
Total hardness	Mean 81.25 mg $CaCO_3$/L (SD ± 11.70)
Alkalinity	Mean 71.28 mg $CaCO_3$/L (SD ± 5.00)
Conductivity	Mean 143.69 µS/cm (SD ± 2.74)

Study design :

Experimental conditions:

The bioaccumulation of [^{14}C]-ethofumesate (purity 97.5%) in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 0.56 mg/L. Following pre-equilibration of the test system for 88.5 hours, fish with a mean wet weight of 1.533 g and an average length of 43.57 mm were exposed to ethofumesate for 28 days followed by a 14 days depuration period. 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 (100 µg/L) only. The fish were fed daily during the study.

Observations:

Daily observations of fish were made for behavioural and/or physiological abnormalities. Any fish mortalities were recorded and the fish removed from the vessel.

Measurements of temperature, pH and dissolved oxygen were made daily in both the treated and control vessel. Total hardness, alkalinity and conductivity of the dilution water were monitored at the start of the study, and at regular intervals throughout the study period.

Aliquots of the [^{14}C]-ethofumesate and control exposure solutions were taken daily from the midpoint of each vessel and radioactivity levels were analysed by liquid scintillation counting (LSC). At time 0 and on fish sampling days during the bioconcentration phase approximately 1 L of the exposure solution was removed for characterising radioactivity by thin layer chromatography (TLC) and on day 0, 10 and 28 additionally by high performance liquid chromatography (HPLC).

Five fish were randomly selected after 6 hours, 1, 3, 7, 10, 14, 21 and 28 days of exposure and at 6 hours, 1, 3, 7 and 14 days of the depuration phase from both the treated and control vessels for analysis. The fish were rinsed in clean water, blotted dry, weighed, measured and then dissected into 3 portions; flesh including the skin (edible); viscera (alimentary tract and associated internal organs) and carcass including fins, head and gills. Each of the dissected tissue samples was then analysed for total radioactivity content. To characterise the radioactivity in fish tissues, the twenty-four fish remaining on day 28 post selection of the fish for the depuration phase were analysed.

Calculations:

Bioconcentration data was subjected to statistical analysis of variance and the mean bioconcentration factors were calculated as follows:
Bioconcentration factor (BCF) = Concentration of [^{14}C] residues in whole fish mg/kg (wet weight) / Concentration of [^{14}C] residues in water mg/L.

Rate constants were determined using the Dow BIOFAC Computer Program (Blau & Agin, 1978)[1]. The BCF at steady state, the time to reach 90% of steady state and the time to achieve 50% of clearance (depuration) were calculated from the estimated rate constants based on the whole fish analysis values.

Results and Discussion:
Validity:
The study was conducted prior to adoption of the latest version of OECD 305 (2012). However, all validity criteria according to OECD 305 (2012) were fulfilled by meeting the following criteria:

- Temperature variation was less than ± 2°C (20.9 – 23.2 °C).
- The concentration of dissolved oxygen did not fall below 60% saturation (90 – 102%).
- The variation of the test substance concentration during exposure was maintained within ± 20% of the mean of the measured values during the uptake phase (82 – 116%), except for one measurement on day 24 (78%) due a blockage in the toxicant feed tube. It is considered that this blockage did not reduce the concentration of the test material for a significant period of time, and thus did not affect the overall outcome of the study.
- The concentration of the test substance was below its limit of solubility in water.
- The mortality in both control and treated fish was less than 10% at the end of the test (≤ 4%).

Exposure concentration:
The mean measured concentration of [^{14}C]-ethofumesate equivalents in the exposure concentration was determined as 0.5648 mg/L (SD ± 0.0526). TLC analysis verified ethofumesate to be present at a mean concentration of 93.22%.

Residue in fish:
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^{-1}.

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 per 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%).

The results based on total radioactivity are summarised as follows:

[1] Blau, Gary E. and Agin, G.L. 1978. A Users Manual for BIOFAC: A computer program for characterising the rates of uptake and clearance of chemicals in aquatic organisms.

Table 5-58: Results based on total radioactivity

Parameter	Description	Value
k_1	Uptake rate constant [L/kg/day]	251 (SD ± 30.07)
k_2	Depuration rate constant [day]	3.49 (SD ± 0.4307)
Cf_{SS}	Concentration in fish at steady state [mg/kg]	37.8
Cw	Concentration in water at steady state [mg/L]	0.5648 (SD ± 0.0526)
BCF_{SS}	Steady-state bioconcentration factor [L/k^1]	67
BCF_K	Kinetic BCF [L/kg]	72 (SD ± 12.4)
CT_{50}	Clearance time [days]	0.199
Depuration after 14 days	Depuration after 14 days [%]	> 99%

Mortality was 4% and 3% in the treatment and the control, respectively. No behavioural or physiological abnormalities were observed in the [^{14}C]-ethofumesate treated fish compared to the controls and fish in both vessels showed a normal increase in weight over the 28 day exposure period.

Conclusion:
The bioaccumulation of [^{14}C]-ethofumesate (purity > 97%) in bluegill sunfish (*Lepomis macrochirus*) was investigated in a flow-through system at a nominal exposure concentration of 0.56 mg L^{-1}. In whole fish, a steady-state was achieved after 1 day of exposure to the test material. The steady-state bioconcentration factor for whole fish was 67 based on total radioactivity. Based on one compartment kinetics for whole fish a BCF of 72 was determined. After exposure, a rapid depuration was observed (99% within 3 days).

Comment RMS: The fish bioconcentration study was conducted according to none specified test guideline. According to the current valid OECD test guideline 305 (2012) the study is considered valid.
Even though some information is missing in the study report (lipid content of fish) the results of the study are acceptable to be used in the risk assessment.

5.3.2 Summary and discussion of aquatic bioaccumulation

Ethofumesate has a log P_{OW} of 2.7 and therefore a fish bioconcentration study is not triggered. However, two fish bioconcentration studies are available for the active substance Ethofumesate. Hence, the results of these two studies were included in the CLH report as additional information. Based on the fish bioaccumulation studies (Caley et.al., 1992 and Barret & Lattimore, 1991) with *L. macrochirus* BCF values (whole fish) between 67 and 144 were determined, which indicate a moderate potential to bioaccumulate in the aquatic food chain.

The active substance was extensively metabolized in fish and the residues were eliminated quickly (CT_{50} < 3 d).

The major residues were NC8493, NC 20645 and NC 9607.

The bioaccumulation potential of all major metabolites in water and sediment (NC8493, NC20645) is also assumed to be low, due to log P_{OW} values clearly lower than 3. Thus, it can be concluded that the risk of bioaccumulation of the major metabolites in the aquatic ecosystem is acceptable.

5.4 Aquatic toxicity

Table 5-59: Summary of relevant information on aquatic toxicity

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OECD 203 (1984), US EPA guideline (1985)	*Lepomis macrochirus* Bluegill sunfish	Semi-static	96 hr	nom	Mortality	15.0	21.2	Barrett, K.L., 1991b Study No. A83373 Reference No. M-155641-01-1
OECD 203 (1984), US EPA guideline (1985)	*Cyprinodon variegatus* Sheepshead minnow	Static	96 hr	nom	Mortality	12.0	25.0	Schupner, J.K., Stachura, J.B., 1992 Study No. A833384 Reference No. M-155652-01-1
US EPA guideline (Guideline E, Subdivision 72-1)	*Oncorhynchus mykiss* Rainbow trout	Semi-static	96 hr	mm	Mortality	4.125	11.91	Caley, C.Y., Cameron, B.D., Chapleo, S., Knoght, B., 1989 Study No. A87614 Reference No. M-161551-01-1
US EPA guideline (Guideline E, Subdivision 72-1)	*Cyprinus carpio* Mirror carp	Semi-static	96 hr	mm	Mortality	6.51	10.92	Cameron, B.D. et al., 1989 Study No. A83349 Reference No. M-155618-01-1
OECD 203, EEC Directive 79/831, Annex V	*Oncorhynchus mykiss* Rainbow trout	Semi-static	96 hr	nom	Mortality	9.7	26.5	Thun, S., 1991 * Study No. A87614 Reference No. M-352116-01-1
OECD 203, EEC Directive 79/831, Annex V	*Leuciscus idus* Golden orfe	Static	96 hr	nom	Mortality	9.3	22.0	Thun, S., 1993 * Study No. 80-91-2312-01-93 Reference No. M-352126-01-1
OECD 210 (1992), OECD 215 (2000), OECD draft guideline « Fish 2-generation test « (2002)	*Danio rerio* Zebrafish	Flow-through	FFLC	nom	Growth	0.156	-	Teigeler, M., 2013 Study No. EBADL027 Reference No. M-464613-01-1
US EPA guideline 72-4	*Pimephales promelas* Fathead minnow	Flow-through	FELS	mm	Growth	4.17	-	Fagella, G.A., 1991 & Meller, M.. Bruns, E., 2013 Study No. A83372 Reference No. M-155640-01-1 and M-470756-01-1

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OECD 202 (1984), US EPA 540/9-85-005 (1985)	*Daphnia manga* Water flea	Static	48 hr	nom	Immobilisation	8.55	13.52	Barber, I., 1991 Study No. A83370 Reference No. M-155638-01-1
OECD 202 (1984), EEC Directive 79/831, Annex V	*Daphnia manga* Water flea	Static	48 hr	nom	Immobilisation	13.0	28.1	Thun, S., 1993 * Study No. 80-91-2312-02-93 Reference No. M-352128-01-1
FIFRA Guideline 72-3	*Mysidopsis bahia* Mysid shrimp **	Static	96 hr	mm	Mortality	< 2.5	5.4	Schupner, J.K., Stachura, B.J., 1992 Study No. A83389 Reference No. M-155657-01-1
OECD 202 (Part 2, 1984)	*Daphnia manga* Water flea	Semi-static	21 d	nom	Reproduction	0.32	0.77	Douglas, M.T., James, C.M., Macdonald, I.A., 1990 Study No. A87619 Reference No. M-161558-01-1
OECD 202 (Part 2, 1984)	*Daphnia manga* Water flea	Semi-static	21 d	mm	Reproduction	1.06	2.7	Bellmann, W., 1992 Study No. 40730.315-202-II Reference No. M-352134-01-1
OECD 202 (Part 2, 1984)	*Daphnia manga* Water flea	Semi-static	21 d	mm	Reproduction	0.25	1.2	Adema, D.M.M., de Rulter, A., 1989 Study No. A83345 Reference No. M-155614-01-1
BBA guideline	*Chironomus riparius* Sediment-dwelling midge	Static	28 d	im	Emergence	5.33	-	Mattock, S.D., 1998 Study No. A91783 Reference No. M-168438-01-1
OECD 219 (draft, 2001)	*Chironomus riparius* Sediment-dwelling midge	Static	28 d	im	Emergence	3.82	-	Desmares-Koopmans, M.J.E., 2002 Study No. 324089 Reference No. IDD00073
OECD 219 (draft, 2000), BBA guideline (1995)	*Chironomus riparius* Sediment-dwelling midge	Static	28 d	im	Emergence	14.05	-	Stabler, D., 2003 Study No. 20021050/01-ASCr Reference No. IDD00074
US EPA – FIFRA CFR 40 – Series 72-3	*Crassostrea virginica* Eastern Oyster **	Flow-through	96 hr	mm	Shell growth	< 0.81	1.7	Yurk, J.J., Ache, B.W., 1992 Study No. A83386 Reference No. M-155654-01-1

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OECD 201 (2006)	*Pseudolirchneriela subcapitata* Green alga	Static	72 hr	mm	Growth rate Yield	5.91	16.347 9.683	Bruns, E., Dorgerloh, M., 2008 Study No. E 323 3418-4 Reference No. M-302092-03-1
OECD 201 (2006), FIFRA guideline 123-2 (1982), OPPTS guideline 850-5400 (1996 draft)	*Anabaena flos-aquae* Blue-green alga	Static	96 hr	nom	Growth rate Yield	20.0	> 20.0	Banman, C.S., Daly, R.A., Lam, C.V., 2009a Study No. EBADL008 Reference No. M-349150-01-1
OECD 201 (2006), FIFRA guideline 123-2 (1982), OPPTS guideline 850-5400 (1996 draft)	*Skeletonema costatum* Saltwater diatom **	Static	96 hr	nom	Growth rate Biomass	5.0 (72 hr) 2.5 (72 hr)	> 20.0 14.5 (72 hr)	Banman, C.S., Daly, R.A., Lam, C.V., 2009b Study No. EBADL009 Reference No. M-347965-01-1
ASTM guideline E 1415-91 (1991)	*Lemna minor* Duckweed	Semi-static	14 d	mm	Growth rate Biomass	4.3	> 52.8 50.4	Scheerbaum, D., 1998 Study No. A91865 Reference No. M-168516-01-1
ISO guideline (2000), OECD 221 guideline (draft, 1999)	*Lemna minor* Duckweed	Semi-static	7 d	mm	Growth rate Biomass	26.0 17.0	> 42 35.0	Bogers, M., 2001 Study No. 324078 Reference No. IDD00077
OECD 221 (2006)	*Myriophyllum spicatum* Water milfoil	Static	14 d	mm	Growth rate Yield	0.036	0.479 0.25	Banman, C.S., 2013 Study No. EBADL019-1 Reference No. M-411454-02-1

nom…nominal, mm…mean measured, im…initially measured
* Parts of the study are considered not reliable, should be used as additional information only.
** Marine species

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference:	**The acute toxicity of [^{14}C]-ethofumesate to bluegill sunfish (*Lepomis macrochirus*) under semi-static conditions**
Author(s), year:	Barrett, K. L., 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 (*Lepomis macrochirus*)
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
Applied concentrations:	
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
Test conditions:	
Water quality:	Dilution water, hardness: 73.5 – 79.33 mg /L as $CaCO_3$, alkalinity: 69.17 – 79.0 mg/L as $CaCO_3$
Conductivity:	142.9 – 165.2 µs/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:	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).

	At the initiation of the study ten fish were allocated at random to each test vessel. The test solutions were renewed after 48 hours to ensure oxygen concentrations were not significantly depleted, and to ensure maintenance of test solution concentrations.
Test parameters:	All test vessels were monitored for mortality and sub-lethal effects after 3, 6, 24, 48, 72 and 96 hours. 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.
Analytical measurements:	At the start and the end of the test samples of the stock and test solutions were taken for quantitative and qualitative analysis. 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).
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.

Findings:

Analytical data: The mean over the 96 hour study period ranged from 93.7 to 115.4% of nominal. Therefore for the purpose of the LC_{50} calculations nominal values were used.

Table 5-60: 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 sheepshead minnow (*Cyprinodon variegatus*) in a static system**
Author(s), year:	Schupner, J.K. and Stachura, J.B., 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:	Sheepshead minnow (*Cyprinodon variegatus*)
Holding of fish :	Test medium: Synthetic sea water (salinity of 17 $^{o}/_{oo}$) 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:	$17^{o}/_{oo}$ (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.
<u>Findings:</u>	
Analytical data:	The mean over the 96 hour study period ranged between the 80 and 120% of the nominal test concentration.

Table 5-61: 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 nominal concentrations.

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 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 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.

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:	**Ethofumesate: Determination of acute toxicity (LC_{50}) to rainbow trout (96 h semi-static)**
Author(s), year:	Caley, C.Y., Cameron, B.D., Chapleo, S., and Knight, B., 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 (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*)
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
Applied concentrations:	
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.
<u>Findings:</u>	
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 5-62: 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 LC_{50} 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:	**Technical ethofumesate: Determination of acute toxicity (LC_{50}) to mirror carp (96 h semi-static) and the analysis of ethofumesate in water samples**
Author(s), year:	Cameron, B.D. et al., 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 (*Cyprinus carpio*)
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 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 24 h intervals.
Test parameters:	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.
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 12.0 and 73.2% of the nominal test concentration.
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.

Biological effects: 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.

Table 5-63: 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 LC_{50} 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 (*Salmo gairdneri*)**
Author(s), year:	Thun, S., 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 (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*)
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:	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 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.
Biological effects	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. 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.

Table 5-64: 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:	Thun, S., 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 (*Leuciscus idus*)
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 ± 2°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
Applied concentrations:	
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
Test conditions:	
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 5-65: 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

Nominal test concentration [mg ai/L]	**Mortality [%]**				
	2-4 h	**24 h**	**48 h**	**72 h**	**96 h**
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 LC_{50} 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.

5.4.1.2 Long-term toxicity to fish

Reference:	**Zebrafish (*Danio rerio*), Life Cylce test - Flow through conditions**
Author(s), year:	Teigeler, M., 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:	- 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. The changes of the study protocol had no impact on the study integrity.
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 (*Danio rerio*)
Holding of fish :	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 Feeding of adult fish: Daily ad libitum with TetraMin® Hauptfutter and brine shrimp nauplii (*Artemia salina*) Feeding of fish larvae: Breeding food 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 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)
Applied concentrations:	
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
Test conditions:	
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 (*Artemia salina*) 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 F_1-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 5-66: 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 $NOEC_{post\text{-}hatch\ survival}$ = 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 5-67: 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 5-68: 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 F_1 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 5-69: 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 (F_1-generation):

Early life stage: The F_1 generation was prepared by sampling eggs from the parental fish and keeping them for hatching.
Hatch of the F_1 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 5-70: 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

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 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 ± 7.2*	72.5 ± 8.3*	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

Table 5-71: Summary of all endpoints including the MDD values

Life phase		Endpoint	Parameter	Nominal concentration [mg/L]		MDD [%]	Remark
				NOEC	LOEC		
P	Early life stage	Population	Time to hatch	2.5	5.0 [a]	- 13.792	Reduction
			Hatching	5.0	> 5.0	- 8.120	Reduction
			Post hatch survival, day 28	1.25	2.5	- 11.252	Reduction
		Growth	Length	0.156	0.313	- 6.417	Reduction
	Juvenile growth	Population	Survival, day 56	5.0	> 5.0	- 11.251	-
		Growth	Length, day 56	0.625	1.25	- 3.804	Reduction
			Pseudo specific growth rate, day 28-56	5.0	> 5.0	n.a.	- [b]
	Adult	Reproduction	Time to first spawning	2.5	> 2.5	7.554	-
			Egg number per day and female	2.5	> 2.5	- 24.635	-
			Fertilisation rate	2.5	> 2.5	- 4.499	-
		Termination	Sex ratio	2.5	> 2.5	18.886	-
			Survival	2.5	> 2.5	- 13.944	-
		Growth	Length, males	2.5	> 2.5	n.a.	- [c]
			Length, females	2.5	> 2.5	n.a.	- [c]
			Weight, males	0.156	0.313	- 17.296	Reduction
			Weight, females	0.156	0.313	- 17.901	Reduction
F_1	Early life stage	Population	Time to hatch	2.5	> 2.5	- 15.805	-
			Hatching	1.25	2.5	- 15.688	Reduction
			Post hatch survival, day 28	2.5	> 2.5	21.357	-
		Growth	Length	2.5	> 2.5	4.904	-
			Weight, group dry	2.5	> 2.5	- 36.924	-

Life phase	Endpoint	Parameter	Nominal concentration [mg/L]		MDD [%]	Remark
			NOEC	LOEC		
		weight				
		Weight, single dry weight	2.5	> 2.5	29.211	-

n.a…not applicable, MDD…Minimum detectable difference of NOEC concentration level and control (in percent of control)

[a] A slight delay in hatch could be observed at day 6 post fertilisation. However, hatch was > 90 % in all treatments at day 8 and thus, this effect should have no negative impact on the fish population.

[b] A statistically significant increase could be observed at ≥ 0.625 mg a.s./L (MDD: 10.136%). Since an increased growth rate cannot be considered as a negative impact on the exposed fish, the NOEC was determined to be ≥ 5.0 mg a.s/L.

[c] A statistically significant difference in comparison to the control was observed at ≥ 0.313 mg a.s./L for male (MDD: -3.960 to -4.252%) and ≥ 0.156 mg a.s./L for female fish (MDD: -3.335 to -3.619%). However, the calculated differences of all treatment levels compared to the control were found to be < 10 % in all test concentration. The difference of the treatment level ≤ 1.25 mg/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.

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 F_1 (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 F_1-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 ± 1.5°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 ± 2°C according OECD 210, 1992 and 26 ± 1.5°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 ± 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 ± 1°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 (21-25 °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 F_1 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 (*Pimephales promelas*) early life stage toxicity test**
Author(s), year:	Fagella, G.A., 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 (*Pimephales promelas*) by Fagella 1991**
Author(s), year:	Meller, M. and Bruns, E., 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 (*Pimephales promelas*)
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 (*Artemia salina*) 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
Applied concentrations:	
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
Test conditions:	
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 µS
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. <u>NOEC Determination:</u> 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 5-72: 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-sided

Table 5-73: Summary of all endpoints including the MDD values

Endpoint	NOEC (% MDD)	LOEC (% MDD)	EC_{10} (CI 95%)
	[mg a.s./L]		
Hatching success / Embryo survival (day 4)	23.2 (- 17.3)	> 23.2 (- 17.3)	> 23.2 (n.d.)
Larval / Fry survival (day 11 and 18)	13.3 (- 16.2)	23.2 (- 16.3)	12.2 (7.44 – 15.31)
Larval / Fry survival (day 25 and 28)	13.3 (- 14.1 - -21.4)	23.2 (-14.1 - -21.5)	12.2 (7.34 – 15.28)
Larval / Fry standard length (day 28)	4.17 (- 4.989)	7.04 (- 5.092)	7.31 (6.35 – 8.08)
Larval / Fry standard wet weight (day 28)	4.17 (- 14.008)	7.04 (- 14.296)	4.93 (62.96 – 6.27)

nd…not determined due to mathematical reasons or inappropriate data, MDD…minimum detectable difference to control (in percent of control), CI 95%...95% confidence interval

Conclusion: 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-EC_{10} 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 mire than ± 1.5°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.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference:	**Determination of the acute toxicity of [^{14}C]-ethofumesate to *Daphnia magna***
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:	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 (*Daphnia magna*)
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:	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 continuously.
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).

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 5-74: 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 EC_{50} 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). However, one neonate in the solvent control was accidentally killed by the operator. Under regular test conditions it can be assumed that this individual would have survived. Thus, immobility in the solvent control does not exceed 10%, fulfilling the validity criteria with respect to control immobility.
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 [^{14}C]-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 be used for the risk assessment.

Reference:	**Acute toxicity in *Daphnia magna* – 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:	Not reliable

Material and methods:	
Test substance:	Ethofumesate, technical grade, batch no.: 20/03/93
Test species:	Water flea (*Daphnia magna*)
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:	$K_2Cr_2O_7$ (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:	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.
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 5-75: 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 EC_{50} 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 study is not considered reliable.

Reference:	**The acute toxicity of ethofumesate technical to the mysid shrimp, *Mysidopsis bahia* 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 (*Americamysis bahia*, formerly known as *Mysidopsis bahia*)
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 $^{o}/_{oo}$,
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 *artemia nauplii* 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 5-76: 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 LC_{50} 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.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:	**An assessment of the effects of ethofumesate on the reproduction of *Daphnia magna***
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 (*Daphnia magna*)
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:	The live and dead Daphnia of the "parental" (P_1) generation were counted daily and recorded together with observations on the general condition and size of the Daphnia as compared with the controls. At each test media renewal the numbers of live and dead "filial" (F_1) Daphnia were recorded. The number of Daphnia with eggs or young in the brood pouch plus the number of discarded unhatched eggs was also determined at this time. Each vessel received approximately 5 ml of a mixed unicellular algal culture supplemented with fry fish food (Liguifry®), daily. Temperature was recorded daily for each flask. Dissolved oxygen, pH and temperature were measured before and after- each test media renewal.
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:	EC_{50} values for immobilisation (mortality) of the parental Daphnia were calculated according to the method of Thompson and Weil. EC_{50} 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 $P_{1:}$	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 EC_{50} values at Day 14 and 21.
Sub-lethal effects on P_1:	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 5-77: 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 F_1:	The number of dead young daphnids was insignificant in all treatment and control groups (< 1 dead young/female).
Statistical power:	Under Regulation (EU) No. 1107/2009 an assessment of the statistical power of the NOEC derived from studies that have been designed to generate a NOEC shall be carried out. In the *Daphnia magna* study no differences to the control were observed at the lowest concentration level of 0.32 mg/L (difference < 0.2%) and the NOEC was statistically derived to be 0.32 mg/L. The statistical power at the NOEC level was retrospectively analysed to be ≥ 0.042 (please refer to Zhenglei Gao, M-466590-01-1). However, the statistical power at all other concentration levels (including the LOEC level of 1 mg/L) was analysed to be 1.0, indicating that the used test design and the applied statistical tests were adequate to statistically detect the adverse effects observed in the study.

Conclusion:	Prolonged exposure of *Daphnia magna* to Ethofumesate resulted in progressive mortality of parental P_1 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:	Acceptable

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:	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. The pH, temperature, and O_2 concentrations were measured at the beginning and at the end of each renewal period.
Analytical measurements:	The analytical control measurements were performed by means of GC analysis.
Statistics:	For the determination of the EC_{50} values the method by Spearman-Kärber was used. 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.
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:	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. 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.

Table 5-78: 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 EC_{50} value for the reproduction was 2.7 mg ai/L and the EC_{50} 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 notifier TFE submitted additional information considering the re-calculation of the chronic endpoint taking into account measured concentrations.
Based on the analytical measurements a NOEC of 1.06 mg ai/L based on geometric mean measured concentrations was determined. The endpoint is considered valid and should be used in the risk assessment.

Reference:	**The chronic toxicity of ethofumesate to *Daphnia magna***
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:	Acceptable

Material and methods:

Test substance:	Ethofumesate techn., batch no.: not given, purity: not specified
Test species:	Waterflea (*Daphnia magna*)
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 (*Chlorella pyrenoidosa*) 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 Daphnia 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 LC_{50} values and their confidence interval were calculated by means of a parametric model developed by Kooijman. The EC_{50} 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 5-79: 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 EC_{50} value for the reproduction was 1.2 mg ai/L and the EC_{50} value for the immobilisation was 13.5 mg ai/L. The NOEC based on reproduction was determined to be 0.32 mg ai/L. The results are based on nominal concentrations.

Comment RMS: The study was conducted according to the OECD 202 (Part II, 1984). The validity criteria given in the test guidelines are met.
The mortality in the control groups was below 20% (being: 12.5%) at the end of the test. The dissolved oxygen was greater than 60% of the air saturation throughout the test duration. The average cumulative number of young per female in the controls after three broods should be greater than 20 at a temperature of 20 ± 1 °C. The number of offspring per female was greater than 20 on day 12 and 14 of the test.
The first young should have been born in the controls after a maximum of nine days (being: 7 days).
Based on the validity criteria the study is considered valid.

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.

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

5.4.3 Algae and aquatic plants

Reference:	***Pseudokirchneriella subcapitata* 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, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
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), 0.179, 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)
Test conditions:	
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:	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. The pH and the temperature was measured at each observation time in all test levels and the controls. 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.
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.

Findings:

Analytical data: 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.

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

Table 5-80: 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 on 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:	**Toxicity of ethofumesate technical to the blue green algae *Anabaena flos-aquae***
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 x 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.
Findings:	
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 5-81: 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

Conclusion: 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

Comment RMS: 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 *Skeletonema costatum***
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 x 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 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.
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 5-82: 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 5-83: 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.

Reference:	***Lemna minor*: 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:	*Lemna minor*, 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:	EC_{50}-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 5-84: 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 *Lemna minor* 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 peeked 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
O_2 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 inly 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 5-85: 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 frons was discoloured at the end of the test

[b] Compared to the mean value of the treatment control (solvent control).

Conclusion:	7 d E_rC_{50} > 42 mg ai/L 7 d E_rC_{10} = 20 mg ai/L (95% C.I. = 8.6 – 48 mg ai/L) NOE_rC = 26 mg ai/L

7 d E_bC_{50} = 35 mg ai/L (95% C.I. = 14 – 86 mg ai/L)
7d E_bC_{10} = 8.8 mg ai/L (95% C.I. = 3.1 – 25 mg ai/L)
NOE_bC = 17 mg ai/L

Based on mean measured concentrations

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, *Myriophyllum spicatum* (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:	*Myriophyllum spicatum* (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
Applied concentrations:	
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
<u>Findings:</u>	
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 5-86: 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_yC_{50} = 0.25 mg ai/L (95% CI: 0.128-0.348 mg ai/L), NOEC = 0.036 mg ai/L Wet weight: E_yC_{50} > 4.13 mg ai/L, NOEC = 4.13 mg ai/L Dry weight: E_yC_{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 5-87: 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
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 (*Lemna* 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.

5.4.4 Other aquatic organisms (including sediment)

Reference:	**Ethofumesate: Chronic toxicity to the sediment dwelling organisms *Chironomus riparius* (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:	- 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. Neither of the deviations are considered likely to have had any impact on the outcome of this study.
Validity:	Acceptable

Material and methods:	
Test substance:	Unlabelled test material: Ethofumesate techn., batch no.: CR 19291/02/940701, purity: 97.7% Radio-labelled test material: [Benzene ring-U-^{14}C] ethofumesate, batch no.: 901B-1, purity: > 98%
Test species:	Midge (*Chironomus riparius*)
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
Applied concentrations:	
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
Test conditions:	
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).

<u>Findings:</u>

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.

Statistical power: Under Regulation (EU) No. 1107/2009 an assessment of the statistical power of the NOEC derived from studies that have been designed to generate a NOEC shall be carried out. This requirement is not appropriate for the limit study with *Chironomus riparius*, since no adverse effects were observed in the treatment (emergence rate 92%) in comparison to the controls (emergence rate 86%).

Table 5-88: 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 ± 1.°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, the NOEC should be based on initially measured concentrations according to the OECD test guideline. Hence, the NOEC was determined to be 5.33 mg ai/L.

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:	- 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. - One test vessel of the solvent control was broken on day 27. Thereafter no more observations were made. - 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. The deviations were considered to have no effect on the outcome of the study.
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 (*Chironomus riparius*)
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. He 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 5-89: 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 5-90: 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 teat 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 ± 1°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 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, the NOEC should be based on initially measured concentrations according to the OECD test guideline. Hence, the NOEC was determined to be 3.82 mg ai/L.

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, *Chironomus riparius* with laboratory test method**
Author(s), year:	Stäbler, D., 2003
Report/Doc. number:	Study no. 20021050/01-ASCr, Reference no. 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 (*Chironomus riparius*)
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 n 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 5-91: 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 was determined. The EC_{50} (emergence, development) was determined to be greater than 100 mg/L.

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 ± 1°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 NOEC should be based on initially measured concentrations according to the OECD test guideline. Hence, the NOEC was determined to be 14.05 mg ai/L.

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

Reference:	**Effects of ethofumesate technical on new shell growth in the Eastern oyster (*Crassostrea virginica*) 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 (*Crassostrea virginica*), 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 $^{o}/_{oo}$
Light regime:	16 hours light / 8 hours darkness
Test system:	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. 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.
Test parameter:	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 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 EC_{50} 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 5-92: 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 EC_{50} = 1.7 mg ai/L (95% C.I. = 0.81 – 5.6 mg ai/L) based on new shell growth
96 h LC_{50} > 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.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Endpoint	Classification Criteria (criteria in bold) CLP (2nd ATP)	Evidence for Ethofumesate
Degradation Ethofumesate	Ethofumesate is not readily biodegradable, and does not meet the criterion for rapid degradation in a water/sediment study with a DT50 whole system of 170 days (geometric mean). Based on available data a non-rapid degradation is proposed for Ethofumesate.	Hydrolytic degradation of Ethofumesate pH 5, 7 and 9: stable at 20°C Ethofumesate showed no to slow (DT_{50} 331d) aerobic mineralization in surface water (OECD 309) The classification as **chronic aquatic hazardous** according to Regulation EC 1272/2008is based on the fact that the active substance is **not considered as ready biodegradable/rapid degradable.**
Bioaccumulation Ethofumesate	**Log K_{ow} is < 4** BCF = 144 (steady-state) Ethofumesate Log K_{ow} = 2.7 at pH 6.44 and 25 °C	The measured log P_{OW} is 2.7 (at pH 6.44 and 25 °C) and is below the classification criteria of 4 (CLP). In addition, the BCF of 144 is below the classification criteria of 500. Therefore Ethofumesate is considered to have **a low bioaccumulation potential.**
Acute aquatic toxicity Ethofumesate	**E_rC_{50} < 1 mg/L (aquatic macrophytes)** E_rC_{50} > 1 mg/L (algae) LC_5EC_{50} > 1 mg/L (fish and aquatic invertebrates)	Ethofumesate is of low toxicity to algae (E_rC_{50} = 16.3 mg/L), but of moderate toxicity to aquatic macrophytes (E_rC_{50} = 0.479 mg/L, *Myriophyllum spicatum*). In addition, the active substance is of low toxicity to fish (LC_{50} = 10.92 mg/L) and aquatic invertebrates (EC_{50} = 5.4 mg/L). The criteria for the proposed classification as **H400** according to Regulation EC 1272/2008 are met. The M-factor is 1.
Chronic aquatic toxicity Ethofumesate	**For not rapidly degradable substances: NOEC ≤ 0.1 mg/L** **NOEC < 0.1 mg/L (aquatic macrophytes)** NOEC > 1 mg/L (fish, daphnids and algae)	Ethofumesate is of moderate chronic toxicity to fish and aquatic invertebrates with a NOEC of 0.156 mg/L and 0.25 mg/L, respectively. In addition, the active substance is of low chronic toxicity to algae with a NOEC of 5.91 mg/L. However, Ethofumesate is of high chronic toxicity to aquatic macrophyte (*Myriophyllum spicatum*) with a NOEC of 0.036 mg/L. Therefore Ethofumesate fulfills the criteria for the proposed classification as **H410** according to Regulation EC 1272/2008. The M-factor is 1.

Endpoint	Classification Criteria (criteria in bold) CLP (2nd ATP)	Evidence for Ethofumesate
SUMMARY	**H400 (M = 1) / H410 (M = 1)**	**PROPOSED CLASSIFICATION**

Conclusion of environmental classification according to Regulation EC 1272/2008

Pictogram: GHS 09

Signal word: Warning

Aquatic Acute 1, M = 1

Aquatic Chronic 1, M = 1

H400 'Very toxic to aquatic life'

H410 'Very toxic to aquatic life with long lasting effects'

Justification for the proposal

H400 follows from the toxicity of the active substance Ethofumesate to aquatic macrophytes (*Myriophyllum spicatum*, E_rC_{50} = 0.479 mg/L, Banman, C.S., 2013).

H410 follows from the toxicity of the active substance Ethofumesate to aquatic macropyhtes (*Myriophyllum spicatum*, NOEC = 0.036 mg/L based on growth rate, Banman, C.S., 2013).

In addition, the active substance is not readily biodegradable (Bogers, M., 1993 and Douglas, M.T. & Sewell, I.G., 1989) and not rapidly degradable (Heintze, A., 2003). In the water-sediment study a DT_{50} of 170 days (geomean) was determined for the whole system. Also Ethofumesate does not meet the criterion of rapid degradation > 70 % within a 28-day period the aquatic environment.

Based on the fish bioaccumulation study (Caley et al., 1992) with *L. macrochirus* a BCF (whole fish) of 144 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain. The substance Ethofumesate does not meet the CLP criteria (BCF ≥ 500) based on the measured fish BCF.

Ethofumesate fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Ethofumesate was hydrolytically stable at environmental temperature at pH 5 and 7 but at pH 9, degradation to BCS-CW 35117 and subsequently to NC 8493, NC 9607 and NC 20645 occurred. Ethofumesate was photolysed in aqueous solution, with experimental DT_{50} values of 15.6 days.

Ethofumesate is not readily biodegradable and cannot be classified as rapidly degraded in water sediment systems since less than 70 % is degraded within 28 days ($DT_{50whole\ system}$ of 170 days, geometric mean).

Ethofumesate has a moderate potential of bioaccumulation in aquatic system because of a measured fish BCF of 144 (Caley et.al., 1992).

Ethofumesate is acute and chronic toxic to aquatic macrophytes (*Myriophyllum spicatum*) with an E_rC_{50} < 1 mg/L and a NOEC value of 0.036 mg/L (Banman, C.S., 2013).

Hazard pictogram		Environment

Hazard class and category:	Hazardous to the aquatic environment Acute Hazard Category 1(M = 1) Chronic Hazard Category 1 (M = 1)	
Signal word	Warning	
Hazard statement:	H400	Very toxic to aquatic life
	H410	Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Ethofumesate, an active substance for plant production products (herbicide), is currently classified under Annex VI of the CLP Regulation (Regulation (EC) 1272/2008) with hazard class Aquatic Chronic 2. In view of new information and after a re-evaluation of the existing studies, the DS proposes to classify ethofumesate as Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=1).

Degradation

A hydrolysis study conducted according to OECD TG 111 and in compliance with GLP at pH 4, 7 and 9 at 50 °C for 5 days, showed that ethofumesate is hydrolytically stable.

Two studies of the photodegradation of ethofumesate in water were submitted. The first study with radio-labelled ethofumesate in water was conducted according to the guidelines OECD TG 316, US EPA OCSPP Test Guideline No 835.2240, and Japanese MAFF New Test Guidelines Annex No 2-6-2, simultaneously. This study, in compliance with GLP, was carried out at 25 °C in a sterile phosphate buffer solution (pH 7) with continuous artificial light. The study resulted in an experimental DT_{50} of 15.6 d (environmental DT_{50} (Phoenix, Arizona, USA) = 53.2 d). A multitude of transformation products was formed; none of them exceeding 10 % AR. A similar degradation pattern is observed in the second study investigating the photolysis of ethofumesate in water. A large number of minor metabolites were formed, one of them occurred at 9.57 % after 12 hours. The direct phototransformation in water was also investigated in another study whose results showed that environmental half-life relevant to Central Europe was above 1 year and that therefore photochemical degradation of ethofumesate might play a minor role under such conditions.

Two ready biodegradability studies, both carried out according to OECD TG 301D and in compliance with GLP, indicated that ethofumesate is not readily biodegradable. In the first one, ethofumesate was added to the inoculum from the secondary effluent of a municipal sewage treatment plant at a concentration of 1 and 3 mg/L over a period of 28 days at 20 ± 1 °C. The degree of biodegradation expressed as ThOD was -14 % and -4 % within 28 days, in contrast to the reference substance, sodium acetate, which degraded to 65 %. In the second study, ethofumesate was added to the inoculum (activated sludge from a

sewage plant treating predominantly domestic sewage) at a concentration of 3 mg/L over a period of 28 days at 20 ± 1 °C. The degree of biodegradation was 10 % after 28 days, in contrast to the reference substances, sodium benzoate and aniline, which degraded to 88 % and 68 %, respectively.

Two aerobic mineralisation in surface water studies were performed according to OECD TG 309 and in compliance with GLP. In the first study, ethofumesate was found to be stable in natural surface water until day 62 of incubation and the mineralisation was marginal with a maximum of 1.1 % (high-dose test) and 0.8 % (low-dose test) at the end of the incubation period. The second study showed ethofumesate was degraded slowly. After a lag-phase of 60 days a degradation of ethofumesate was observed: the remaining amounts of ethofumesate after 88 days were 58.3 % AR and 79.3 % AR in the low- (10 µg/L) and high-dose (100 µg/L) experiment, respectively. The main metabolite formed was NC20645 (ethofumesate carboxylic acid) with a maximum amount of 18.3 % AR. The metabolite identified as BCS CW35117 (ethofumesate acetic acid) was formed at 13.4 % AR and 2.4 % AR in the low-dose and high-dose experiment, respectively. The formation of carbon dioxide due to mineralisation was low (0.9 % and 0.8 % for the low and high concentration, respectively).

The aerobic transformation of radiolabelled ethofumesate was investigated in two water/sediment studies, according to BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) Guideline Part IV, 5-1, 1990. In the first study, after 103 days of incubation, 32 and 27 % AR (13 and 18 % parent compound) was recovered in the river and pond water phase, respectively, while 57 and 64 % (37 and 41 % parent compound) was associated to the sediments. In the second one, after 234 days of incubation in Waldwinkel and 225 days in Ruckhaltebecken, 5.1 and 26 % (1.5 and 21 % parent compound) of applied radioactivity was recovered in the water phase, while 81 and 58 % (53 and 30 % parent compound) was associated to the sediments. The dissipation half-lives of ethofumesate from the water phases in Waldwinkel and Ruckhaltebecken were 7 and 50 days, respectively. For the whole systems, the half-lives were extrapolated to 285 and 242 days. In other two more recent water/sediment studies, mineralisation of ethofumesate ranged between 1.2 % AR and 15.3 % AR after 103 and 125 days, respectively. Non-extractable residues in the sediment compartment ranged between 14.2 % AR and 43.2 % AR at study end. Whole system half-lives ranged between 89 and 294 days (geomean 170 d; n = 8).

Based on the information above, the DS concludes that ethofumesate is not considered to be rapidly degradable.

Bioaccumulation

Based on experimental data, ethofumesate has a measured log K_{ow} of 2.7 (method OECD TG 107, 25 °C and pH 6.44).

In a bioaccumulation study, carried out according to U.S. EPA guideline 165-4 and in compliance with GLP, bluegill sunfish (*Lepomis macrochirus*) were continuously exposed to radio-labelled ethofumesate at a nominal concentration of 0.124 mg/L for 28 days in a flow-through system and thereafter the depuration of radioactivity followed in untreated water for 14 days. In whole fish, apparent steady-state was achieved after 24 h of exposure to the test material. The steady-state bioconcentration factor for whole fish was 144 L/kg based on total radioactivity. After exposure, a rapid depuration was observed (> 99 % within 3 days).

The actual bioaccumulation of [^{14}C]-ethofumesate in bluegill sunfish (*Lepomis macrochirus*) was also investigated in a flow-through system at a nominal exposure concentration of 0.56 mg/L (no test guideline). In whole fish, a steady-state was achieved after 1 day of exposure to the test material. The steady-state bioconcentration factor for whole fish was 67 L/kg based on total radioactivity. Based on one compartment kinetics for whole fish, a BCF of 72 was determined. After exposure, a rapid depuration similar to the first study was observed (99 % within 3 days).

Based on this information, the DS concludes that ethofumesate does not bioaccumulate.

Aquatic toxicity

Several acute and chronic aquatic toxicity data are available for all three trophic levels. New, valid ecotoxicological data are available.

The ecotoxicological test results are summarised in the following tables (the key data are highlighted in bold).

Method	Test organism	Test system	Results			Test [c]	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
OECD TG 203 (1984), US EPA guideline (1985) GLP	*Lepomis macrochirus*	Semi-static 96 h	Mortality	21.2		nom	Anonymous, 1991b
OECD TG 203 (1984), US EPA guideline (1985) GLP	*Cyprinodon variegatus*	Static 96 h	Mortality	25.0		nom	Anonymous, J.B., 1992
US EPA guideline (Guideline E, Subdivision 72-1) GLP	*Oncorhynchus mykiss*	Semi-static 96 h	Mortality	11.91		mm	Anonymous, 1989
US EPA guideline (Guideline E, Subdivision 72-1) GLP	*Cyprinus carpio*	Semi-static 96 h	Mortality	10.92		mm	Anonymous, 1989
OECD TG 203, EEC Directive 79/831, Annex V GLP	*Oncorhynchus mykiss*	Semi-static 96 h	Mortality	26.5		nom	Anonymous, 1991*

OECD TG 203, EEC Directive 79/831, Annex V GLP	*Leuciscus idus*	Static 96 h	Mortality	22.0		nom	Anonymous, 1993*
OECD TG 210 (1992), OECD TG 215 (2000), OECD draft guideline "Fish 2-generation test" (2002) GLP	*Danio rerio*	Flow-through 28 d	Growth		0.156	nom	Anonymous, 2013
US EPA guideline 72-4 GLP	*Pimephales promelas*	Flow-through 28 d	Growth		4.17	mm	Anonymous, 2013
OECD TG 202 (1984), US EPA 540/9-85-005 (1985) GLP	*Daphnia magna*	Static 48 h	Immobilisation	13.52		nom	Barber, I., 1991
OECD TG 202 (1984), EEC Directive 79/831, Annex V GLP	*Daphnia magna*	Static 48 h	Immobilisation	28.1		nom	Thun, S., 1993*
FIFRA Guideline 72-3 GLP	*Mysidopsis bahia*	Static 96 h	Mortality	5.4		mm	Schupner, J.K., Stachura, B.J., 1992
OECD TG 202 (Part 2, 1984) GLP	*Daphnia magna*	Semi-static 21 d	Reproduction		0.32	nom	Douglas, M.T., James, C.M., Macdonald, I.A., 1990
OECD TG 202 (Part 2, 1984) GLP	*Daphnia magna*	Semi-static 21 d	Reproduction		1.06	mm	Bellmann, W., 1992
OECD TG 202 (Part 2, 1984) GLP	*Daphnia magna*	Semi-static 21d	Reproduction		0.25	mm	Adema, D.M.M., de Rulter, A., 1989
OECD TG 201	*Pseudokirchneriella subcapitata*	Static 72 h	Growth rate	16.347	5.91	mm	Bruns E. and Dorgerloh M., 2008
OECD TG 201	*Anabaena flos-aquae*	Static 96 h	Growth rate	> 20	20	nom	Banman C.S. et al., 2009a

OECD TG 201	*Skeletonema costatum*	Static 96 h	Growth rate	> 20 (72 h)	5 (72 h)	nom	Banman C.S. et al., 2009b
ASTM guideline E 1415-91	*Lemna minor*	Semi-static 14 d	Growth rate	> 52.8	4.3	mm	Scheerbaum D., 1998
ISO guideline (2000) and draft OECD TG 221	*Lemna minor*	Semi-static 14 d	Growth rate	> 42	26	mm	Bogers M., 2001
higher tier study based on OECD TG 221	*Myriophyllum spicatum*	Static 14 d	Growth rate	**0.479**	**0.036**	mm	Banman C.S., 2013
mm – mean measured concentration im – initial measured concentration nom – nominal concentration							

Four acute toxicity studies in fish are reported by the DS. All of the provided reliable LC_{50} values, ranging from 10.92 to 25.0 mg/L, are above the cut-off of 1 mg/L for classification as Aquatic Acute 1. The other studies, by Anonymous (1991) and Anonymous (1993), respectively, are reported as unreliable and marked in the CLH Report as additional information only, due to the deficiencies concerning data on mean measured concentrations in the study reports.

Two chronic toxicity studies to fish are included in the CLH report and are assessed as appropriate and acceptable by the DS. The study by Anonymous (2013) was conducted according to three different test guidelines, OECD TG 210 (1992), OECD TG 215 (2000) and the OECD draft guideline "Fish 2-generation test" (2002); for the evaluation of this study the validity criteria of all used test guidelines were considered. Based on the most sensitive endpoint (growth of parental early life stages and adults) the overall NOEC was 0.156 mg a.i/L, (nominal concentration). In the early life stage toxicity test study by Anonymous (1991) the overall chronic 28-day-NOEC observed was 4.17 mg/L (mean measured concentrations), based on the most sensitive endpoint for growth (standard length and wet weight); a statistical re-evaluation of the original study data performed by Meller M. and Bruns, E. (2013) was reported by the DS, indicating the full reliability of study results from Anonymous (1991).

Three acute toxicity studies for aquatic invertebrates are available and included in the CLH report. The study by Thun S. (1993) is reported as unreliable by the DS as some validity criteria with respect to analytical measurement of the test concentrations are not met. In the fully acceptable study by Schupner, J.K., Stachura, B.J. (1992), a 96 h LC_{50} value of 5.4 mg/L was determined under static conditions, based on mean measured concentrations.

Three 21 d semi-static *D. magna* studies are reported by the DS, all with NOEC values > 0.1 mg/L.

In total, six toxicity studies to aquatic algae and plants are reported by the DS. All five studies using monocotyledononus aquatic plants (*Lemna* sp.) and algal species providing

reliable E_rC_{50} values, ranging from 16.347 to > 52.8 mg/L, give values above the cut-off of 1 mg/L for classification as Aquatic Acute 1. Also, the NOEC values from these studies are all > 0.1 mg/L.

From the available aquatic acute toxicity data, the sixth study shows that aquatic macrophytes are the most sensitive trophic group with E_rC_{50} values < 1 mg/L. In particular, the most sensitive species tested is *Myriophyllum spicatum*, that was exposed to the test substance in a static test system for 14 d. The E_rC_{50} of 0.479 mg/L is based on mean measured concentrations.

Also, for chronic aquatic toxicity, the most sensitive species tested is *Myriophyllum spicatum* with a NOE_rC of 0.036 mg/L (14 d static test), based on mean measured concentrations.

Toxicity studies on other aquatic organisms (one study on *Crassostrea virginica* and three studies on *Chironomus riparius*) were also reported by the DS as reliable information available on ethofumesate. The studies on *C. riparius* provide NOEC values ranging from 3.82 to 14.05 mg/L based on initial measured concentrations. The study on *C. virginica* provides acute and chronic toxicity values (96 h EC_{50} = 1.7 mg/L; NOEC < 0.81 mg/L, both based on new shell growth).

Comments received during public consultation

Three MSCAs commented on the proposed environmental classification. Two of them agreed with the DS's proposal. For one MSCA, it was unclear if the *M. spicatum* 14 days study endpoints were relevant for both acute and chronic classification and in addition it was noted that a sediment phase was included in the study which made interpretation of endpoints difficult. The DS responded by agreeing with the uncertainties raised. They further indicated that *M. spicatum* is commonly used For classification and labelling purposes and that whilst no sediment measures for ethofumesate were made, the concentrations in the aquatic phase were 74 – 83 % of nominal.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider ethofumesate as not rapidly degradable. The substance is hydrolytically stable, not readily biodegradable, and not ultimately degraded to a level greater than 70% over 28 days in surface water and water/sediment simulation studies.

Bioaccumulation

The measured BCF (for total radioactivity) in whole fish of 144 L/kg is below the decisive CLP criterion (BCF ≥ 500). Therefore, RAC agrees with the DS's proposal to consider that the actual bioaccumulation of ethofumesate is low.

Aquatic toxicity

Myriophyllum spicatum, a rooted macrophyte species, may be considered the target aquatic plant species for ethofumesate. From the other aquatic plants or algae studies reported by the DS, *Lemna* sp. and algae are less sensitive to ethofumesate.

Although the study with *M. spicatum* was conducted according to OECD TG 221 (*Lemna* growth inhibition test) which foresees an exposure period of 7 days, in this case the exposure time was 14 days as recommended by OECD TG 239 (water-sediment *M. spicatum* toxicity test), which is a valid time period to calculate both acute and chronic endpoints. Moreover the study fulfils the validity test criteria reported in OECD TG 239.

Regarding sediment, the OECD TG 239 recommends to determine the concentration at the beginning and the end of the test, at least at the highest test concentrations, unless the water concentration is > 80 % of the nominal. In this study, the condition is not completely verified but the measured concentrations (74% - 83 % of the nominal) are not too far from this limit. Moreover, at the highest test concentrations the concentrations are > 80 % of the nominal. RAC therefore considers that this study is valid and that *M. spicatum* is an appropriate organism for the classification of ethofumesate.

Acute aquatic hazard

Ethofumesate is of low acute toxicity to fish and aquatic invertebrates with reliable $L(E)C_{50}$ values > 1 mg/L. Instead, the available acute toxicity data on aquatic macrophytes show E_rC_{50} values < 1 mg/L. The most sensitive species tested is *Myriophyllum spicatum* with an E_rC_{50} of 0.479 mg/L, based on mean measured concentrations.

Chronic aquatic hazard

Ethofumesate is of moderate chronic toxicity to fish and aquatic invertebrates with NOEC values of 0.156 mg/L and 0.25 mg/L, respectively. The most sensitive species tested is *Myriophyllum spicatum,* (14 d static test) with a NOE_rC of 0.036 mg/L, based on mean measured concentrations.

Conclusion on the classification

Ethofumesate is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation. The lowest acute toxicity value falls in the range of $0.1 < L(E)C_{50} \leq 1$ mg/L and the lowest chronic toxicity value lies in the toxicity range of $0.01 < NOEC \leq 0.1$ mg/L.

RAC agrees with the DS that ethofumesate fulfils the CLP criteria for classification as **Aquatic Acute 1; H400** with an **M-factor of 1** and **Aquatic Chronic 1; H410** with an **M-factor of 1.**

6 OTHER INFORMATION

7 REFERENCES

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
KCA 8.2.1	Barrett, K. L.	1991b	THE ACUTE TOXICITY OF [14C]-ETHOFUMESATE TO BLUEGILL SUNFISH (*Lepomis macrochirus*) UNDER SEMI-STATIC CONDITIONS Schering AG, Berlin, Germany 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
KCA 8.2.1	Caley, C. Y.; Cameron, B. D.; Chapleo, S.; Knight, B.	1990a	DETERMINATION OF ACUTE TOXICITY (LC50) TO RAINBOW TROUT (96H, SEMI-STATIC) Ethofumesate Inveresk Research Int. Ltd., Tranent, Scotland 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
KCA 8.2.1	Caley, C. Y.; Cameron, B. D.; Chapleo, S.; Knight, B.; Wright, J. G.	1990b	DETERMINATION OF ACUTE TOXICITY (LC50) TO BLUEGILL SUNFISH (96H, SEMI-STATIC) Ethofumesate Inveresk Research Int. Ltd., Tranent, Scotland 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
KCA 8.2.1	Cameron, B. D.; Caley, C. Y.; Chapleo, S.; McKenzie, J.; McGuire, G. M.	1989	TECHNICAL ETHOFUMESATE - DETERMINATION OF ACUTE TOXICITY (LC50) TO MIRROR CARP (96 HOURS, SEMISTATIC) AND THE ANALYSIS OF ETHOFUMESATE IN WATER SAMPLES Inveresk Research Int. Ltd., Tranent, Scotland 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

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**
KCA 8.2.1	Schupner, J. K.; Stachura, B. J.	1992a	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL TO THE SHEEPSHEAD MINNOW (*Cyprinodon variegatus*) IN A STATIC SYSTEM Nor-Am Chemical Company, Pikeville, NC, USA Bayer CropScience, Report no.: A83384, Edition Number: M-155652-01-1 EPA MRID no.: 42409301 Date: 1992-06-12 GLP/GEP: yes, unpublished	Y	Y	Needed for risk assessment	Bayer CropScience
KCA 8.2.1	Thun, S.	1991a	Acute toxicity in rainbow trout (*Salmo Gairdneri*) test article: Ethofumesate IBR Forschungs GmbH, Walsrode, Germany 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)
KCA 8.2.1	Thun, S.	1993a	Acute toxicity in golden orfe (*Leuciscus Idus*) - Test article: Ethofumesate techn. IBR Forschungs GmbH, Walsrode, Germany 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)
KCA 8.2.2	Knacker, T.; Schallnass, B.; Zietz, E.; Diehl, T.	1990	A STUDY OF THE PROLONGED TOXICITY TO FISH (*Salmo gairdneri*) OF ETHOFUMESATE TECHNICAL Battelle-Institut e.V., Frankfurt am Main, Germany Bayer CropScience, Report no.: A83355, Report includes Trial Nos.: 78B BE-ET-12-89-02-F1P-2 Edition Number: M-155624-01-1 Date: 1990-05-29 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience
KCA 8.2.2	Thun, S.	1991b	Prolonged toxicity test in rainbow trout (*Salmo Gairdneri*) - Test article: Ethomumesate IBR Forschungs GmbH, Walsrode, Germany 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)
KCA 8.2.2	Wuethrich, V.	1993	21-DAY PROLONGED TOXICITY STUDY IN THE RAINBOW TROUT UNDER FLOW-THROUGH CONDITIONS Ethofumesate RCC Umweltchemie AG, Itingen, Switzerland Bayer CropScience, Report no.: A87616,	Y	N	-	Bayer CropScience

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			Edition Number: M-161553-01-1 Date: 1993-04-27 GLP/GEP: yes, unpublished				
KCA 8.2.2.1	Bruns, E.; Meller, M.	2013	Ethofumesate technical: Statistical Re-evaluation of the fish early life stage toxicity study with fathead Minnow (*Pimephales promelas*) by Faggella 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
KCA 8.2.2.1	Faggella, G. A.	1991	ETHOFUMESATE - FATHEAD MINNOW (*Pimephales promelas*) EARLY LIFE STAGE TOXICITY TEST Nor-Am Chemical Company, Pikeville, NC, USA 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
KCA 8.2.2.2	Teigeler, M.	2013	Zebra fish (*Danio rerio*), life cycle test, flow through conditions - Ethofumesate Fraunhofer Institut, Schmallenberg, Germany 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
KCA 8.2.2.3	Barrett, K. L.; Lattimore, A. E.	1991	DETERMINATION OF THE ACCUMULATION AND ELIMINATION OF [14C]-ETHOFUMESATE IN BLUEGILL SUNFISH (*Lepomis macrochirus* L.) Schering AG, Berlin, Germany 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
KCA 8.2.2.3	Caley, C. Y.; Cameron, B. D.; Chapleo, S.; Hall, B. E.; Wright, J. G.	1992	BIOACCUMULATION TEST IN BLUEGILL SUNFISH 14C-Ethofumesate Inveresk Research Int. Ltd., Tranent, Scotland 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

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KCA 8.2.4.1	Thun, S.	1993b	Acute toxicity in *Daphnia Magna* - Test article: Ethofumesate techn. IBR Forschungs GmbH, Walsrode, Germany Feinchemie Schebda , Report no.: 80-91-2312-02-93, Edition Number: M-352128-01-1 Date: 1993-03-15 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)
KCA 8.2.4.2	Schupner, J. K.; Stachura, B. J.	1992b	THE ACUTE TOXICITY OF ETHOFUMESATE TECHNICAL TO THE MYSID SHRIMP *Mysidopsis bahia* IN A STATIC SYSTEM Nor-Am Chemical Company, Pikeville, NC, USA Bayer CropScience, Report no.: A83389, Edition Number: M-155657-01-1 EPA MRID no.: 42364502 Date: 1992-06-12 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	Bayer CropScience
KCA 8.2.5.1	Adema, D. M. M., de Ruiter, A.	1989	THE CHRONIC TOXICITY OF ETHOFUMESATE TO *Daphnia magna* 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	N	N	-	Bayer CropScience
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 Schebda , Report no.: OFC00004891, Edition Number: M-352134-01-1 Date: 1992-09-21 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)
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 *Daphnia magna* 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

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**
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*
KCA 8.2.5.4	Mattock, S. D.	1998	Chronic toxicity to the sediment dwelling organism *Chironomus riparius* (BBA method) Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report no.: A91783, Report includes Trial Nos.: 194/183 Envir 208B Edition Number: M-168438-01-1 Date: 1998-03-30 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience
KCA 8.2.5.4	Stäbler, D.	2003	Assessment of side effects of Ethofumesate Technical on the larvae of the midge, *Chironomus riparius* 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
KCA 8.2.6.1	Bruns, E.	2008	*Pseudokirchneriella subcapitata* growth inhibition test with 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	N	Y	Needed for risk assessment	TaskForce Ethofumesate
KCA 8.2.6.2	Banman, C. S.; Daly, R. A.; Lam, C. V.	2009a	Toxicity of ethofumesate technical to the blue green algae *Anabaena flos-aquae* Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL008, Edition Number: M-349150-01-1 Date: 2009-06-10 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	TaskForce Ethofumesate
KCA 8.2.6.2	Banman, C. S.; Daly, R. A.; Lam, C. V.	2009b	Toxicity of ethofumesate technical to the saltwater diatom *Skeletonema costatum* 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	N	Y	Needed for risk assessment	TaskForce Ethofumesate

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**
KCA 8.2.7	Banman, C. S.	2011	Toxicity of ethofumesate technical to the aquatic macrophyte, *Myriophyllum spicatum* (amended final report) Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report no.: EBADL019-1, Edition Number: M-411454-02-1 Date: 2011-07-25 **...Amended: 2013-05-22** GLP/GEP: yes, unpublished	N	Y	**	TaskForce Ethofumesate
KCA 8.2.7	Bogers, M.	2001	A 7-Day Aquatic Plant Toxicity Test using *Lemna minor* with Ethofumesate AgriChem B.V., 324078 Notox B.V, 5231 DD 's-Hertogenbosch, The Netherlands GLP: yes Published: no	N	N	-	ACM*
KCA 8.2.7	Scheerbaum, D.	1998b	Ethofumesate - Substance technical 98.8 percent w/w - *Lemna minor*: Semi static phytotoxicity test - Code: AE B049913 00 1D97 0002 Dr. U. Noack-Laboratorium fuer Angewandte Biologie, Sarstedt, Germany Bayer CropScience, Report no.: A91865, Report includes Trial Nos.: ENVIR/211B TLA5699-TLA56991 Edition Number: M-168516-01-1 Date: 1998-05-28 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience
KCA 8.2.8	Yurk, J. J.; Ache, B. W.	1992	EFFECT OF ETHOFUMESATE TECHNICAL ON NEW SHELL GROWTH IN THE EASTERN OYSTER (*Crassostrea virginica*) UNDER FLOW-THROUGH TEST CONDITIONS Environmental Science and Engineering, Inc., Gainsville, FL, USA Bayer CropScience, Report no.: A83386, Report includes Trial Nos.: 507B Edition Number: M-155654-01-1 EPA MRID no.: 42388101 Date: 1992-05-28 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment	Bayer CropScience

* 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

** 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.

8 ANNEXES