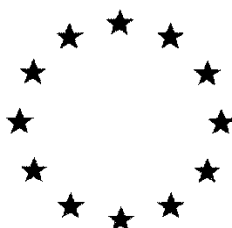


# ***European Commission***



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

## **ETHOFUMESATE**

### **Volume 3 – B.5 (PPP) – Ethofumesate SC 500**

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

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## Version History

When	What
1998	Initial DAR
2000	Addendum 8 to Vol.3 rev. 2
2015/01	DRAR

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## **B.5. METHODS OF ANALYSIS**

### **B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA**

The references of all risk assessment methods were located in the respective sections. Please note that the reliabilities of the corresponding methods are considered in the relevant sections of the risk assessment, if necessary.

#### **B.5.1.1. Section Ecotoxicology**

<b>Reference:</b>	<b>Toxicity of the formulation ethofumesate SC 500A G(ethofumesate 500 g/L) to the aquatic macrophyte, <i>Myriophyllum spicatum</i></b>
Author(s), year:	Banman, C.S., 2013
Report/Doc. number:	M-437702-02-1
Guideline(s):	
GLP:	yes

#### **Principle of method**

Test solutions from the study were analyzed by LC-MS/MS (APCI pos.) to determine the concentrations of ethofumesate. Ethofumesate-ethoxy-d5 is taken as internal standard.

Column: Gemini 5µm C18, 50 x 2.00 mm; gradient mobile phase: C = 100% Methanol, D = 10 mM NH<sub>4</sub>CO<sub>3</sub> in HPLC-grade water; MS-transitions: Ethofumesate: 287.1/ 259.2, Ethofumesate-ethoxy-d5: 292.2/ 260.1.

#### **Validation**

##### Specificity

The blank values of all control samples were below 0.3 x LOQ.

##### Linearity

A 5-point standard calibration curve was prepared. Linearity for instrument response versus relative response of Ethofumesate / Ethofumesate-ethoxy-d5 internal standard between 0.0008- and 0.100-mg/L standard concentrations. The correlation coefficient R<sup>2</sup> is > 0.99. Plot and equation of the calibration curve is available.

##### Accuracy

See Table B.5.1.1-1

##### LOQ

0.001 mg/L

##### Repeatability

See Table B.5.1.1-1

Validation is summarized in Table B.5.1.1-1

#### **Conclusion**

The analytical method is considered acceptable covering the LOQ mentioned.

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<b>Reference:</b>	<b>Effect on vegetative vigor of non-target terrestrial plants (Tier II) AE 049913: Suspension Concentrate (43.8% w/w)</b>
Author(s), year:	Christ, M.T. & Abedi J., 2003
Report/Doc. number:	M-241176-01-1
Guideline(s):	
GLP:	yes

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**Principle of method**

Samples were immediately analyzed for AE B049913 by diluting the samples to the appropriate concentration and analyzing the samples by high performance liquid chromatography (HPLC) on a non-polar stationary phase with UV detection at 225 nm. Separation was achieved using acetonitrile and 0.1% acetic acid in deionized water (v:v) under gradient conditions on a Kromasil C8 250X 4.6 mm, 5 µm particle size (Keystone Scientific). Quantitation was performed by external standard.

**Validation**Specificity

The blank value of the control sample is below 0.3 x LOQ.

Linearity

The calibration range is between 5.0-12.0 mg/L (n = 7).  $R^2 = 1$ , Equation and plot of the graph are enclosed to the study.

Accuracy

See Table B.5.1.1-1

LOQ

5.5 mg/L

Repeatability

See Table B.5.1.1-1

**Conclusion**

The analytical method is considered acceptable covering the LOQ mentioned.

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<b>Reference:</b>	<b>Effect on seedling emergence of non-target terrestrial plants (Tier II) AE 049913: Suspension Concentrate (43.8% w/w)</b>
Author(s), year:	Christ, M.T. & Abedi J., 2003
Report/Doc. number:	M-241177-01-1
Guideline(s):	
GLP:	yes

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**Principle of method**

Samples were immediately analyzed for AE B049913 by diluting the samples to the appropriate concentration and analyzing the samples by high performance liquid chromatography (HPLC) on a non-polar stationary phase with UV detection at 225 nm. Separation was achieved using acetonitrile and 0.1% acetic acid in deionized water (v:v) under gradient conditions on a Kromasil C8 250X 4.6 mm, 5 µm particle size (Keystone Scientific). Quantitation was performed by external standard.

**Validation**Specificity

The blank value of the control sample is below 0.3 x LOQ.

Linearity

The calibration range is between 5.0 - 12.0 mg/L (n = 7).  $R^2 = 0.9999$ , Equation and plot of the graph are enclosed to the study.

Accuracy

See Table B.5.1.1-1

LOQ

5.5 mg/L

Repeatability

See Table B.5.1.1-1

**Conclusion**

The analytical method is considered acceptable covering the LOQ mentioned.

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<b>Reference:</b>	<b>Ethofumesate SC 500 g/L: Effect on the seedling emergence and growth of ten species of non-target terrestrial plants (Tier 2)</b>
Author(s), year:	Gosch, H., 2009
Report/Doc. number:	M-358944-01-1
Guideline(s):	
GLP:	yes

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No validation of the analytical method used is appended to the study.

**Table B.5.1.2.6-1: Validation results**

References	Analyte	Detection method	Matrix	LOQ [mg/L] or [mg/kg]	Fortification level [mg/L] or [mg/kg]	Mean recovery [%]	RSD [%]	n
Banman, C.S., 2013 M-437702- 02-1	Ethofumesate	HPLC	aqueous solution	0.001	0.001	102.3	9.3	3
					0.005	105.3	5.5	3
					0.05	89	-	2
					2	87	-	1
Christ, M.T. & Abedi J., 2003 M-241176-01-1	Ethofumesate	HPLC	aqueous solution	5.5	5.5	98	2.0	3
					10	99	0.6	3
Christ, M.T. & Abedi J., 2003 M-241177-01-1	Ethofumesate	HPLC	aqueous solution	5.5	5.5	97	1.0	3
					10	98	1.0	3



**B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES****B.5.2.1. Analysis of the plant protection product (CP 5.1.1)**

Additional studies were performed, which were not submitted during the Annex I inclusion process. These studies will be summarized below. For all studies submitted during the frame of the Annex I inclusion, please refer to grey tables below and to the corresponding section in the Monograph.

<b>Report:</b>	<a href="#">KCP 5.1.1 /01;Johnson, M. K.;1991;M-157966-01</a>
<b>Title:</b>	THE DETERMINATION OF ETHOFUMESATE IN TECHNICAL ETHOFUMESATE AND ITS EMULSIFIABLE AND SUSPENSION CONCENTRATION FORMULATIONS BY GAS CHROMATOGRAPHY (GC)
<b>Report No:</b>	A85639
<b>Document No:</b>	<a href="#">M-157966-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	no
<b>Report:</b>	<a href="#">KCP 5.1.1 /02;Johnson, M. K.; Shaw, B.;1991;M-157962-01</a>
<b>Title:</b>	AN ASSESSMENT OF THE ACCURACY AND PRECISION OF THE ANALYTICAL METHOD AM 1100/17/1 FOR THE DETERMINATION OF ETHOFUMESATE IN: ETHOFUMESATE TECHNICAL PRODUCT, NORTRON EMULSIFIABLE CONCENTRATE, NORTRON SUSPENSION CONCENTRATE
<b>Report No:</b>	A85637
<b>Document No:</b>	<a href="#">M-157962-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	yes
<b>Report:</b>	<a href="#">KCP 5.1.1 /03;Juppo, A.;1992;M-161441-01</a>
<b>Title:</b>	DETERMINATION OF ETHOFUMESATE IN TECHNICAL MATERIAL AND LIQUID FORMULATIONS
<b>Report No:</b>	A87543
<b>Document No:</b>	<a href="#">M-161441-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	no
<b>Report:</b>	<a href="#">KCP 5.1.1 /04;De Vries, R.;1993;M-161429-01</a>
<b>Title:</b>	DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD OF ETHOFUMESATE
<b>Report No:</b>	A87534
<b>Document No:</b>	<a href="#">M-161429-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	yes
<b>Report:</b>	<a href="#">KCP 5.1.1 /05;Johnson, M. K.;1998;M-188813-01</a>
<b>Title:</b>	The determination of ethofumesate in the technical grade active ingredient and its suspension and emulsifiable concentrate formulations by high performance liquid chromatography : analytical method Ethofumesate Code: AE B049913
<b>Report No:</b>	C004901
<b>Document No:</b>	<a href="#">M-188813-01-1</a>
<b>Guidelines:</b>	EU (=EEC): 91/414 Ann. II-4.1 & Ann. III - 5.1;Deviation not specified
<b>GLP/GEP:</b>	no

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<b>Report:</b>	<a href="#">KCP 5.1.1 /06;Johnson, M. K.; Lyon, C. F.;1997;M-188815-01</a>
<b>Title:</b>	The validation of AM1100/29/01 for the determination of ethofumesate in the technical grade active ingredient and its suspension and emulsifiable concentrate formulations by high performance liquid chromatography Ethofumesate Code: AE B0499
<b>Report No:</b>	C004902
<b>Document No(s):</b>	Report includes Trial Nos.: AM1100/29/01 <a href="#">M-188815-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	no

#### B.5.2.1.1. Methods for the determination of the active substance in the preparation

The analytical method AM016709FF1 is used for the determination of ethofumesate in the preparation.

<b>Reference:</b>	<b>Determination of desmedipham, ethofumesate, metamitron and phenmedipham in formulations - Assay HPLC, external standard</b>
Author(s), year:	Michel, A.; 2009
Report/Doc. number:	AM016709FF1/M-358630-01-1
Guideline(s):	EU 91/414/EEC Annex III 5.1;not specified
GLP:	no

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<b>Reference:</b>	<b>Validation of HPLC-method AM016709FF1 - Determination of ethofumesate in formulations - Ethofumesate SC 500 g/L</b>
Author(s), year:	Michel, A.;2009
Report/Doc. number:	VB1-AM016709FF1/M-358634-01-1
Guideline(s):	EU Directive 91/414/EEC, Annex III 5.1 SANCO/3030/99 rev. 4;not specified
GLP:	no

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#### Principle of the method

Active substances are separated from components on a reverse phase column using isocratic solution. After UV detection at 225 nm (column: Hypersil ODS 125 mm x 4.0 mm, 3µm), the quantitative evaluation is carried out by comparing the area peak areas with those of reference substances, using an external standard.

The HPLC method AM016709FF1 for the determination of ethofumesate in the preparation has been completely validated by checking the parameters linearity, precision, accuracy, specificity and interference.

**Validation**

Linearity	11 concentrations (single injection); range 0.9 – 14.5 mg/100 mL correlation coefficient $r_K = 1$ ; regression equation: $y = 4.2756x + 0.056$ A graph of the calibration curve is given. The function is linear in the operating range.
Precision Repeatability	6 samples (single injection) from one batch RSD: 0.43 % (Horwitz value: 1.51%)
Accuracy	6 samples of laboratory-prepared synthetic formulation containing known weight of analyte; statistical assessment of the recovery results; calculation confidence interval. Mean recovery : 100.1 %; RSD: 0.18 %
Specificity Interference	The UV-spectra of active substance and reference item show no spectral difference; the retention times are identical. No interferences are found.

**Conclusion**

The method AM016709FF1 for the determination of ethofumesate in the preparation is found to be valid.

**B.5.2.1.2. Methods for the determination of degradation products in the preparation**

There is no decrease of the content in the active substance in the preparation after storage therefore an analytical method and validation is not needed.

**B.5.2.1.3. Methods for the determination of relevant impurities identified in the technical material or which may be formed during manufacture of the preparation or from degradation of the preparation during storage**

Ethofumesate technical material produced by the manufacturer Bayer CropScience AG does not contain any relevant impurities such as EMS (ethyl-methanesulfonate) and iBMS (isobutyl-methanesulfonate). But as required by the guidelines, an analytical method for their determination in the preparation has been developed.

The analytical method AM039414FP1 is used for the determination of EMS (ethyl-methanesulfonate) and iBMS (isobutyl-methanesulfonate) in the preparation.

<b>Reference:</b>	<b>Analytical method - Determination of traces of AE C639174 (ethyl-methanesulfonate) and AE C639170 (isobutyl-methanesulfonate) in ethofumesate formulations by derivatization with trimethyl amine followed by liquid chromatography - mass spectrometry with selected-ion-recording (LC-MS/SIR)</b>
Author(s), year:	Zoellner, P.; Fey, G.; 2014
Report/Doc. number:	AM039414FP1/M-484464-01-1
Guideline(s):	EC 1107/2009; EU 283/2013 ; EPA OCSPP 830.1800
GLP:	no

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<b>Reference:</b>	<b>Validation of the analytical method AM039414FP1 for the determination of AE C639174 (ethyl-methanesulfonate) and AE C639170 (isobutyl-methanesulfonate) in AE B049913 (ethofumesate) formulations by derivatization with trimethyl amine followed by liquid chromatography - mass spectrometry with selected-ion-recording (LC-MS/SIR)</b>
Author(s), year:	Zoellner, P.; Fey, G.; 2014
Report/Doc. number:	PA14/002/M-484461-01-1
Guideline(s):	EC 1107/2009; EU 283/2013 ; EPA OCSPP 830.1800
GLP:	yes

### Principle of the method

After derivatization with trimethyl amine the relevant impurities are determined in two separate analytical runs by liquid chromatography-mass spectrometry (LC-MS) combined with electrospray ionization (ESI).

Quantification is carried out by selected ion recording (SIR) and an internal standard calibration protocol with stable isotope labelled AE C639174

(EMS\*, BCS-CY10447) and AE C639170 (IBMS\*; BCS-CY10464).

### Validation

Linearity	<p>8 reference solutions of each analyte were prepared.</p> <p>concentrations range : 0.040 – 3.001 mg/kg of AE C639174</p> <p>concentrations range : 0.0396 – 3.962 mg/kg of AE C639170</p> <p>considering a test item solution concentration of 1000 mg/10 mL</p> <p>AE C639174 (EMS) : correlation coefficient <math>r_K = &gt; 0.999</math></p> <p>AE C639170 (iBMS) : correlation coefficient <math>r_K = &gt; 0.999</math></p> <p>Chromatograms are given. The function is linear in the operating range.</p>
Precision Repeatability	<p>5 samples of the test item were spiked with AE C639174 and AE C639170 at concentration levels of 0.04 mg/kg and 0.1 mg/kg.</p> <p>AE C639174 (EMS) : RSD for 0.1 mg/kg : 5.1 % ; RSD for 0.04 mg/kg : 4.1 %</p> <p>AE C639170 (iBMS) : RSD for 0.1 mg/kg : 1.4 % ; RSD for 0.04 mg/kg : 2.3 %</p> <p>Values are given for the nominal concentration of AE B049913-test item solution of approx.. 100 mg/mL.</p> <p>Horwitz value for 0.04 mg/kg: 17.4%</p>

Accuracy	<p>5 samples of the test item were spiked with AE C639174 and AE C639170 at concentration levels of 0.04 mg/kg and 0.1 mg/kg.</p> <p>AE C639174 (EMS)      Mean recovery for 0.04 mg/kg : 80.4 %  Mean recovery for 0.1 mg/kg : 86.4 %</p> <p>AE C639170 (iBMS)    Mean recovery for 0.04 mg/kg : 99.4 %  Mean recovery for 0.1 mg/kg : 101.4 %</p> <p>Values are given for the nominal concentration of AE B049913-test item solution of approx.. 100 mg/mL.</p>
Specificity Interference	<p>The molecular ions of the trimethyl amine derivatives of EMS, IBMS and for each internal standard were monitored. No interferences in any of the investigated ion traces of the analytes or the internal standards were visible in the LC-MS chromatograms of the unspiked ethofumesate formulation. From these observations it can be concluded that this LC-MS method is selective for EMS and IBMS in Ethofumesate formulations.</p>
LOQ	<p>0.04 mg/kg  (min. LOQ required: 0.0443 mg/kg related to a formulation containing 44.3 % w/w ethofumesate)</p>

### Conclusion

The method AM039414FP1 for the determination of EMS (ethyl-methanesulfonate) and iBMS (isobutyl-methanesulfonate) in Ethofumesate SC 500 is found to be valid.

#### B.5.2.1.4 Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities.

With respect to toxicological, eco-toxicological or environmental aspects the product does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

#### B.5.2.1.5 Applicability of existing CIPAC methods

The CIPAC method available for the determination of ethofumesate in such preparation can be found in the FAO specification 233/SC/M/3 (CIPAC Handbook J, p.48, 2000).

**B.5.3. REFERENCES RELIED ON**

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebra te study Y/N	Data protection claimed Y/N	Justificat ion if data protectio n is claimed	Owner	Previous evaluatio n
KCP 5.1.1 /07	Michel, A.	2009	Determination of desmedipham, ethofumesate, metamitron and phenmedipham in formulations - Assay HPLC, external standard Bayer CropScience, Report No.: AM016709FF1, Edition Number: M-358630-01-1 Date: 2009-11-10 GLP/GEP: no, unpublished	N	Y	Revised analytical method	Bayer CropScience	Submitte d for the purpose of renewal (2014)
KCP 5.1.1 /08	Michel, A.	2009	Validation of HPLC-method AM016709FF1 - Determination of ethofumesate in formulations - Ethofumesate SC 500 g/L Bayer CropScience, Report No.: VB1-AM016709FF1, Edition Number: M-358634-01-1 Date: 2009-11-10 GLP/GEP: no, unpublished	N	Y	Validatio n according to SANCO /3030	Bayer CropScience	Submitte d for the purpose of renewal (2014)
KCP 5.1.1 /09	Zoellner, P.; Fey, G.	2014	Analytical method - Determination of traces of AE C639174 (ethyl-methanesulfonate) and AE C639170 (isobutyl-methanesulfonate) in ethofumesate formulations by derivatization with trimethyl amine followed by liquid chromatography - mass spectrometry with selected-ion-recording (LC-MS/SIR) Bayer CropScience, Report No.: AM039414FP1, Edition Number: M-484464-01-1 Date: 2014-04-28 GLP/GEP: no, unpublished	N	Y	Validatio n according to SANCO /3030	Task Force Ethofumesate	Submitte d for the purpose of renewal (2014)
KCP 5.1.1 /10	Zoellner, P.; Fey, G.	2014	Validation of the analytical method AM039414FP1 for the determination of AE C639174 (ethyl-methanesulfonate) and AE C639170 (isobutyl-methanesulfonate) in AE B049913 (ethofumesate) formulations by derivatization with trimethyl amine followed by liquid chromatography - mass spectrometry with selected-ion-recording (LC-MS/SIR) Bayer CropScience, Report No.: PA14/002, Edition Number: M-484461-01-1 Date: 2014-04-28 GLP/GEP: yes, unpublished	N	Y	Validation according to SANCO /3030	Task Force Ethofumesate	Submitte d for the purpose of renewal (2014)