

# ***European Commission***



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

**ETHOFUMESATE**

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

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

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### Version History

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|---------|------|
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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>Acute toxicity of ethofumesate 500 SC (500 g/L) against <i>Oncorhynchus mykiss</i></b> |
| Author(s), year:    | ██████████ 1997   |
| Report/Doc. number: | 5548  |
| Guideline(s):       |   |
| GLP:                | yes   |

Within the study is stated that the validation of the analytical method is described in a separate report no. 5579.

The study was submitted on request.

#### **Principle of method**

GC-MS (SIM). The fragment ions ( $m/z = 286, 161$  and  $212$ ) were chosen. Ion  $161$  was used as qualifier ion to confirm the identity of the Ethofumesatew, while ion  $286$  was used to generate the response for the calibration curve for Ethofumesate and ion  $212$  was used to generate the responsefor the internal standard (Pyren dlo).

#### **Validation**

##### Specificity

The blank value of the control sample cannot be assessed due to different dimension of scaling compared to the chromatograms of the spiked sample. A blank with same scaling dimension is required.

##### Linearity

The quantification was carried out by internal calibration. A series of 5 standard solutions of Ethofumesate, with concentrations ranging from  $0.05 \text{ mg/l}$  to  $2.5 \text{ mg/l}$  ( $r^2 = 1$ ), was used to obtain a calibration curve and determine the linear range. The response ratio for each level was plotted against the corresponding concentration ratio of the standard solutions. By curve fitting linear regression, was found to be the most suitable, and the calibration curve was obtained. No calibration curve is available although it is stated in the table of content page 22 is empty.

##### Accuracy

see Table B.5.1.1-1

##### LOQ

$0.50 \text{ mg/L}$

##### 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.

|                     |  |
|---------------------|--|
| <b>Reference:</b>   | <b>The effect of ethofumesate 500 SC (500 g/L) on the Daphnia magna acute test</b> |
| Author(s), year:    | Pors, J., 1997   |
| Report/Doc. number: | 5591   |
| Guideline(s):       |  |
| GLP:                | yes  |

Within the study is stated that the validation of the analytical method is described in a separate report no. 5579. The study was submitted on request.

### Principle of method

GC-MS (SIM). The fragment ions ( $m/z = 286, 161$  and  $212$ ) were chosen. Ion 161 was used as qualifier ion to confirm the identity of the Ethofumesate, while ion 286 was used to generate the response for the calibration curve for Ethofumesate and ion 212 was used to generate the response for the internal standard (Pyren dlo).

### Validation

#### Specificity

The blank value of the control sample cannot be assessed due to different dimension of scaling compared to the chromatograms of the spiked sample. A blank with same scaling dimension is required.

#### Linearity

The quantification was carried out by internal calibration. A series of 5 standard solutions of Ethofumesate, with concentrations ranging from 0.05 mg/l to 2.5 mg/l ( $r^2 = 1$ ), was used to obtain a calibration curve and determine the linear range. The response ratio for each level was plotted against the corresponding concentration ratio of the standard solutions. By curve fitting linear regression, was found to be the most suitable, and the calibration curve was obtained. No calibration curve is available although it is stated in the table of content page 22 is empty.

#### Accuracy

see Table B.5.1.1-1

#### LOQ

0.50 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>The effect of ethofumesate 500 SC (500 g/L) on the growth rate of the alga <i>Chlorella vulgaris</i></b> |
| Author(s), year:    | Pors, J., 1997  |
| Report/Doc. number: | 5600  |
| Guideline(s):       |   |
| GLP:                | yes   |

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Within the study is stated that the validation of the analytical method is described in a separate report no. 5579. The study was submitted on request.

#### **Principle of method**

GC-MS (SIM). The fragment ions ( $m/z = 286, 161$  and  $212$ ) were chosen. Ion  $161$  was used as qualifier ion to confirm the identity of the Ethofumesatew, while ion  $286$  was used to generate the response for the calibration curve for Ethofumesate and ion  $212$  was used to generate the responsefor the internal standard (Pyren dlo).

#### **Validation**

##### Specificity

The blank value of the control sample cannot be assessed due to different dimension of scaling compared to the chromatograms of the spiked sample. A blank with same scaling dimension is required.

##### Linearity

The quantification was carried out by internal calibration. A series of 5 standard solutions of Ethofumesate, with concentrations ranging from  $0.05$  mg/l to  $2.5$  mg/l ( $r^2 = 1$ ), was used to obtain a calibration curve and determine the linear range. The response ratio for each level was plotted against the corresponding concentration ratio of the standard solutions. By curve fitting linear regression, was found to be the most suitable, and the calibration curve was obtained. No calibration curve is available although it is stated in the table of content page 22 is empty.

##### Accuracy

see Table B.5.1.1-1

##### LOQ

$0.50$  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>Toxicity of Ethofol 500 SC (HBX01) to the aquatic plant Lemna gibba in a static growth inhibition test</b> |
| Author(s), year:    | Hoffmann, K. & Deierling, T., 2010  |
| Report/Doc. number: |   |
| Guideline(s):       |   |
| GLP:                | yes   |

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**Principle of method**

HPLC method, detection at 230 nm

Column: US ES RP 18 250 x 4 mm; isocratic mobile phase 40% A Water + 0.1% phosphoric acid, 60% B acetonitrile + 5% water.

**Validation**Specificity

The blank value of the control sample cannot be assessed due to different dimension of scaling compared to the chromatograms of the spiked sample. A blank with same scaling dimension is required.

Linearity

Calibration range from 0.25 mg/L to 5 mg/L (n= 6). The correlation coefficient is 1. Plot and equation of the calibration curve is available.

Accuracy

see Table B.5.1.1-1

LOQ

0.75 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.

A blank with same scaling dimension as the spiked chromatograms is required.

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|                     |   |
|---------------------|---|
| <b>Reference:</b>   | <b>Phytotoxicity test with the rooted submerge aquatic macrophyte <i>Myriophyllum aquaticum</i>, static, 7-10 d</b> |
| Author(s), year:    | Scheerbaum, D., 2013  |
| Report/Doc. number: |   |
| Guideline(s):       |   |
| GLP:                | yes   |

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**Principle of method**

LC-MS/MS determination; ESI pos.; two transitions  $m/z$  287  $\rightarrow$  121 and  $m/z$  287  $\rightarrow$  259

LC-column: Waters Acquity UPLC BEH C18, 1.7  $\mu$ m, 50 x 2.1 mm; gradient mobile phase A: water + 1% formic acid, B: methanol + 1% formic acid

**Validation**Specificity

LC-MS/MS using two transitions is highly specific. The blank value of all control samples was below 0.3 x LOQ.

Linearity

The linearity were determined for ethofumesate (both transitions) in the concentration ranges from 2.0  $\mu$ g/L to 30.0  $\mu$ g/L (n= 6). The correlation coefficients were >0.992. Plots and equation of the calibration curves are available.

Accuracy

see Table B.5.1.1-1

LOQ

0.00593 mg ethofumesate/kg water

0.0237 mg ethofumesate/kg sediment

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>Honeybee - Acute oral toxicity test with ethofumesate 500 SC (ethofumesate: 500 g/L)</b> |
| Author(s), year:    | Mallikarjunappa, S., 1998   |
| Report/Doc. number: | 2295/97   |
| Guideline(s):       |   |
| GLP:                | yes   |

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

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|                     |  |
|---------------------|--|
| <b>Reference:</b>   | <b>Evaluation of the phytotoxicity of ethofol formulation (500 g/L ethofumesate) (based on OECD guideline 208 B) vegetative vigour test terrestrial non target</b> |
| Author(s), year:    | Dodd, J., 2004   |
| Report/Doc. number: |  |
| Guideline(s):       |  |
| GLP:                | yes  |

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### Principle of method

HPLC method, detection at 280 nm

Column: Intersil ODS2 250 x 4.6 mm; isocratic mobile phase: acetonitrile/water 70/30 v/v

### Validation

#### Specificity

No chromatogram of a blank sample is submitted.

#### Linearity

Calibration range from 20.08/L to 100.4 mg/L (n= 5). The correlation coefficient is 1. Plot and equation of the calibration curve is available.

#### Accuracy

Only three recoveries at 5000 mg/L were investigated at a single fortification is reported.

#### LOQ

5000 mg/L based on a single fortification.

#### Repeatability

see Table B.5.1.1-1

Validation is summarized in Table B. B.5.1.1-1

### Conclusion

No evaluation of specificity can be made and the second fortification is missing..

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|                     |   |
|---------------------|---|
| <b>Reference:</b>   | <b>Evaluation of the phytotoxicity of ethofol formulation (500 g/L ethofumesate) (based on OECD guideline 208 A) seedling emergence terrestrial non target plants - GLP study</b> |
| Author(s), year:    | Bramby-Gunary, J., 2004   |
| Report/Doc. number: |   |
| Guideline(s):       |   |
| GLP:                | yes   |

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**Principle of method**

HPLC method, detection at 280 nm

Column: Intersil ODS2 250 x 4.6 mm; isocratic mobile phase: acetonitrile/water 70/30 v/v

**Validation**Specificity

No chromatogram of a blank sample is submitted.

Linearity

Calibration range from 20.08/L to 100.4 mg/L (n= 5). The correlation coefficient is 1. Plot and equation of the calibration curve is available.

Accuracy

Only one recovery at 10690 mg/L was investigated, see Table B.5.1.1-1. No second fortification is reported.

LOQ

-

Repeatability

see Table B.5.1.1-1

Validation is summarized in Table B.5.1.1-1

**Conclusion**

The analytical method is not according to guidance document 3029/99 since only one recovery is determined and a 2<sup>nd</sup> fortification is missing and no evaluation of specificity can be made.

Table B.5.1.2.6-1: Validation results

| References                               | Analyte      | Detection method | Matrix           | LOQ<br>[mg/L] or<br>[mg/kg] | Fortification level<br>[mg/L] or [mg/kg] | Mean<br>recovery<br>[%] | RSD<br>[%] | n |
|--|--------------|------------------|------------------|-----------------------------|--|-------------------------|------------|---|
| Hoffmann, K. &<br>Deierling, T.,<br>2010 | Ethofumesate | HPLC             | aqueous solution | 0.75                        | 0.75                                     | 101.25                  | 1.69       | 4 |
|  |              |                  |                  |                             | 10                                       | 104.5                   | 1.24       | 4 |
|  |              |                  |                  |                             | 100                                      | 103                     | 1.12       | 4 |
| 1997<br>5548                             | Ethofumesate | GC-MS            | water            | 0.5                         | 0.5                                      | 88                      | 11         | 6 |
|  |              |                  |                  |                             | 1.0                                      | 101                     | 5.5        | 3 |
|  |              |                  |                  |                             | 5.0                                      | 98                      | 4          | 3 |
| Pors, J., 1997<br>5591                   | Ethofumesate | GC-MS            | water            | 0.5                         | 0.5                                      | 88                      | 11         | 6 |
|  |              |                  |                  |                             | 1.0                                      | 101                     | 5.5        | 3 |
|  |              |                  |                  |                             | 5.0                                      | 98                      | 4          | 3 |
| Pors, J., 1997<br>5600                   | Ethofumesate | GC-MS            | water            | 0.5                         | 0.5                                      | 88                      | 11         | 6 |
|  |              |                  |                  |                             | 1.0                                      | 101                     | 5.5        | 3 |
|  |              |                  |                  |                             | 5.0                                      | 98                      | 4          | 3 |
| Scheerbaum, D.,<br>2013                  | Ethofumesate | HPLC             | aqueous solution | 0.00593                     | 0.00593                                  | 83                      | 12         | 5 |
|  |              |                  |                  |                             | 0.456                                    | 98                      | 3.1        | 5 |
|  |              |                  | sediment         | 0.0237                      | 0.0237                                   | 105                     | 4.0        | 5 |
|  |              |                  |                  |                             | 0.237                                    | 105                     | 7.3        | 5 |
| Dodd, J., 2004                           | Ethofumesate | HPLC             | aqueous solution | -                           | 5000                                     | 104                     | 0.96       | 3 |
| Bramby-Gunary,<br>J., 2004               | Ethofumesate | HPLC             | aqueous solution | -                           | 10690                                    | 107                     | -          | 1 |

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

|                     |   |
|---------------------|---|
| <b>Reference:</b>   | <b>HPLC Determination of Ethofumesate in Technical Material and Formulations – For the Ethofol 500 g/L SC Formulation. G.C. Laboratories Ltd, Analytical Chemistry Centre, Stotfold, UK.</b>  |
| Author(s), year:    | White, G., 2003   |
| Report/Doc. number: | Report No. J14405   |
| Guideline(s):       | PSD Guidelines for the Validation of Analytical Methods for Pesticides (PRD 2400), Commission Directive 96/46/EC and SANCO/3030/99 rev.4 'Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, section 4) and Annex III (part A, section 4 ) of Directive 91/414. |
| GLP:                | yes   |

**Principle of the method**

The principle of the method for determination of Ethofumesate in technical material and formulations is by the use of reversed phase high performance liquid chromatography employing dipropyl phthalate as an internal standard.

A sample of the formulation or technical material to be analysed is measured or weighed out into a suitable receptacle to which the internal standard (3.5g dipropyl phthalate/1000 mL acetonitrile) and acetonitrile are added. Samples are fully dissolved by sonication for 10 minutes and any undissolved solids are removed by filtration. Samples are then injected into the HPLC using a degassed acetonitrile/water mobile phase and fitted with UV detector set to record at 227 nm. (Column : Prodigy ODS2 250 mm × 4.6 mm)

**Validation**Specificity

No interference affecting the chromatographic peak of the Ethofumesate and internal standard was observed.

Linearity

6 standards were prepared in the range of approx. 50% - 150% nominal, correlation coefficient  $r^2 > 0.999$ ; plot and equation and plot of the calibration curve are available.

Accuracy

The mean recovery was 100.4% (n = 6) with a RSD = 1.016%

Repeatability ((Precision)

Mean Ethofumesate content: 45.98% (w/w).

The relative standard deviation (RSD) at six replicates was 0.967% and so within guideline requirements for Horwitz RSD ( $\leq 1.506\%$ ).

**Conclusion**

The method for the determination of ethofumesate in Ethofol 500 g/L SC is found to be valid.

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

No method is required since according to the shelf life study the content of ethofumesate did not decrease.

**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**

No impurities of toxicological, ecotoxicological or environmental relevance are present in the technical active material or expected to form during manufacture of the plant protection product or from degradation of the plant protection product during storage. However, a method is available to confirm that the possibly relevant impurities Ethyl methanesulfonate (EMS) and Methanesulfonic acid, 2-methylpropyl ester (IBMS), which have already been shown not to occur in relevant amounts in the technical (refer to Volume 4, Point CA 1.10), do not occur in the plant protection product Ethofol 500 SC (code HBX01). The validated method and proof of the absence of EMS and IBMS are reported below.

|                     |   |
|---------------------|---|
| <b>Reference:</b>   | <b>Re-validation of SOP DLA-262.2 “Ethofumesate TC GC-MS determination of trace impurities”</b>   |
| Author(s), year:    | Bos, M.W.S., 2014   |
| Report/Doc. number: | Report No. DL 14-127  |
| Guideline(s):       | SANCO/3030/99 rev.4 ‘Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, section 4) and Annex III (part A, section 4 ) of Directive 91/414 |
| GLP:                | yes   |

**Principle of the method**

The principle of the method for determination of the two chemical species EMS and IBMS in the product Ethofol is based on gas chromatography coupled to a mass spectrometer detector (GC-MS) in the Single Ion mode with external standardisation.

A sample of the formulation or blank to be analysed is weighed into a 10 mL volumetric flask and, where needed, spiked with relevant standard solution. The dispersion is then diluted to 10 mL with ethyl acetate. 1 µL is finally injected into the GC (column: 30 m DB-624, 0.32 mm i.d. and 1.8 µm film thickness; MS-SIM: Plot 1 ion: m/z 79.00; Plot 2 ion: m/z 95.00) and measured.

The area of the resulting peaks is then correlated to the analytes’ concentration using the results obtained by calibration.

**Validation**Specificity

No interfering peaks were found at the retention time of EMS and IBMS. The chromatograms are recorded in TIC (total ion chromatogram).

### Linearity

Six standard solutions were prepared in ethyl acetate and injected in the GC-MS to establish the linearity response between 0.005 and 1 mg/L. Correlation coefficients > 0.999 for both analytes. Plots and equations of the calibration curve are available.

### Accuracy

#### EMS

fortification at 0.1 mg/kg (related to ethofumesate) mean recovery was 86% (n = 5)

fortification at 1.2 mg/kg (related to ethofumesate) , mean recovery was 104% (n = 5)

#### IBMS

fortification at 0.1 mg/kg (related to ethofumesate) mean recovery was 83% (n = 5)

fortification at 1.3 mg/kg (related to ethofumesate) mean recovery was 118% (n = 5)

### Repeatability ((Precision)

Mean EMS content: 0.292 µg. The relative standard deviation (RSD) at five replicates was 3.57% and so within guideline requirements for Horwitz RSD ( $\leq 12.90\%$ ).

Mean IBMS content: 0.351 µg. The relative standard deviation (RSD) at five replicates was 4.47% and so within guideline requirements for Horwitz RSD ( $\leq 12.55\%$ ).

### LOQ

EMS = 0.1 mg/kg compared to ethofumesate content (0.05 mg/kg to formulation)

IBMS = 0.1 mg/kg compared to ethofumesate content (0.05 mg/kg to formulation)

### **Conclusion**

The analytical method for the determination of EMS (ethyl-methanesulfonate) and IBMS (isobutyl-methanesulfonate) in Ethofol 500 SC 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.**

No methods are required for co-formulants or components of co-formulants.

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

A CIPAC method is available for the determination of ethofumesate in SC formulations.

**B.5.3. REFERENCES RELIED ON**

| Data Point      | Author(s)      | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not  | Vertebra<br>te study<br>Y/N | Data<br>protection<br>claimed<br>Y/N | Justificat<br>ion if<br>data<br>protectio<br>n is<br>claimed  | Owner | Previous<br>evaluatio<br>n                                  |
|-----------------|----------------|------|--|-----------------------------|--------------------------------------|---|-------|---|
| KCP<br>5.1.1/01 | White, G.A.    | 2003 | HPLC DETERMINATION OF<br>ETHOFUMESATE IN TECHNICAL<br>MATERIALS AND FORMULATIONS -<br>FOR THE ETHOFOL 500G/L SC<br>FORMULATION<br>United Phosphorus Ltd., J14405<br>G.C. Laboratories Ltd, Analytical<br>Chemistry Centre, Stotfold, UK<br>GLP: yes<br>Published: no | N                           | Y                                    | New<br>data for<br>active<br>ingredie<br>nt, not<br>previous<br>ly<br>submitte<br>d nor<br>evaluate<br>d    | UPL   | Submitte<br>d for the<br>purpose<br>of<br>renewal<br>(2014) |
| KCP<br>5.1.1/02 | Bos,<br>M.W.S. | 2014 | RE-VALIDATION OF SOP DLA-262.2<br>“ETHOFUMESATE TC GC-MS<br>DETERMINATION OF TRACE<br>IMPURITIES<br>United Phosphorus Ltd., DL 14-127<br>Cerexagri B.V., Vondelingenplaat, The<br>Netherlands<br>GLP: yes<br>Published: no   | N                           | Y                                    | New<br>data for<br>existing<br>formulat<br>ion, not<br>previous<br>ly<br>submitte<br>d nor<br>evaluate<br>d | UPL   | Submitte<br>d for the<br>purpose<br>of<br>renewal<br>(2014) |