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ETHOFUMESATE

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

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B.5. METHODS OF ANALYSIS

INTRODUCTION

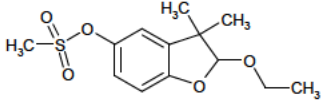
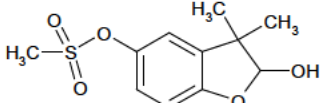
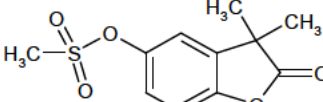
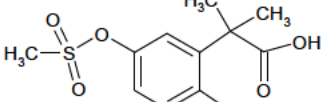
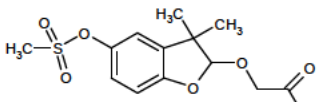
Ethofumesate is an herbicidal active substance and was included into Annex I of Directive 91/414 in 2002 (Directive 2002/37/EC, dated 3rd of May 2002, Entry into Force 1st of March 2003).

This dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of ethofumesate and were, therefore, not evaluated during the first EU review of this compound. All other studies, which were already submitted by the Task Force Ethofumesate for the first Annex I inclusion, are contained in the Monograph and in the baseline dossier (D-008920/8989) provided by the Task Force Ethofumesate. These studies are summarized in tables written in grey typeface in the dossier.

Ethofumesate is a racemic mixture of (two) enantiomers. The synthesis process for ethofumesate does not involve chiral auxiliaries and therefore the resulting isomeric mixture has always been a racemic one (1:1). The herbicidal activity of the two enantiomers has been shown to be equivalent and not different from the racemic mixture. In degradation studies (non-guideline lysimeter study and in a water sediment study) no significant changes in the ratio of the racemate (1:1) were observed, indicating that the degradation and distribution of both enantiomers is the same in the environment. Therefore it was considered adequate that all studies on the active substance were performed using the racemic mixture.

The presented and submitted studies used different synonyms and codes for the active substance ethofumesate, its metabolites and reference compounds used. In order to present a common basis for the evaluation the following list summarizes all names used. In the present dossier section generally the name or the corresponding “NC-code” (e.g. ethofumesate-2-hydroxy or NC 8493) were used.

List of synonyms and codes relevant for analytical methods

Code Number	Synonym	Chemical name	Structure
Ethofumesate	NC 8438, AE B049913	(<i>RS</i>)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate (IUPAC) 5-Benzofuranol, 2-ethoxy-2,3-dihydro-3,3-dimethyl-, methanesulfonate (CAS) [CAS No.: 26225-79-6]	
Ethofumesate-2-hydroxy	NC 8493, AE C508493, BCS-BB94377, hydroxy-derivative, 2-hydroxy-ethofumesate, Fumesate	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate (IUPAC)	
Ethofumesate-lactone	NC 9607, AE C509607, 2-keto-Ethofumesate, Ethofumesate-2-keto, Oxo-derivative, Fumesate lactone	2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulfonate (IUPAC)	
Ethofumesate-carboxylic acid	NC 20645, AE C520645, BCS-AV65501, RO 9607 (“ring-open 9607”), “Hydrolyzed AE C509607” [res. method no. 01116/M001], Ethofumesate- γ -hydroxy-carboxylic acid, open-ring-2-keto-ethofumesate, ring opened lactone ----- AE C639175 (potassium salt) BCS-CU88901 (sodium salt)	2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methyl propionic acid (IUPAC)	
Ethofumesate-acetic acid	BCS-CW35117	({3,3-dimethyl-5-[(methylsulfonyl)oxy]-2,3-dihydro-1-benzofuran-2-yl} oxy)acetic acid (IUPAC)	

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA (CA 4.1)**B.5.1.1. Methods for the analysis of the active substance as manufactured (CA 4.1.1)****TASKFORCE****BAYER CROPSCIENCE**

Method **AM022208FP2** is validated for the determination of ethofumesate in technical grade active substance.

Methods **AM020008FP3** and **AM022108FP2** are validated for the determination of significant impurities as well as for additional possibly occurring process impurities.

Method **AM037113FP1** is validated for the determination of both (relevant) impurities, EMS (ethyl-methanesulfonate, AE C639174) and iBMS (isobutyl-methanesulfonate, AE C639170), in ethofumesate technical grade active substance.

Comment:

All methods are sufficiently validated and reported in detail in Volume 4.

ADAMA

The analytical methods are evaluated in the course of equivalence assessments to the reference source (Bayer CropScience DAR 1998).

Comment:

All methods for the determination of the active substance (including ratio of isomers), significant impurities as well as EMS and iBMS are sufficiently validated and reported in detail in Volume 4.

UPL

The analytical methods were evaluated in the course of equivalence assessments prepared by UK in August 2010, amended by DE in July 2012 and September 2012, respectively to the reference source (Bayer CropScience DAR 1998).

Comment:

All methods are sufficiently validated and reported in detail in Volume 4.

Applicability of existing CIPAC methods

The CIPAC method available for the determination of ethofumesate in technical grade active substance can be found in the FAO specification 233/TC/M/3 (CIPAC Handbook J, p.44, 2000).

B.5.1.2. Methods for risk assessment (CA 4.1.2)

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.5.1.2.1. *Section Residue*

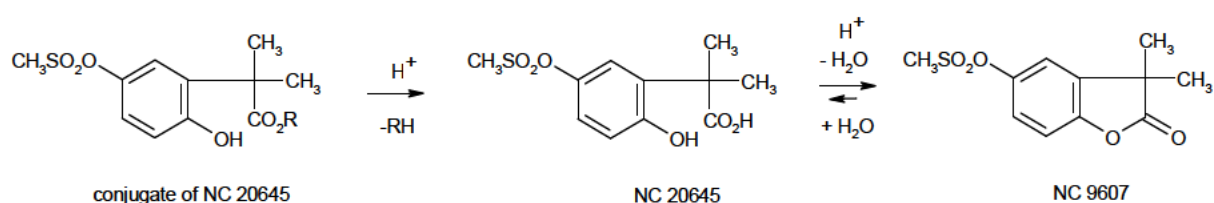
Following (non-radiolabelled) analytical methods used in risk assessment studies provided for AIR 3 procedure were evaluated in this chapter.

PLANT MATRICES

TASKFORCE

For ethofumesate residues, the agreed residue definition for risk assessment and enforcement in target plants (primary crops) and rotational crops (succeeding crops) is the sum of parent compound ethofumesate and the metabolite NC 9607 (ethofumesate-lactone), expressed in ethofumesate equivalents (cf. Reg (EU) No 149/2008 and 524/2011, as well as EFSA Journal 2010; 8(11):1901). However, monitoring of the relevant ethofumesate residue is only possible if NC 9607 is included in the residue definition as *common moiety* which comprises the determination of the metabolite NC 20645 (free and conjugated form) and metabolite NC 9607 itself. Therefore the current residue definition is not sufficiently precise and should be changed accordingly.

The common moiety NC 9607 is formed from metabolite NC 9607 (ethofumesate-lactone) itself and from the main plant metabolite, a conjugate of metabolite NC 20645 (ethofumesate-carboxylic acid), which is cleaved under acidic conditions and immediately transformed to NC 9607 due to an intramolecular condensation reaction. Minor amounts of non-conjugated metabolite NC 20645, also present in the samples, are also converted to the common moiety NC 9607.



The conditions to cleave the conjugate of metabolite NC 20645 were determined in the plant metabolism studies (cf. MCA 6.2.1). In contrast to many other conjugates formed in plants, the main conjugate in hand was quite stable and rather harsh hydrolysis conditions were necessary to release the exocon: Treatment with 6 M HCl for 6 hours at 95 °C was successful, but also less harsh conditions (3 M HCl for 1 hour at 60 °C) cleaved the conjugate (as shown by radio-analysis).

Due to the fact that the residue definition comprises the common moiety product as analytical target, all residue analytical methods for plant matrices need to include an acidic hydrolysis step.

Original Annex II submission

In the scope of the original Annex II submission in 1996, several analytical methods were provided for the determination of parent ethofumesate and its relevant metabolites (comprising metabolites NC 9607 and NC 20645, as well as a conjugate of metabolite NC 20645) in plants and plant products. The principle of all methods is based on the cleavage of the conjugate by an acidic hydrolysis step and the conversion of the formed exocon to the common moiety product NC 9607. Available amounts of non-conjugated metabolite NC 20645 are also converted to NC 9607. Parent ethofumesate and the common moiety NC 9607 are determined by GC-FPD. The limit of quantitation (LOQ) in all of the methods ranged between 0.02 mg/kg and 0.05 mg/kg for each of the two analytes. The plant matrices under investigation were sugar beet roots and tops (leaves), grass and tobacco (green and dried leaves).

The determination of the transient metabolite NC 8493 and its conjugates, which are found only in young/immature plants, was also included in some methods; however these metabolites are not part of the residue definition.

Dossier update

Additional residue analytical methods were submitted in 2005 to support the inclusion of ethofumesate in the Annex I of Directive 91/414/EEC. The methods are based on the same principle as the proceeding methods: An acidic hydrolysis is conducted to receive the common moiety, which can be analysed besides parent ethofumesate by GC-MSD. The limit of quantitation (LOQ) was 0.05 mg/kg for each of the two analytes. Peas and chickpeas were additional plant matrices under investigation.

For all studies submitted during the frame of the first Annex I inclusion please refer to the table in grey typeface below plus the corresponding section in the Monograph and in the baseline dossier (D-008920) provided by the Task Force Ethofumesate.

Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:

Report:	<u>KCA 4.1.2 /09;Manley, J. D.; Reeve, M. D.; Snowdon, P. J.;1986;M-155352-01</u>
Title:	ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES IN GRASS AND SUGARBEET (IMPROVED METHOD)
Report No:	A83075
Document No(s):	Report includes Trial Nos.: 041/01/017 <u>M-155352-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	<u>KCA 4.1.2 /10;Crofts, M.; Whiteoak, R. J.;1974;M-155262-01</u>
Title:	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET
Report No:	A82985
Document No:	<u>M-155262-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	<u>KCA 4.1.2 /11;Reary, J. B.; Browne, P. M.;1979;M-155320-01</u>
Title:	ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES IN GREEN LEAF TOBACCO
Report No:	A83043
Document No:	<u>M-155320-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	<u>KCA 4.1.2 /12;Browne, P. M.; Reary, J. B.;1980;M-155329-01</u>
Title:	ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES IN DRIED TOBACCO
Report No:	A83052
Document No:	<u>M-155329-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	<u>KCA 4.1.2 /13;Crofts, M.; Whiteoak, R. J.;1974;M-155264-01</u>
Title:	ANALYTICAL METHOD FOR NORTON RESIDUES - INTERFERENCE BY OTHER PESTICIDES
Report No:	A82987
Document No:	<u>M-155264-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	<u>KCA 4.1.2 /14;Crofts, M.;1975;M-155287-01</u>
Title:	A CONFIRMATORY METHOD FOR NORTON RESIDUES BY HIGH PRESSURE LIQUID CHROMATOGRAPH
Report No:	A83010
Document No:	<u>M-155287-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	<u>KCA 4.1.2 /15;Knoch, E.;1994;M-161444-01</u>
Title:	METHOD VALIDATION - DETERMINATION OF RESIDUES OF PHENMEDIPHAM, ETHOFUMESATE, AND THE OXO-METABOLITE OF ETHOFUMESATE IN/ON SUGAR BEET
Report No:	A87545
Document No:	<u>M-161444-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	<u>KCA 4.1.2 /16;Godfrey, T. L.;1996;M-165212-01</u>
Title:	Ethofumesate and metabolite analytical grades AE B049913 and AE C509607 (NC 8438 and NC 9607) Analytical method for the determination of active substance and major metabolite in sugar beet (roots and tops) by GC/MSD
Report No:	A89687
Document No(s):	Report includes Trial Nos.: 041/07/001 <u>M-165212-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	<u>KCA 4.1.2 /17;Wrede, A.;1997;M-165538-01</u>
Title:	Ethofumesate AE B049913 (Hoe 082551, ZK 49913) Analytical method for the determination of residues of ethofumesate and its metabolite NC 9607 (AE C509607) in sugar beets and chickpeas by GC
Report No:	A89866
Document No(s):	Report includes Trial Nos.: CR 96/021 <u>M-165538-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	<u>KCA 4.1.2 /18;Wrede, A.;2000;M-199547-01</u>
Title:	Validation of the method AL 081/96-0 in peas and sugar beet roots by GC-MSD - ethofumesate - Code: AE B049913
Report No:	C009934
Document No:	<u>M-199547-01-1</u>
Guidelines:	Deviation not specified
GLP/GEP:	yes

Report: KCA 4.1.2 /19;Fuchsbichler, G.;1989;M-463306-01
Title: Ethofumesat und 2-oxo-2,3-dihydro-3,3-dimethyl-benzofurane-5-yl-methansulfonat (ethofumesat-2-keto), Bestimmung in Zuckerrueben
Report No: HVA 10/89
Document No: M-463306-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Report: KCA 4.1.2 /20;Fuchsbichler, G.;1992;M-357851-01
Title: Ethofumesate; SC500; sugar beet; Germany; BBA
Report No: HVA 14/91
Document No(s): Report includes Trial Nos.:
FSG 3189-H-RI-A
FSG 3189-H-RI-B
FSG 3189-H-RIV-A
FSG 3189-H-RIV-B
M-357851-01-2
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

Report: KCA 4.1.2 /21;Schneider, E.;1991;M-463329-01
Title: Bestimmung von Ethofumesat und Ethofumesat-2-keto in Spinat und Mais - Gaschromatographische Methode mit massenselektivem Detektor
Report No: M-463329-01-1
Document No: M-463329-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Report: KCA 4.1.2 /22;Krebs, G.; Schneider;1992;M-463318-01
Title: Determination of ethofumesate and phenmedipham in sugar beet plants high-pressure liquid chromatography
Report No: DrK063
Document No: M-463318-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Since all residue analytical methods were only validated for parent compound ethofumesate and the common moiety NC 9607, an additional validation study was conducted in sugar beet roots to prove that the exocon NC 20645 (which is released by acidic cleavage from its conjugated form as shown in the plant metabolism studies) is transformed into the common moiety NC 9607 when applying the acidic conditions of the methods.

The recovery results for all three analytes were summarized in one report (Tew, E. L.; Cole, M.; 2001; M-237088-01) and a method number was assigned (XB/01/01) to the validation study. The report refers to data described in an additional report (Cole, M. G.; 2000; M-187353-01).

Both reports were already submitted and evaluated and are therefore included in the baseline dossier (indicated by the grey background). They are mentioned here only to allow for an easier traceability of the argumentation.

Reference:	Analytical method for the determination of Ethofumesate and its metabolites NC 9607, NC 8493 and NC 20645 in sugar beet roots and tops
Author(s), year:	Tew, E. L.; Cole, M. 2001
Report/Doc. number:	C045437/M-237088-01-1
Guideline(s):	Deviation not specified
GLP:	no

Reference:	Validation of an analytical method for the residues of NC 20645 in sugar beet roots and whole milk, USA, 1998 Code: AE C639175 00 1B97 0001
Author(s), year:	Cole, M. G.;2000
Report/Doc. number:	C004116/M-187353-01
Guideline(s):	USEPA (=EPA): OPPTS 860.1500;Deviation not specified
GLP:	yes

The recoveries for NC 20645 show that the conversion of the exocon into the common moiety (and analytical target) is almost quantitative when using the following conditions: hydrolysis with hydrochloric acid at 80 °C for 2.5 hours (concentrated HCl is added to the aqueous phase in order to conduct the hydrolysis with approx. 6 M HCl). . The recovery results for all three compounds are summarized in table B.5.1.2.1-1.

An independent laboratory validation confirmed the conversion of the exocon NC 20645 to the common moiety NC 9607 and therefore the feasibility of the method for all constituents of the residue definition (Eckert, J. A.;2001; M-240796-01). However, according to guidance document SANCO 3029/99 no ILV is required.

Conclusion

The additional validation experiment showed that the method is capable to analyse for all constituents of the residue definition and is therefore valid for data generation and risk assessment

New data for AIR (in black typeface):

Reference:	Formation of 2-keto-ethofumesate (AE C509607) by acidic extraction of plant matrices containing open-ring-2-keto-ethofumesate (AE C520645) - (sugar beet (leaf), sugar beet (body), orange (fruit), wheat (grain))
Author(s), year:	Schulte, G.; 2013
Report/Doc. number:	MR-13/061/ M-459805-01
Guideline(s):	not specified, in agreement with <ul style="list-style-type: none"> – EU Guidance Document for residue analytical methods SANCO/825/00 rev. 8.1 – EU Guidance Document for residue analytical methods SANCO/3029/99 – OECD Guidance Document on pesticide residue analytical methods ENV/JM/Mono (2007)
GLP:	yes

Analytical method used:

Reference:	Analytical method 00955/M002 for the determination of ethofumesate and its metabolite AE C509607 in three different plant groups (sugar beet, leaf and body and orange)
Author(s), year:	Konrad S. 2012
Report/Doc. number:	M-438402-01-1
Guideline(s):	REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16 OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/ Mono (2007); 2007-08-13 US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method;not specified
GLP:	yes

Residues of ethofumesate and its metabolite AE C509607 were determined by GC-MS according to method 00955/M002. The analytical method 00955/M002 is evaluated below.

Reference:	ANALYTICAL METHOD FOR RESIDUE IN SUGAR BEET TREATED WITH NORTRON
Author(s), year:	Whiteoak, R. J.; Crofts, M.; Harris, R. J.; 1973
Report/Doc. number:	A83491/ M-155727-01
Guideline(s):	Deviation not specified
GLP:	no

Reference:	ANALYTICAL METHOD FOR RESIDUES IN SUGARBEET TREATED WITH NORTRON
Author(s), year:	Whiteoak, R. J.; Crofts, M.; Harris, R. J.; 1976
Report/Doc. number:	A83492/ M-155728-01
Guideline(s):	Deviation not specified
GLP:	no

Principle of method

Both residue analytical methods were developed as data collection methods for the determination of ethofumesate (parent compound), the metabolite NC 8493 in free or conjugated form, metabolite NC 9607, and as well as the conjugate of its ring-open form NC 20645 in/on plant material of sugar beets.

Ethofumesate related residues are extracted in a two-part process and quantified using GC-FPD in the sulphur mode. In a first step, the non-conjugated residues are extracted from plant material by maceration using a mixture of methanol/dichloromethane, which is subsequently washed by water. The organic extract is separated from the aqueous phase and subjected to a clean-up step. Since metabolite NC 8493 is not amenable to direct determination by GC analysis, an acetylation step has to be conducted (if the analysis for the transient metabolite NC 8493 is not omitted). The acetylation does not affect either the parent or metabolite NC 9607.

In a second step, the aqueous phase and the remaining matrix are heated with a methanol/water mixture to liberate bound residues and to extract the water soluble conjugates. Addition of hexane reduces frothing. The supernatant is subjected to an acidic hydrolysis. The sample is boiled under reflux with hydrochloric acid (approx. 6 M HCl in final hydrolysis solution) for 75 minutes to cleave the conjugates of NC 20645 and NC 8493. Under the acidic conditions, the common moiety NC 9607 is formed from the exocon NC 20645 by an intramolecular condensation. The residues are extracted with diethyl ether. The dried organic phase is subjected to a silica gel column and the percolate is concentrated in a Kundera-Danish evaporator before acetylation (only needed if the transient metabolite NC 8493 has to be determined) and GLC analysis.

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Recovery data have been obtained concurrently to the analysis of several field samples.

Control samples were fortified with either ethofumesate, NC 9607 or NC 8493. Because the conjugates of NC 20645 and NC 8493 have not been unambiguously identified, it was not possible to synthesise them. Recovery data for these conjugates are therefore obtained by spiking the aqueous extracts before acid hydrolysis with the non-conjugated chemicals (in case of NC 20645, the ring-closed form NC 9607 was spiked). The exocons cannot be added earlier because of the difference in solubility compared to the conjugates. Evidence for

the efficiency of extraction of the conjugates is based on the metabolism studies with radiolabelled active substance. The recoveries for the available reference compounds are summarized in Table B.5.1.2.1-1.

Except for the conjugated form of NC 8493 (which was a minor metabolite in mature plants only), mean recoveries for all analytes ranged between 88.2% and 97.2%. However, the variation of the single values was rather pronounced as indicated by the high standard deviations.

Limit of quantification

The limit of quantification (LOQ), was according to the lowest level of fortification and differs from analyte to analyte and matrix. Please refer to table B.5.1.2.1-1.

Conclusions

Methods RESID/73/18/1 & 2 meet the performance requirements to determine the relevant ethofumesate residues (comprising ethofumesate, the metabolite NC 9607, its ring-open form NC 20645 and the respective conjugate) in plant materials, with an LOQ of 0.02 mg/kg. Mean recoveries were all in an acceptable range for the relevant analytes, however the single recoveries showed a rather high variability with values below 70% and above 110%. To improve the variation of the single recoveries for all analytes, the method was slightly modified.

Reference:	ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES IN SUGAR BEET (IMPROVED METHOD)
Author(s), year:	Manley, J. D.; Snowden, P. J.; 1984
Report/Doc. number:	A83493/M-155729-01-1
Guideline(s):	Deviation not specified
GLP:	no

Principle of method

Residue analytical method RESID/84/42 was developed as data collection methods for the determination of ethofumesate (parent compound), the metabolite NC 8493 in free or conjugated form, metabolite NC 9607, and as well metabolite NC 20645 in free and conjugated form in/on plant matrices of sugar beets. In principle, it is based on the procedure applied in method RESID/73/18/1 and RESID/73/18/2 but contains some modifications.

Ethofumesate related residues are extracted in a two-part process and quantified using GC-FPD in the sulphur mode. In a first step, the non-conjugated residues are extracted from plant material by maceration using a mixture of methanol/dichloromethane. The extract is filtered, washed with water, dried and concentrated. Since metabolite NC 8493 is not amenable to direct determination by GC analysis, an acetylation step has to be conducted (if the analysis for the transient metabolite NC 8493 - which was mainly found in immature plants - is not omitted). The acetylation does not affect either the parent or metabolite NC 9607.

In a second step, the aqueous phase and the remaining matrix are heated with water for one hour. Hexane can be added to reduce frothing, if necessary. The liquid phase is filtered warm through a Buchner funnel and the solids are rinsed with methanol. The combined filtrates are subjected to an acidic hydrolysis step. The sample is boiled

under reflux with hydrochloric acid (approx. 6 M HCl in the final hydrolysis solution) for 75 minutes to cleave the conjugates of NC 20645 and NC 8493. Under the acidic conditions, the common moiety NC 9607 is formed from the exocon NC 20645 by an intramolecular condensation. The residues are extracted with diethyl ether. The dried organic phase is subjected to a silica gel column and the percolate is concentrated in a Kundera-Danish evaporator before acetylation (only needed if the transient metabolite NC 8493 has to be determined) or an additional clean-up step using a Florisil column. The residues are eluted with a mixture of dichloromethane and ethyl acetate, concentrated in a Kundera-Danish evaporator and re-dissolved in ethyl acetate after addition of the GLC marker compound for GLC analysis.

Validation

Specificity

Chromatogram of untreated roots contains residues of 0.012 mg/kg ethofumesate but not detectable for NC 9607. This can be accepted if the LOQ is 0.05 mg/kg for ethofumesate.

Linearity

A curve of the form $y = ax^n$ (where n is 1.5 to 2.0) is applicable over the tested range of 1 to 5 µg/mL ethofumesate, NC 9607 and NC 8493 (measured as NC 8906) i.e. approximately 5 to 25 ng (or equivalent, for NC 8493). No calibration curve is provided.

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates in 82 tests conducted during April and May 1984. Control samples were fortified with either ethofumesate, NC 9607 or NC 8493.

When testing conjugate recoveries, fortifications were made using the corresponding 'free' metabolites NC 9607 or NC 8493, as the specific conjugated materials are not available for this purpose. These tests therefore assume that the acid hydrolysis of conjugates is quantitative, based on radiolabelled studies, as well as a quantitative transformation of metabolite NC 20645 to the common moiety NC 9607 occurs.

The mean recoveries per sample matrix and analyte were between 70-110% and the relative standard deviation was <20%. Any apparent residues in the respective control (untreated) samples were subtracted to give the results summarized in Table B.5.1.2.1-1.

Limit of quantification

The peak obtained from a 1 µg standard of any of the analytes gives the smallest peak which can be measured with reasonable accuracy; such a peak represents a residue of 0.03 mg/kg and corresponds therefore to the limit of detection. This level was also used as the lowest fortification level. A level of 0.05 mg/kg was considered to be an appropriate limit of quantification for all compounds determined. A LOQ is questionable since mostly 1 sample is investigated at single fortification levels.

Precision (repeatability)

The precision and repeatability of the method can be assessed on the basis of the determined relative standard deviations (RSD) for the mean values of the recovery rates for each analyte.

In total 6 to 11 recoveries were conducted per analyte at 3 to 6 fortification levels using method RESID/84/42 for the sugar beet matrices immature plant, mature sugar beet root and mature sugar beet leaves (tops). The determined overall RSD values for all sugar beet-related matrices ranged between 5.1% and 17.1% per analyte.

Conclusion

Method RESID/84/42 meets the performance requirements to determine the relevant ethofumesate residues (comprising ethofumesate, the metabolite NC 9607, its ring-open form NC 20645 and the respective conjugate) in plant materials. Mean recoveries over all fortifications (0.07 to 0.33 (or 1.0) mg/kg) were all in an acceptable range for the relevant analytes in all matrices. The mean RSD values in sugar beet related matrices were below 20% for all analytes. According to guidance document 3029/99 a minimum of 3 samples have to be analysed. This is not the case for any fortification. Therefore the recoveries achieved in this study based on a insufficient method validation.

Subsequent to the first Annex I inclusion, additional data has been generated to validate the already submitted method 00955 (see Wrede, A.; 1997; M-165538-01) in additional plant matrices. For this purpose, a modification of the method was prepared (method 00955/M002, see Konrad, S.; 2012; M-438402-01). In a separate study (Schulte, G.; 2013; M-459805-01) data has been generated to prove that metabolite NC 20645 is converted to the common moiety NC 9607 and can be analysed as such.

In addition, some of the residue analytical methods applied in the supervised residue studies (Whiteoak, R. J.; et al.; 1973; M-155727-01 ; Whiteoak, R. J.; et al.; 1976; M-155728-01 and Manley, J. D.; Snowdon, P. J.; 1984; M-155729-01) - used to support the intended uses in sugar beet, fodder beet and beetroot – have never been submitted since they were preliminary methods being superseded by a final method (see Manley, J. D.; et al.; 1986; M-155352-01) which was submitted to support the Annex I inclusion of ethofumesate. The principle of the method did not change significantly, however the methods are briefly described here to confirm their validity.

In addition to the analytical methods used in the supervised residue studies, an analytical method was evaluated to monitor the storage stability of metabolite NC 20645 in different plant matrices.

Reference:	STORAGE STABILITY OF OPEN-RING-2-KETO ETHOFUMESATE (AE C520645) IN PLANT MATRICES FOR 24 MONTHS - PHASE REPORT AFTER 6 MONTHS
Author(s), year:	Schulte, G.; 2013
Report/Doc. number:	M-459806-01
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Analytical method 01343 for the determination of residues of open-ring-2-keto-ethofumesate (AE C520645) in/on plant matrices by HPLC-MS/MS - Method for storage stability
Author(s), year:	Schulte, G.;2013
Report/Doc. number:	MR-12/056/M-448288-01
Guideline(s):	European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection, 2004-03-17 US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method;not specified
GLP:	yes

Principle of method

Analytical method 01343 was developed for the determination of ethofumesate-carboxylic acid (NC 20645) in/on plant materials. NC 20645 was extracted from sugar beet leaf (high water content), sugar beet root (high starch content), rape seed (high oil content), bean pod (high protein content), and orange fruit (high acid content) with acetonitrile/water (4/1, v/v) using a shaker. After filtration of the extract, the sodium salt of the stable isotopically labelled metabolite NC 20645 was added as internal standard. The internal standard is hydrolyzed during analysis to the corresponding phenyl-13C6 NC 20645. The solution was made up to volume, filtered and subjected to reversed phase HPLC-MS/MS in negative ion mode without further clean-up. Residues were quantified using an internal stable labelled standard.

ValidationSpecificity

Apparent residues in control samples were below 30% of the LOQ. The recoveries were not corrected for interferences. Two MRM transitions were monitored for NC 20645 in each plant matrix tested: m/z 272.8 → 148.8 for quantitation and m/z 272.8 → 134.9 for confirmation. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

Linearity

The internal standard procedure, using stable isotopically labelled internal standard was used for calibration. During each set of analyses, a calibration curve was established for the analyte with at least five concentration levels in each matrix in the range between 0.460 mg/L and 18.5 mg/L. The correlation coefficients of the 1/x weighted linear regressions were always above 0.99.

Accuracy

The method was validated by conducting recovery experiments with five plant materials, representing the different commodity categories.

Recovery rates were determined for five replicates per sample material spiked with the sodium salt of metabolite NC 20645 at 0.01 mg NC 20645/kg (=LOQ), and 0.1 mg NC 20645/kg. The mean recovery value per spiking level ranged from 93% to 102% (n=5) for rape seed, from 87% to 99% (n=5) for sugar beet leaf, from 82% to

99% (n=5) for sugar beet root, from 88% to 94% (n=5) for bean pod, and from 90% to 93% for orange fruit (considering the mass transition for quantification).

For confirmation of the individual residues a 2nd MRM transition was used. Results of the confirmation procedure showed that the mean recovery rates were also between 70 to 110% and the RSD were below 20% at fortification levels of 0.01 and 0.1 mg/kg for all tested materials.

LOQ

The lowest fortification level, corresponding to the limit of quantification (LOQ), was 0.01 mg analyte/kg for all tested matrices.

Repeatability

Full sets of validation recovery determinations (5 repetitions at 2 fortification levels) were run using method 01343 for five different plant matrices, demonstrating satisfactory repeatability of the method (RSDs between 1.6-6.3% for all matrices, n=5 per fortification level and matrix). Thus, the RSD values for each matrix were well below 20%, thus the precision/repeatability of the method can be considered to be acceptable. The results are summarised in Table B.5.1.2.1-1.

Stability of analytes

The stability of NC 20645 (spiked as the sodium salt of NC 20645) in plant extracts was investigated in the conduct of the present method validation for all matrices. The analytical solutions were stored in a refrigerator ($4 \pm 3^{\circ}\text{C}$) and were reanalysed after a storage period of 9 to 15 days. The results obtained for stability in all matrices are given in the table below. These data demonstrate that the residues in the analytical solutions are stable for at least 9 and up to 15 days under refrigerated conditions.

Stability of NC 20645 in plant extracts

Sample material	Days after first measurement	Fortification level [mg/kg]	Recovery rate [%]							RSD [%]	Deviation [%]	
			individual values					min	max			mean
Rape, seed	0	0.1	95	100	107	102	104	95	107	102	4.4	11
	15	0.1	87	93	94	95	83	83	95	90	5.7	
Sugar beet, leaf	0	0.1	99	101	97	103	97	97	103	99	2.6	8-9
	14	0.1	94	88	91	88	92	88	94	91	2.9	
Sugar beet, body	0	0.1	95	99	100	102	97	95	102	99	2.7	9.7
	13	0.1	89	89	89	85	93	85	93	89	3.2	
Bean, pod	0	0.1	91	91	93	98	99	91	99	94	4.1	3.2
	9	0.1	96	96	96	96	103	96	103	97	3.2	
Orange, fruit	0	0.1	93	94	95	93	91	91	95	93	1.6	3.4
	9	0.1	94	100	98	97	93	93	100	96	3.0	

Fortification as: NC 20645* Determination as: NC 20645 Calculated as: NC 20645

* NC 20645 (ethofumesate-carboxylic acid) is available as the corresponding sodium salt (BCS-CU88901)

Quantifier Mass Transition (272.8 - 148.8)

In general, the stability of the residues during the whole analytical procedure is proven by performing concurrent recovery experiments with each sample set.

The stability of NC 20645 (sodium salt = BCS-CU88901) in stock and standard solutions was tested in the conduct of the respective storage stability study (Schulte, G.; 2013; M-459806-01).

Conclusion

Residue method 01343 was validated for the metabolite NC 20645 by conducting recovery experiments with plant matrices representing the five different crop commodities. The data presented in this chapter are applicable to the crops covered in this dossier (sugar beets). All results are in accordance with the general requirements for residue analytical methods. Therefore, the analytical method 01343 has been successfully validated.

Reference:	Ethofumesate and lenacil suspension concentrate 300 + 120 g/l AE B049913 02 SC 37 A101 and AE B049913 02 WP42 A101 Ethofumesate and lenacil SC compared with a WP formulation in sugar beet; determination of residues in sugar beet roots and tops following one pre-emergence application; Italy, 1995;
Author(s), year:	Helgers, A.;1997, Amended: 1997-02-27
Report/Doc. number:	M-165366-02-1
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Ethofumesate and metabolite analytical grades AE B049913 and AE C509607 (NC 8438 and NC 9607) Analytical method for the determination of active substance and major metabolite in sugar beet (roots and tops) by GC/MSD
Author(s), year:	Godfrey, T. L.; 1996
Report/Doc. number:	A89687/M-165212-01-1
Guideline(s):	Deviation not specified
GLP:	yes

Principle of method

After extraction of ethofumesate and NC 9607 by homogenisation with acetone, water is added to facilitate the removal of the acetone, leaving an aqueous extract. Addition of base allows the partition of ethofumesate into hexane prior to clean-up through a silica Sep-pak cartridge and determination by gas chromatographic mass selective detection (GC/MSD). The metabolite NC 9607 remaining in the aqueous extract and the solids from the acetone extraction are then hydrolysed with acid to liberate the metabolite from conjugation. After partition into diethyl ether the extracts are cleaned-up by elution through a florisil gravity column before determination by GC/MSD.

Validation

Specificity

GC-MS in TIC (total ion chromatogram) mode was used for ethofumesate and the metabolite NC 9607

Linearity

A curve of the form $y = ax^2 + bx + c$ (2nd order quadratic, forced through the origin) is applicable over the tested range of 1.0 to 10.0 µg/mL ethofumesate and NC.

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

LOQ

0.05 mg/kg both analytes

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Subsequent to the first Annex I inclusion, additional data has been generated to validate the already submitted method 00955 (see Wrede, A.;1997; M-165538-01) in additional plant matrices. For this purpose, a modification of the method was prepared (method 00955/M002, see Konrad, S.; 2012; M-438402-01). In a separate study (Schulte, G.; 2013; M-459805-01) data has been generated to prove that metabolite NC 20645 is converted to the common moiety NC 9607 and can be analysed as such.

In addition, some of the residue analytical methods applied in the supervised residue studies (Whiteoak, R. J.; et al.; 1973; M-155727-01 ; Whiteoak, R. J.; et al.; 1976; M-155728-01 and Manley, J. D.; Snowdon, P. J.; 1984; M-155729-01) - used to support the intended uses in sugar beet, fodder beet and beetroot – have never been submitted since they were preliminary methods being superseded by a final method (see Manley, J. D.; et al.; 1986; M-155352-01) which was submitted to support the Annex I inclusion of ethofumesate. The principle of the method did not change significantly, however the methods are briefly described here to confirm their validity.

Reference:	Amendment no. 1 to report no: 10-2109 - Determination of the residues of ethofumesate in/on sugar beet after spray application of ethofumesate SC 500 in the field in Spain, Italy and Greece
Author(s), year:	Schulte, G.; 2013 Amended: 2013-07-09
Report/Doc. number:	M-444836-02
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Analytical method 00955/M002 for the determination of ethofumesate and its metabolite AE C509607 in three different plant groups (sugar beet, leaf and body and orange)
Author(s), year:	Konrad S. 2012
Report/Doc. number:	M-438402-01-1
Guideline(s):	REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16 OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/ Mono (2007); 2007-08-13 US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method;not specified
GLP:	yes

Principle of method

Residue analytical method 00955/M002 was developed as a data collection method for the determination of the residues of ethofumesate (parent compound) and the common moiety NC 9607 (which comprises the metabolites NC 9607 and NC 20645 and conjugates of NC 20645) in/on plant matrices.

Ethofumesate related residues are extracted in a two-part process and quantified using GC-MS. In a first step, the residues are extracted under reflux from plant material using a mixture of ethyl acetate/n-hexane. The organic extract is separated from the aqueous phase and the remaining matrix and dried with anhydrous sodium-sulphate. In a second step, the aqueous phase and the remaining matrix are subjected to an acidic hydrolysis. The sample is boiled under reflux with hydrochloric acid (approx. 6 M HCl in final hydrolysis solution) for one hour to release bound residues and to cleave the conjugates of NC 20645. Under the acidic conditions, the common moiety NC 9607 is formed from the exocon NC 20645 by an intramolecular condensation. After pH adjustment, the common moiety is extracted using again a mixture of ethyl acetate/n-hexane. The dried organic phases from both extraction steps are combined and subjected to a clean-up procedure (silica gel cartridge followed by a C18-cartridge). The final eluate is re-dissolved in ethyl acetate for GC-MS analysis.

Extraction, hydrolysis and clean-up procedures of the original method 00955 were followed exactly, however additional washing steps might be included during the clean-up procedure. In addition, the initial weight of the samples and the final sample volume for analysis can be adapted, if applicable.

Validation

Specificity

Apparent residues in control samples were below $0.3 \times \text{LOQ}$.

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with either ethofumesate or NC 9607 at concentrations of 0.01 and 0.1 mg/kg. Mean recoveries per fortification level for ethofumesate for all matrices were in a range of 77-102%, using the primary conditions ($m/z = 286$ amu). For NC 9607, good recoveries (85-109%) were achieved at the fortification level of 0.01 mg/kg for all matrices ($m/z = 256$ amu) and at the fortification level of 0.1 mg/kg for the sugar beet matrices (77-81%). The recovery levels were significantly lower for orange, and the mean recovery accounted for 61% only. However, considering the mean of the two fortification levels, recoveries above 70% were achieved for all matrices. Moreover, no use of ethofumesate in orange is registered or intended and therefore no data collection method is needed for this matrix.

Although this method is used only for data collection in Europe, three ions were monitored for ethofumesate (m/z 286, 207 or 161*) and the common moiety NC 9607 (m/z 256 and 177 or 121**) in each matrix tested; one ion was used for quantification and one of the two additionally monitored ions was used for confirmation.

Linearity

The linearity of the detector response was tested for ethofumesate and the common moiety NC 9607 in pure solvent and standard in matrix over the range of 10/ 12.5 ng/L to 1000 ng/mL ($n = 7$). A good linear relation between injected amount and peak areas was observed over the entire range. The correlation coefficient of the regression line was between 0.9878 and 0.9919 for standard in solvent, weighed 1/x. Correlation coefficients for the matrix matched standards were in the same range or even better. Therefore it was decided to use matrix matched standards ($n = 5$) for all matrices in order to compensate all possible matrix effects.

Plots of the graphs and equation parameters are available.

Limit of quantification

The lowest fortification level, corresponding to the limit of quantification (LOQ), was 0.01 mg/kg for all tested matrices.

Precision (repeatability)

The precision and repeatability of the method can be assessed on the basis of the determined relative standard deviations (RSD) for the mean values of the recovery rates.

Full sets of validation recovery determinations (5 repetitions at 2 fortification levels) were run using method 00955/M002 for sugar beet leaves and sugar beet body, and as well for orange fruit, demonstrating satisfactory repeatability of the method (RSDs between 0.6-16.6%, $n=5$) for the relevant sugar beet matrices. The RSDs for both components in orange ranged between 5.9-28.4%; however only the RSD for the common moiety NC 9607 at a fortification level of 0.1 mg/kg was above the required level of 20%.

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

The data for NC 9607 in orange at 10 x LOQ are not according to the guideline, however, matrix orange is not a representative use.

Reference:	Amendment No. 1 to Report No: 10-2501 - Determination of the residues of ethofumesate in/on the field rotational crop barley, carrot, lettuce and wheat after spray application of ethofumesate SC 500 on sugar beet and soil in the field, in the Netherlands, Italy, Spain and Germany
Author(s), year:	Schulte, G.; Diehl, P.;2013 Amended: 2013-09-13
Report/Doc. number:	M-463906-02
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Analytical method 00955/M002 for the determination of ethofumesate and its metabolite AE C509607 in three different plant groups (sugar beet, leaf and body and orange)
Author(s), year:	Konrad S. 2012
Report/Doc. number:	M-438402-01-1
Guideline(s):	REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
	European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99
	Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16
	OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/ Mono (2007); 2007-08-13
	US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method;not specified
GLP:	yes

The analytical method 00955/M002 is evaluated above.

UPL

Reference:	DETERMINATION OF THE STORAGE STABILITY OF ETHOFUMESATE AND ITS METABOLITE NC20645 IN SUGAR BEET MATRICES DURING STORAGE AT < OR = TO -18°C FOR A PERIOD OF 12 MONTHS
Author(s), year:	Hamberger, R. (2013)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	
Author(s), year:	De Bredelaar
Report/Doc. number:	BV Study R04-16-NF-09/ GAB-IFU Project Number 20031150/06-RSB
Guideline(s):	
GLP:	yes

Principle of method

Sugar beet specimens (whole young plants with roots, leaves, tops and roots) were extracted with acetone. Water was added to the extract and the solvent evaporated to the aqueous remainder. The aqueous phase was made alkaline, resulting in conversion of NC9607 into its open form (opening of the cyclic ester, this is metabolite NC20645). In the following extraction step with hexane, only Ethofumesate was extracted, whereas the metabolite NC20645 remain in the alkaline aqueous phase. The hexane extract was dried over sodium sulphate and the volume of the sample reduced using a rotary evaporator and finally brought to a defined volume with n-hexane.

The remaining plant material from the acetone extraction step and the alkaline aqueous phase from the hexane extraction step were combined. The sample was adjusted to a neutral pH, 10 % acetone was added and the sample cooked under reflux. The sample was filtered and the filtrate subjected to an acid hydrolysis step. The conjugated bound residues of the metabolite were converted into their free form at this step. Finally, the hydrolyzed sample matrix was applied onto a Lichrolut EN solid phase cartridge and the analytes eluted with a methanol/acetonitrile mixture. After evaporation, the eluate was brought to a defined volume with ethyl acetate.

Final solutions 1 (containing Ethofumesate) and 2 were combined; an additional SPE cleanup with EnviCarb™ was made at this step and analyzed using capillary gas chromatography with mass selective detection (GC-MSD) Column: Phenomenex ZB-35, 30 m x 0.25 mm i.d., 0.25 µm film thickness Part No. 7HG-G003-11:

MS (EI-SIM): Ethofumesate : m/z 207.0 (quantifier), 286.0 (qualifier 1) 161.0 (qualifier 2); NC20645 determined as NC9607: 149.0 (quantifier) 150.0 (qualifier 1) 256.0 (qualifier 2)

ValidationSpecificity

MS detection using 3 fragmentation ions for each analyte. Analysis of control specimens used for recovery experiments of sugar beet whole plant with roots (early growth stage), leaves with tops and roots with GC/MS using three characteristic fragment ions with m/z > 100 per analyte yielded no significant residues of Ethofumesate and its metabolites above 30 % of the LOQ indicating that no significant interferences were present.

Linearity

The linearity of the detector response was confirmed by injecting five matrix-matched standard solutions covering the working range of 30 µg/L to 1500 µg/L with correlation coefficients of $r^2 \geq 0.995$ (respectively $r \geq 0.997$). Calibration graphs and equations are available.

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

LOQ

0.1 mg/kg

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable for the LOQ mentioned although no recoveries/validation of a second fortification validated was found.

Reference:	Frozen storage stability of residues of ethofumesate metabolite NC 20645 in sugar beet (roots and tops with leaves)
Author(s), year:	Schlewitz P. (2014)
Report/Doc. number:	No R B1312
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Validation of the Method of Analysis of the residues of Ethofumesate and its metabolite 2-keto ethofumesate (free and conjugated form) in Sugar Beets
Author(s), year:	Perny A. (2002)
Report/Doc. number:	AO019
Guideline(s):	
GLP:	yes

Principle of method

Free residues are extracted from crop samples with a dichloromethane/methanol mixture. Conjugated residues are extracted from the residual filter cake by digestion with aqueous methanol followed by acidic hydrolysis to yield free metabolite. Extracts are purified by liquid partitions followed by chromatography on a Florisil column. Residues are determined by GC-MSD.

Ions monitored: m/z 149; 177; 207; 161 Ions used for quantitation (m/z): 207 (ethofumesate); 177 (2-keto-ethofumesate)

Validation

Specificity

The specificity of the method can be assessed on the basis of the absence of interference observed on the control samples used for spiking and analyzed in duplicate within this study. No chromatograms are available to demonstrate this.

Linearity

The method was found to be linear in the concentration range 125 ng/ml to 2500 ng/ml. (squared coefficient of correlation > 0.99) for both analytes. No graph, levels, and equation are available to demonstrate this.

Accuracy

A good accuracy was observed for both analytes and both matrices, since average recoveries were well into the range 70-110 % for two fortification levels. (see Table B.5.1.2.1-1).

LOQ

0.05 mg/kg both analytes

Repeatability

The repeatability of the method, estimated as relative standard deviation obtained on the fivefold determination at each spiking level, was well below 20 % (see tables above). (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned. However, amendments concerning specificity and linearity/calibration are required.

Reference:	Determination of residues of Ethofumesate, Phenmedipham and Desmedipham after one application of Ethofol 500SC or three applications of Betasana Trio SC in sugar beet (outdoor) at 4 sites in Northern Europe 2009
Author(s), year:	Tandy, R. (2012a)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Validation of the Method of Analysis of the residues of Ethofumesate and its metabolite 2-keto ethofumesate (free and conjugated form) in Sugar Beets
Author(s), year:	Perny A. (2002)
Report/Doc. number:	A0019
Guideline(s):	
GLP:	yes

The analytical method A0019 is evaluated above.

Reference:	Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Northern Europe (The Netherlands, Belgium) – 2012
Author(s), year:	Huauilmé, J.-M. (2013a)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	ANALYTICAL PHASE Report - Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Northern Europe (The Netherlands, Belgium) – 2012
Author(s), year:	Hamberger, R. (2012)
Report/Doc. number:	
Guideline(s):	Regulation (EC) 1107/2009; Guidance document SANCO/3029/99 rev. 4; Guidance document SANCO 825/00 rev.8.1
GLP:	yes

Principle of method

The analytical method was fully validated in a separate validation study performed by CIP Chemisches Institut Pforzheim GmbH under study directorship of Rene Hamberger (study No. 11A04042-01-VMSB) according to SANCO guidelines 3029/99 rev. 4 and 825/00 rev.8.1. For sugar beet whole plant with roots (early growth stage), this validation was already extended in BIOTEK Agriculture study BPL 11/380/GC (CIP Phase ID: 11804042-01- RASB) because of having higher residues than 10 fold LOQ in treated field specimens.

The data presented in this report demonstrate that the used method permits the determination of residues of Ethofumesate and its metabolites NC8493, NC9607 and NC20645 in sugar beet whole plant with roots (early growth stage), leaves with tops and roots with specificity, accuracy, precision and repeatability.

The metabolite NC20645 is converted to NC9607 within the analytical method and was finally detected as NC9607. As it is not possible to differentiate these two metabolites at the end, recoveries were done separately for NC20645 in the separate validation study (study No. 11A04042-01-VMSB) to show that the conversion is working.

Including a hydrolysis step, also conjugated residues were extracted.

Three characteristic fragment ions with $m/z > 100$ per analyte ($m/z = 207, 286, 161$ for Ethofumesate, $m/z = 149, 150, 256$ for NC9607, $m/z = 258, 179, 137$ for NC8493) were used.

Validation

Specificity

Analysis of control specimens used for recovery experiments of sugar beet whole plant with roots (early growth stage), leaves with tops and roots with GC/MS using three characteristic fragment ions with $m/z > 100$ per analyte yielded no significant residues of Ethofumesate and its metabolites above 30 % of the LOQ indicating that no significant interferences were present.

Linearity

The calibration graphs for Ethofumesate and its metabolites NC8493 and NC9607 were linear within the range from 30 µg/L to 1500 µg/L with correlation coefficients of $r^2 \geq 0.993$ (respectively $r \geq 0.996$).

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

LOQ

0.01 mg/kg in sugar beet roots for ethofumesate and NC8493, 0.005 mg/kg for NC 9607 (NC 20645).

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Northern Europe (The Netherlands, Belgium) – 2011
Author(s), year:	Chevallier, E. (2012)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Analytical phase Report - Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Northern Europe (The Netherlands, Belgium) – 2011
Author(s), year:	Hamberger, R. (2012)
Report/Doc. number:	
Guideline(s):	Regulation (EC) 1107/2009; Guidance document SANCO/3029/99 rev. 4; Guidance document SANCO 825/00 rev.8.1
GLP:	yes

Principle of method

The analytical method was fully validated in a separate validation study performed by CIP Chemisches Institut Pforzheim GmbH under study directorship of Rene Hamberger (study No. 11A04042-01-VMSB) according to SANCO guidelines 3029/99 rev. 4 and 825/00 rev.8.1. For sugar beet whole plant with roots (early growth stage), this validation was already extended in BIOTEK Agriculture study BPL 11/380/GC (CIP Phase ID: 11804042-01- RASB) because of having higher residues than 10 fold LOQ in treated field specimens.

The data presented in this report demonstrate that the used method permits the determination of residues of Ethofumesate and its metabolites NC8493, NC9607 and NC20645 in sugar beet whole plant with roots (early growth stage), leaves with tops and roots with specificity, accuracy, precision and repeatability.

The metabolite NC20645 is converted to NC9607 within the analytical method and was finally detected as NC9607. As it is not possible to differentiate these two metabolites at the end, recoveries were done separately for NC20645 in the separate validation study (study No. 11A04042-01-VMSB) to show that the conversion is working.

Including a hydrolysis step, also conjugated residues were extracted.

Three characteristic fragment ions with $m/z > 100$ per analyte ($m/z = 207, 286, 161$ for Ethofumesate, $m/z = 149, 150, 256$ for NC9607, $m/z = 258, 179, 137$ for NC8493) were used.

Validation

Specificity

Analysis of control specimens used for recovery experiments of sugar beet whole plant with roots (early growth stage), leaves with tops and roots with GC/MS using three characteristic fragment ions with $m/z > 100$ per analyte yielded no significant residues of Ethofumesate and its metabolites above 30 % of the LOQ indicating that no significant interferences were present.

Linearity

The calibration graphs for Ethofumesate and its metabolites NC8493 and NC9607 were linear within the range from 30 µg/L to 1500 µg/L with correlation coefficients of $r^2 \geq 0.993$ (respectively $r \geq 0.996$).

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

5 replicates are performed only for sugar beet whole plant (early growth stage) at the highest fortification level because of having higher residues than 10 fold LOQ in treated field specimens. The validation (performed in the separate validation study by CIP Chemisches Institut Pforzheim GmbH under study directorship of René Hamberger, study No. 11A04042-01-VMSB) is reported above.

LOQ

The LOQ of 0.01 mg/kg in sugar beet roots for ethofumesate and NC8493, 0.005 mg/kg for NC 9607 (NC 20645) was set in study Hamberger R. (2012) 11A04042-01-VMSB as reported above.

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Determination of residues of ETHOFUMSATE and ETHOFUMESATE-2-KETO, after one or three applications of ETHOFOL 500SC, or three application of BETASANA TRIO SC in sugar beet (outdoor) at 5 sites in Northern Europe and 5 sites in Southern Europe 2010
Author(s), year:	Tandy, R. (2013)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Validation of the Method of Analysis of the residues of Ethofumesate and ist metabolite 2-keto ethofumesate (free and conjugated form) in Sugar Beets
Author(s), year:	Perny A. (2002)
Report/Doc. number:	No A0019
Guideline(s):	
GLP:	yes

The analytical method A0019 was already evaluated above.

Reference:	Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Southern Europe (Italy, Spain)-2012
Author(s), year:	Huauilmé, J.-M. (2013b)
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Analytical method used:

Reference:	Analytical phase Report - Magnitude of residue of Ethofumesate and metabolites in sugar beet raw agricultural commodities after one foliar application of Ethofumesate 500 g/L SC – 4 trials (2 harvest trials and 2 decline curve trials) Southern Europe (Italy, Spain)-2012
Author(s), year:	Hamberger, R. (2012)
Report/Doc. number:	
Guideline(s):	Regulation (EC) 1107/2009; Guidance document SANCO/3029/99 rev. 4; Guidance document SANCO 825/00 rev.8.1
GLP:	yes

Principle of method

The analytical method was fully validated in a separate validation study performed by CIP Chemisches Institut Pforzheim GmbH under study directorship of Rene Hamberger (study No. 11A04042-01-VMSB) according to SANCO guidelines 3029/99 rev. 4 and 825/00 rev.8.1. For sugar beet whole plant with roots (early growth stage), this validation was already extended in BIOTEK Agriculture study BPL 11/380/GC (CIP Phase ID: 11804042-01- RASB) because of having higher residues than 10 fold LOQ in treated field specimens.

The data presented in this report demonstrate that the used method permits the determination of residues of Ethofumesate and its metabolites NC8493, NC9607 and NC20645 in sugar beet whole plant with roots (early growth stage), leaves with tops and roots with specificity, accuracy, precision and repeatability.

The metabolite NC20645 is converted to NC9607 within the analytical method and was finally detected as NC9607. As it is not possible to differentiate these two metabolites at the end, recoveries were done separately

for NC20645 in the separate validation study (study No. 11A04042-01-VMSB) to show that the conversion is working.

Including a hydrolysis step, also conjugated residues were extracted.

Three characteristic fragment ions with $m/z > 100$ per analyte ($m/z = 207, 286, 161$ for Ethofumesate, $m/z = 149, 150, 256$ for NC9607, $m/z = 258, 179, 137$ for NC8493) were used.

Validation

Specificity

Analysis of control specimens used for recovery experiments of sugar beet whole plant with roots (early growth stage), leaves with tops and roots with GC/MS using three characteristic fragment ions with $m/z > 100$ per analyte yielded no significant residues of Ethofumesate and its metabolites above 30 % of the LOQ indicating that no significant interferences were present.

Linearity

The calibration graphs for Ethofumesate and its metabolites NC8493 and NC9607 were linear within the range from 30 µg/L to 1500 µg/L with correlation coefficients of $r^2 \geq 0.993$ (respectively $r \geq 0.996$).

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

5 replicates are performed only for sugar beet whole plant (early growth stage) at the highest fortification level because of having higher residues than 10 fold LOQ in treated field specimens. The validation (performed in the separate validation study by CIP Chemisches Institut Pforzheim GmbH under study directorship of René Hamberger, study No. 11A04042-01-VMSB) is reported above.

LOQ

The LOQ of 0.01 mg/kg in sugar beet roots for ethofumesate and NC8493, 0.005 mg/kg for NC 9607 (NC 20645) was set in study Hamberger R. (2012) 11A04042-01-VMSB as reported above.

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Evaluation of Ethofumesate Herbicide Residues Crop Rotation Study, Cereal, Root and Leafy Vegetable Crops Following Sugar Beet - One Application to Two Trials Initiated in 2012 - NEU (the United Kingdom) and SEU (Italy)
Author(s), year:	Spence, Ch. (2014)
Report/Doc. number:	34890/697614
Guideline(s):	
GLP:	yes

Reference:	Analytical phase Report - Evaluation of Ethofumesate Herbicide Residues Crop Rotation Study, Cereal, Root and Leafy Vegetable Crops Following Sugar Beet - One Application to Two Trials Initiated in 2012 - NEU (the United Kingdom) and SEU (Italy)
Author(s), year:	Hamberger, R. (2014)
Report/Doc. number:	
Guideline(s):	Regulation (EC) 1107/2009; Guidance document SANCO/3029/99 rev. 4; Guidance document SANCO 825/00 rev.8.1
GLP:	yes

Principle of method

The analytical method was fully validated in a separate validation study performed by CIP Chemisches Institut Pforzheim GmbH under study directorship of Rene Hamberger (study No. 11A04042-01-VMSB) according to SANCO guidelines 3029/99 rev. 4 and 825/00 rev.8.1. The data presented in this report demonstrate that the used method permits the determination of residues of Ethofumesate and its metabolites NC9607 and NC20645 in carrot leaves + roots, radish leaves + roots, spinach, cereal forage, cereal grain, cereal hay, cereal straw with specificity, accuracy, precision and repeatability.

The metabolite NC20645 is converted to NC9607 within the analytical method and was finally detected as NC9607. As it is not possible to differentiate these two metabolites at the end, recoveries were done separately for NC20645 in the separate validation study (study No. 11A04042-01-VMSB) to show that the conversion is working.

Including a hydrolysis step, also conjugated residues were extracted.

Three characteristic fragment ions with $m/z > 100$ per analyte ($m/z = 207, 286, 161$ for Ethofumesate, $m/z = 149, 150, 256$ for NC9607, $m/z = 258, 179, 137$ for NC8493) were used.

Validation

Specificity

Analysis of control specimens used for recovery experiments with GC/MS using three characteristic fragment ions with $m/z > 100$ per analyte yielded no significant residues of Ethofumesate and its metabolites above 30 % of the LOQ indicating that no significant interferences were present.

Linearity

The calibration graphs for Ethofumesate and its metabolite NC9607 were linear within the range from 10 µg/L to 1000 µg/L with correlation coefficients of $r^2 \geq 0.99$ (respectively $r \geq 0.995$)

Accuracy

Mean recovery rates were between 70 to 110% (see Table B.5.1.2.1-1).

5 replicates are performed only for sugar beet whole plant (early growth stage) at the highest fortification level because of having higher residues than 10 fold LOQ in treated field specimens. The validation (performed in the

separate validation study by CIP Chemisches Institut Pforzheim GmbH under study directorship of René Hamberger, study No. 11A04042-01-VMSB) is reported above.

LOQ

LOQ was 0.01 mg/kg for each analyte for root and leafy vegetable matrices, 0.05 mg/kg for each analyte for cereal matrices.

Repeatability

The RSD were below 20% (see Table B.5.1.2.1-1).

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Table B.5.1.2.1-1: Recovery results and relative standard deviations from risk assessment method validation concerning plant matrices

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n			
Cole, M. G.;2000 M-187353-01 Taskforce	Ethofumesate	GC-FPD	Sugar beet (roots)	0.05	0.05	89	8.9	4			
					0.08	-	-	1			
			Sugar beet (tops)	0.05	0.05	88	8.9	5			
					0.5	100	2.5	3			
	NC 9607		Sugar beet (roots)	0.05	0.05	91	8.0	4			
					0.08	-	-	1			
					0.16	-	-	1			
			Sugar beet (tops)	0.05	0.05	103	13.2	5			
					4.0	83	4.1	3			
					overall: 90	7.5	5				
	NC 20645		Sugar beet (roots)	0.05	0.05	83	-	2			
					0.1	77	-	2			
					1.0	69	-	2			
			Sugar beet (tops)	0.05	0.05	83	-	2			
4.0		83			4.1	3					
overall: 89		9.0			6						
Whiteoak, R. J.; Crofts, M.; Harris, R. J.; 1973 M-155727-01 Whiteoak, R. J.; Crofts, M.; Harris, R. J.;1976 M-155728-01 Taskforce	Ethofumesate	RESID/73/18/1 GC-FPD	Sugar beet (roots)	0.05	0.05 – 0.50	90.1	28.1	44			
			Sugar beet (tops)	0.02	0.02 – 0.25	90.5	17.7	39			
	NC 8493		Sugar beet (roots)	0.05	0.05 – 0.20	90.1	12.5	21			
			Sugar beet (tops)	0.05	0.05 – 0.10	97.2	11.6	22			
	NC 9607		Sugar beet (roots)	0.05	0.05 – 0.50	88.2	17.3	40			
			Sugar beet (tops)	0.02	0.02 – 0.25	89.5	15.1	40			
	NC 8493 (addition of NC 8493)		Sugar beet (roots)	0.05	0.05 – 0.10	61.2	17.5	20			
			Sugar beet (tops)	0.10	0.10 – 0.20	68.7	25.6	23			
			NC 20645 ((addition of NC 9607)	Sugar beet (roots)	0.05	0.05 – 0.50	89.8	19.4	36		
				Sugar beet (tops)	0.05	0.05 – 2.00	93.8	20.1	44		
			Manley, J. D.; Snowdon, P. J.;1984 M-155729-01-1 Taskforce	Ethofumesate	RESID/84/42 GC-FPD	Sugar beet, immature plant	-	0.13	92	15.3	6
								0.17			
0.33											
1.00											
Sugar beet body	-	0.03		91	12.7	9					
		0.07									
		0.10									
		0.03									
		0.07									
		0.10									

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
					0.17			
				0.33				
			Sugar beet leaves	-	0.03	92	17.1	11
					0.07			
					0.10			
					0.17			
			0.33					
	NC 8493 (free form)		Sugar beet, immature plant	-	0.07	92	11.4	6
			0.10					
			0.17					
			0.33					
			1.00					
	NC 8493 (conjugated form)		Sugar beet, immature plant	-	0.07	94	8.2	6
			0.17					
			0.33					
	NC 9607 (free form)		Sugar beet, immature plant	-	0.07	100	5.1	6
					0.10			
					0.17			
					0.33			
					1.00			
					0.07			
				0.07				
				0.17				
			Sugar beet leaves	-	0.03	83	8.8	9
					0.07			
					0.13			
					0.17			
					0.33			
	NC 20645 (conjugated form)		Sugar beet body	-	0.03	88	15.1	7
					0.07			
					0.17			
			Sugar beet leaves	-	0.03	90	14.7	8
					0.07			
					0.17			
					0.33			

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n	
Schulte, G.; 2013 M-459806-01 and MR-12/056/M- 448288-01 Taskforce	NC 20645 determined as NC 20645 calculated as NC 20645	LC-MS/MS m/z = 272.8 -> 148.8	Rape (seed)	0.01	0.67				
					1.00				
					0.01	93	6.3	5	
					0.1	102	4.4	5	
			Sugar beet (leaf)	0.01	0.01	87	4.3	5	
					0.1	99	2.6	5	
			Sugar beet (roots)	0.01	0.01	82	5.2	5	
					0.1	99	2.7	5	
			Bean (pod)	0.01	0.01	88	3.5	5	
					0.1	94	4.1	5	
			Orange (fruit)	0.01	0.01	90	4.9	5	
					0.1	93	1.6	5	
			LC-MS/MS m/z = 272.8 -> 134.9	Rape (seed)	0.01	0.01	90	7.6	5
						0.1	95	3.2	5
		Sugar beet (leaf)		0.01	0.01	97	3.6	5	
					0.1	94	2.1	5	
		Sugar beet (roots)		0.01	0.01	82	4.4	5	
					0.1	92	3.6	5	
		Bean (pod)		0.01	0.01	91	4.1	5	
					0.1	94	2.8	5	
Orange (fruit)	0.01	0.01	92	3.4	5				
		0.1	92	2.8	5				
Helgers, A.; 1997 M-165366-02-1 and Godfrey, T. L.; 1996 M-165212-01-1 Taskforce	ethofumesate	GC-MS (TIC)	sugar beet roots	0.05	0.05	87	7.9	5	
					0.2	79	0.8	3	
			sugar beet tops	0.05	0.05	81	6.2	5	
					0.2	71	1.4	4	
	NC 9607		sugar beet roots	0.05	0.05	79	11.9	5	
					0.2	105	17.5	3	

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Schulte, G.; 2013 M-444836-02 Konrad S. 2012 M-438402-01-1 Taskforce			sugar beet tops	0.05	0.05	112	9.3	5
					0.2	91	2.5	3
	ethofumesate	00955/M002 GC-MS quantification (m/z = 286 amu)	Sugar beet leaf	0.01	0.01	85	3.7	5
					0.1	80	7.8	5
			Sugar beet body	0.01	0.01	102	9.6	5
					0.1	80	6.9	5
			Orange	0.01	0.01	83	5.9	5
					0.1	77	18.4	5
		00955/M002 GC-MS confirmation (m/z = 207 amu)	Sugar beet leaf	0.01	0.01	82	5.3	5
					0.1	81	8.4	5
			Sugar beet body	0.01	0.01	91	12.5	5
					0.1	81	7.0	5
			Orange	0.01	0.01	94	6.8	5
					0.1	77	17.5	5
	NC 9607	00955/M002 GC-MS quantification (m/z = 256 amu)	Sugar beet leaf	0.01	0.01	109	0.6	5
					0.1	77	6.9	4*
			Sugar beet body	0.01	0.01	93	16.6	5
					0.1	81	4.7	4*
			Orange	0.01	0.01	83	10.6	5
					0.1	61	28.4	5
		00955/M002	Sugar beet leaf	0.01	0.01	102	8.8	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n	
		GC-MS confirmation (m/z = 177 amu)			0.1	73	7.6	4*	
			Sugar beet body	0.01	0.01	93	17.4	5	
					0.1	82	5.5	4*	
			Orange	0.01	0.01	84	10.6	5	
					0.1	63	30.5	5	
Hamberger, R. (2013) UPL	ethofumesate	GC-MS	Sugar beet whole plant with roots (early growth stage)	0.1	0.1	82	13.1	8	
			Sugar beet leaves with tops	0.1	0.1	86	11.5	8	
			Sugar beet roots	0.1	0.1	77	12.3	8	
	NC20645 detected as NC 9607		Sugar beet whole plant with roots (early growth stage)	0.1	0.1	83	14.8	8	
			Sugar beet leaves with tops	0.1	0.1	74	11.7	8	
			Sugar beet roots	0.1	0.1	88	9.9	8	
Schlewitz P. (2014) No R B1312 Tandy, R. (2012a)	ethofumesate	GC-MS	Sugar beet leaves	0.050	0.050	96.0	2.9	5	
					0.500	78.7	1.5	5	
			Sugar beet	0.050	0.050	95.7	3.1	5	

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Perny A. (2002) A0019 UPL	NC20645 extraction for free analyte		roots		0.500	102.3	3.7	5
			Sugar beet leaves	0.050	0.050	90.9	2.8	5
					0.500	85.6	1.1	5
			Sugar beet roots	0.050	0.050	84.7	11.0	5
					0.500	75.5	9.1	5
	NC20645 extraction for conjugated analyte		Sugar beet leaves	0.050	0.050	74.9	6.1	5
					0.500	100.3	8.8	5
			Sugar beet roots	0.050	0.050	75.2	2.7	5
					0.500	80.5	6.3	5
Huaulmé, J.-M. (2013a) Hamberger, R. (2013) UPL	ethofumesate	GC-MS	Sugar beet whole plant with roots (early growth stage)	0.01	0.01	78	13.0	5
					0.1	103		1
					100	82	6.3	5
			Sugar beet leaves with tops	0.01	0.01	87	16.8	6
			Sugar beet roots	0.01	0.01	90	8.7	5
	NC 9607 and NC20645 detected as NC 9607		Sugar beet whole plant with roots (early growth stage)	0.005	0.005	94	9.0	5
					0.05	85		1
					2.5	87	6.3	5
			Sugar beet leaves with tops	0.005	0.005	86	6.2	6
			Sugar beet roots	0.005	0.005	91	16.7	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
	NC8493		Sugar beet whole plant with roots (early growth stage)	0.01	0.01	94	2.1	5
					0.1	88		1
					5	103	3.6	5
			Sugar beet leaves with tops	0.01	0.01	91	10.9	6
			Sugar beet roots	0.01	0.01	91	6.5	5
Chevallier, E. (2012) Hamberger, R. (2012) UPL	ethofumesate	GC-MS	Sugar beet whole plant with roots (early growth stage)	0.01	0.01	88	-	2
					0.1	105	-	1
					100	104	12.2	5
			Sugar beet leaves with tops	0.01	0.01	92	8.6	3
			Sugar beet roots	0.01	0.01	99	4.8	3
	NC20645 detected as NC 9607		Sugar beet whole plant with roots (early growth stage)	0.005	0.005	83		2
					0.05	70		1
					2.5	86	11.8	5
			Sugar beet leaves with tops	0.005	0.005	92	4.1	3
			Sugar beet roots	0.005	0.005	86	10.9	3
	NC8493		Sugar beet whole plant with roots (early growth stage)	0.01	0.01	86	-	2
					0.1	89	-	1
					5	92	7.4	5
			Sugar beet leaves with tops	0.01	0.01	92	7.6	3

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Sugar beet roots		0.01	88	14.0	3
Huaulmé, J.-M. (2013b) Hamberger, R. (2013) UPL	ethofumesate	GC-MS	Sugar beet whole plant with roots (early growth stage)	0.01	0.01	78	13.0	5
					0.1	103	-	1
					100	82	6.3	5
			Sugar beet leaves with tops	0.01	0.01	87	16.8	6
			Sugar beet roots	0.01	0.01	90	8.7	5
	NC 9607 and NC20645 detected as NC 9607		Sugar beet whole plant with roots (early growth stage)	0.005	0.005	94	9.0	5
					0.05	85	-	1
					2.5	87	6.3	5
			Sugar beet leaves with tops	0.005	0.005	86	6.2	6
			Sugar beet roots	0.005	0.005	91	16.7	5
	NC8493		Sugar beet whole plant with roots (early growth stage)	0.01	0.01	94	2.1	5
					0.1	88	-	1
					5	103	3.6	5
			Sugar beet leaves with tops	0.01	0.01	91	10.9	6
			Sugar beet roots	0.01	0.01	91	6.5	5
Spence, Ch. (2014) Hamberger, R. (2014) UPL	ethofumesate	GC-MS	Carrot roots	0.01	0.01	107	4.5	4
					0.1	102	1.0	3
			Carrot leaves	0.01	0.01	110	3.5	4
					0.1	105	2.4	3
			Spinach mature leaves	0.01	0.01	104	6.1	7

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
					0.1	104	6.2	3
			Radish roots	0.01	0.01	105	3.0	4
					0.1	100	1.5	3
			Radish leaves	0.01	0.01	106	1.4	4
					0.1	100	5.0	3
			Cereal grain	0.05	0.05	101	3.3	5
					0.5	94	6.8	5
			Cereal forage	0.05	0.05	102	3.8	5
					0.5	96	0.9	5
			Cereal hay	0.05	0.05	98	8.9	5
					0.5	91	1.9	3
			Cereal straw	0.05	0.05	93	1.2	5
					0.5	98	2.7	5
	NC 9607 and NC20645 detected as NC 9607		Carrot roots	0.005	0.005	92	14.0	4
					0.05	89	4.3	3
			Carrot leaves	0.005	0.005	81	6.2	4
					0.05	79	11.0	3
			Spinach mature leaves	0.005	0.005	78	9.4	7
					0.05	82	2.4	3
			Radish roots	0.005	0.005	81	5.2	4
					0.05	81	5.6	3
			Radish leaves	0.005	0.005	85	4.1	4

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
					0.05	83	2.5	3
			Cereal grain	0.025	0.025	85	17.2	5
					0.25	79	15.5	5
			Cereal forage	0.025	0.025	93	8.5	5
					0.25	95	2.0	5
			Cereal hay	0.025	0.025	87	5.9	5
					0.25	85	1.2	3
			Cereal straw	0.025	0.025	86	7.6	5
					0.25	85	8.1	5

* outlier: discarded for calculation of mean and RSD

Animal matrices

TASKFORCE

For ruminant and pig matrices the sum of parent compound ethofumesate and the *metabolite NC 9607*, expressed in ethofumesate equivalents, is defined as residue definition for risk assessment (cf. Reg (EU) No 149/2008 and 524/2011). However, monitoring of the relevant ethofumesate residue is only possible if NC 9607 is included in the residue definition as *common moiety* which comprises the determination of the metabolites NC 20645 and NC 9607. Therefore the current residue definition is not sufficiently precise and should be changed accordingly.

The more precise proposal (comprising parent ethofumesate and the common moiety NC 9607 or parent ethofumesate and the metabolites NC 20645 and NC 9607) is based on the results of the cattle metabolism studies. In these studies, parent compound ethofumesate is a significant constituent of the residue in milk and edible tissues (with the exception of kidney where parent compound accounted for approx. 2% of the TRR, only) followed by the metabolites NC 20645 and NC 9607 in significant smaller quantities. In kidney, metabolite NC 20645 was detected as main constituent (approx. 71% of the TRR).

A residue definition for poultry matrices was not yet considered necessary since the calculated dietary burden of poultry to ethofumesate residues was below the trigger value of 0.1 mg/kg feed dry matter. Nevertheless, the metabolism studies in laying hen demonstrated that metabolic pathways of ethofumesate in ruminants and poultry are very similar. Thus relevant residues in all livestock can be evaluated with the same residue definition.

The metabolite patterns identified for cows and hens were consistent with the rat metabolism and ethofumesate, NC 20645 and NC 9607 are considered as the major indicator compounds in commodities of livestock origin.

Due to the fact that the metabolites NC 20645 and NC 9607 are interconvertible, both metabolites can serve as analytical target in a residue analytical method. However, in analogy to the plant residue methods, the former methods for livestock matrices include an acidic treatment which converts metabolite NC 20645 into the common moiety NC 9607 before GC analysis.

Original Annex II submission

In the scope of the original Annex II submission in 1996, analytical methods were provided for the determination of parent ethofumesate and its relevant metabolites (comprising metabolites NC 9607 and NC 20645) in cattle and poultry matrices. The methods included the necessary acidic treatment to convert metabolite NC 20645 into the common moiety, however the conversion step was never validated – recoveries are only available for parent ethofumesate and the common moiety NC 9607.

For all studies submitted during the frame of the first Annex I inclusion please refer to the tables below in grey typeface and to the corresponding section in the Monograph and in the baseline dossier (D-008920) provided by the Task Force Ethofumesate.

Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:

Report: KCA 4.1.2 /25/ [REDACTED];1977;M-155301-01
Title: RESIDUES IN MILK AND TISSUES FOLLOWING A 28-DAY FEEDING STUDY WITH ETHOFUMESATE IN DAIRY COWS - PART 1
Report No: A83024
Document No: M-155301-01-1
Guidelines: Deviation not specified
GLP/GEP: no

Report: KCA 4.1.2 /26;Whiteoak, R. J.;1990;M-155384-01
Title: GAS LIQUID CHROMATOGRAPHIC DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES IN MILK AND CATTLE TISSUES
Report No: A83109
Document No: M-155384-01-1
Guidelines: Deviation not specified
GLP/GEP: no

Report: KCA 4.1.2 /27/ [REDACTED];1975;M-155288-01
Title: INVESTIGATION OF TISSUE AND EGG RESIDUES FROM HENS FOLLOWING DIETARY INTAKE OF NC 8438 FOR 21 DAYS
Report No: A83011
Document No: M-155288-01-1
Guidelines: Deviation not specified
GLP/GEP: no

Report: KCA 4.1.2 /28/ [REDACTED] 1999;M-185949-01
Title: Review of analytical methodology for residues in edible animal products (dairy, tissues, fat and offal) Ethofumesate AE B049913
Report No: C003328
Document No: M-185949-01-1
Guidelines: Deviation not specified
GLP/GEP: no

Dossier update

Reference:	Ethofumesate-derived residues in the meat and milk of dairy cows: resulting from oral ingestion of ethofumesate
Author(s), year:	██████████;1994
Report/Doc. number:	B002201/M-237976-01
Guideline(s):	USEPA (=EPA): 171-4(j); Deviation not specified
GLP:	no

Reference:	Validation of an analytical method for the residues of NC 20645 in sugar beet roots and whole milk
Author(s), year:	Cole, M. G. 2000
Report/Doc. number:	C004116/M-187353-01-1
Guideline(s):	USEPA (=EPA): OPPTS 860.1500
GLP:	yes

Since all residue analytical methods were only validated for parent compound ethofumesate and metabolite NC 9607 in the course of the cattle feeding study (see KCA 6.4.2 /29; ██████████ 1994; M-237976-01), an additional validation study was conducted in milk as representative animal matrix to prove that the main metabolite NC 20645 is transformed into the common moiety NC 9607 when applying the acidic conditions of the method (see Cole, M. G.; 2000; M-187353-01). The recovery results for all three compounds are summarized in Table B.5.1.2.1-2.

The additional validation study was already submitted and evaluated and is therefore included in the baseline dossier (indicated by the grey typeface). It is mentioned here only with some data to allow for an easier traceability.

New data for AIR (in black typeface):

Subsequent to the first Annex I inclusion, an additional method has been developed to analyse the matrices of a new cow feeding study conducted in the US. The method description and validation is attached to the report of the cow feeding study (Appendix 4).

Reference:	FREEZER STORAGE STABILITY OF ETHOFUMESATE IN ANIMAL MATRIX SAMPLES - INTERIM REPORT
Author(s), year:	Perez, R.; Schmitt, J. L.; Patel, D., 2013
Report/Doc. number:	M-467206-01, RAADP031
Guideline(s):	Residue Chemistry Test Guidelines: OPPTS 860.1380 Storage Stability Data Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160
GLP:	yes

Analytical method used:

Reference:	Ethofumesate - Magnitude of the residue in dairy cow
Author(s), year:	██████████ 2010
Report/Doc. number:	RAADP014/M-388797-01-1
Guideline(s):	OPPTS 860.1480; DACO 7.5.1; not specified
GLP:	yes

Principle of method

Residue analytical method AD-001-A10-02 was developed as a data collection method for the determination of the relevant residues of ethofumesate (comprising parent compound and the metabolites NC 9607 – measured as NC 20645 – and NC 20645 itself, as well as the minor metabolite NC 8493) in cattle matrices by LC-MS/MS. This current LC-MS/MS method is a simplification of the prior GC-MS residue method (described in the first feeding study, see KCA 6.4.2 /03; ██████████ 1994; M-237976-01). The extraction techniques are essentially identical, including the use of an acidic extraction mixture for milk samples and the direct extraction of muscle, fat and liver with ethyl acetate or methanol, respectively.

The extract solutions were fortified with a mixture of isotopic internal standards and the solvent was evaporated. The evaporated residue was dissolved in hexane and extracted three times with a mixture of acetonitrile/100 mM aqueous KOH solution (3:1 v/v). The extracts were evaporated and cleaned up on a solid phase extraction cartridge. The combined effluents from the cartridge were evaporated to near dryness and dissolved in a mixture of 10 mM aqueous NH_4HCO_3 solution/ acetonitrile (9:1 v/v). Due to the basic conditions metabolite NC 9607 was converted into metabolite NC 20645, which was the analytical target. The resultant samples were analyzed by high performance liquid chromatography-electrospray ionization /tandem mass spectrometry (LC-MS/MS). For each analyte, two mass transitions were detected, one for quantification (primary conditions) and one for confirmation purposes.

Validation**Specificity**

Although this method was used for data collection, two mass transitions were monitored for each analyte: ethofumesate (m/z 287 → 121 and 287 → 259); NC 8493 (257 → 79 and 257 → 178) and the common moiety

NC 20645 (m/z 273 \rightarrow 194 and 273 \rightarrow 149) in each matrix tested; one mass transition was used for quantification and one for confirmation. Thus the method is highly specific for the analytes under investigation.

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with ethofumesate, NC 8493, NC 9607 or NC 20645 at different concentrations. Mean recoveries per fortification level for ethofumesate were in a range of 75-113% using the primary conditions. For NC 8493, good recoveries (75-101%) were achieved for all fortification levels and all matrices, except for liver where the recoveries were slightly lower (61 – 86%) using the primary conditions. Mean recoveries for NC 9607 ranged between 75-111% and for NC 20645 between 64-113% for all fortification levels and all matrices. The lower recoveries for NC 20645 were also detected in liver (64-86%) using the primary conditions.

Linearity

The linearity of the detector response was tested for analytical method AD-001-A10-02. The relative responses of the LC-MS/MS to ethofumesate, NC 8493, and NC 20645 (demonstrated by calibration curves) were linear over the range of 10 to 1000 ng/mL ($n=7$). The correlation coefficients of the linearity curves were all >0.995 .

Limit of quantification

The lowest fortification level, corresponding to the limit of quantification (LOQ), was 0.01 mg/kg for all tested matrices. The limit of detection was $<30\%$ of the LOQ for all analytes in milk, muscle and fat and for ethofumesate and NC 9607 in liver. LODs equal to the LOQ were determined for all analytes in kidney and for NC 8493 and NC 20645 in liver.

Precision (repeatability)

The precision and repeatability of the method can be assessed on the basis of the determined relative standard deviations (RSD) for the mean values of the recovery rates.

Different sets of validation recovery determinations (3-10 repetitions at 2 fortification levels) plus additional concurrent recoveries were run using method AD-001-A10-02 for milk, cream, whey, muscle, fat, liver and kidney demonstrating satisfactory repeatability of the method (RSDs between 1.2-26.3%) for all analytes. Mean RSDs above 20 % were only detected at the fortification level of 0.01 mg/kg in kidney (ethofumesate, NC 9607 and NC 20645) and in liver (NC 8493). Kidney and liver were the only control samples with background residues in the range of the LOQ and thus higher standard deviations can be caused by different interference. For the most important food items (milk, cream, whey, muscle and fat) the recoveries ranged between 80-113% and the RSDs between 1.7-17.8% and were therefore in an acceptable range and thus the precision/repeatability of the method can be considered to be confirmed.

Stability of analytes

During a storage period of 3 months under deep-freezer conditions, ethofumesate, NC 9607 (determined as NC 20645) and NC 20645 were stable in muscle, liver, kidney, fat and milk. In liver, metabolite NC 8493

(which is not a constituent of the residue definition) was found to readily convert to NC 9607 or NC 20645, both of which are detected and measured by the analytical method as NC 20645. Since the common moiety NC 20645 is included in the residue definition, stability for NC 8493 in liver is addressed by measuring NC 20645 (which is a constituent of the residue definition) in the stored liver samples, if existent. These results validate the residue values reported in the cattle feeding study with respect to storage stability of samples frozen prior to analysis.

Reproducibility – independent laboratory

Not required for data collection methods.

Conclusion

Method AD-001-A10-02 meets all necessary performance requirements to determine ethofumesate residues (comprising parent compound, metabolite NC 8493 and metabolites NC 9607 and NC 20645, both analysed as NC 20645) in cattle matrices, with an LOQ of 0.01 mg/kg. The method is valid for data collection and risk assessment.

The recovery results are summarized in Table B.5.1.2.1-2

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Table B.5.1.2.1-2: Recovery results and relative standard deviations from risk assessment method validation concerning animal matrices

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
1994; M-237976-01 Cole, M. G.; 2000; M-187353-01 Taskforce	ethofumesate	GC-FPD	Whole milk	0.01	0.01	87	19.3	5
					0.05	73	-	2
	NC 9607		Whole milk	0.01	0.01	99	27.6	4
					0.05	77	-	2
	NC 20645		Whole milk	0.05	0.05	76	-	2
					0.5	78	14.9	3
					1.0	68	-	2
Perez, R.; Schmitt, J. L.; Patel, D., 2013 M-467206-01 Taskforce 2010	ethofumesate	AD-001-A10-02 LC-MS/MS (ESI positive) m/z 287 → 121 (quantifier)	Cream	0.01	0.01	113	7.5	3
			Whey	0.01	0.01	101	3.6	3
			Milk	0.01	0.01	91	4.0	10
					0.05	90	11.1	3
			Muscle	0.01	0.01	83	3.2	5
					0.05	87	3.0	3
			Fat	0.01	0.01	83	17.8	5
					0.1	80	4.4	3
			Liver	0.01	0.01	95	2.2	3
					0.2	96	4.2	3
			Kidney	0.01	0.01	105	26.3	5
					1.5	75	4.1	3
	NC8493	AD-001-A10-02 LC-MS/MS (ESI negative) m/z 257 → 79	Cream	0.01	0.01	94	4.4	3
			Whey	0.01	0.01	101	3.6	3
			Milk	0.01	0.01	96	4.5	10

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
		(quantifier)			0.05	98	8.3	3
			Muscle	0.01	0.01	90	8.8	5
					0.05	92	2.2	3
			Fat	0.01	0.01	92	4.6	5
					0.1	89	1.7	3
			Liver	0.01	0.01	61	26.0	3
					0.2	86	1.2	3
			Kidney	0.01	0.01	93	16.7	5
					1.5	75	3.4	3
	NC 9607 analysed as NC 20645	AD-001-A10-02 LC-MS/MS (ESI negative) m/z 273 → 194 (quantifier)	Cream	0.01	0.01	102	3.4	3
			Whey	0.01	0.01	98	2.1	3
			Milk	0.01	0.01	96	3.2	10
					0.05	96	8.8	3
			Muscle	0.01	0.01	91	3.4	5
					0.05	98	3.1	3
			Fat	0.01	0.01	91	5.9	5
					0.1	89	4.5	3
			Liver	0.01	0.01	105	9.6	3
					0.2	98	3.1	3
			Kidney	0.01	0.01	111	23.9	5
					1.5	75	1.5	3

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
	NC 20645	AD-001-A10-02 LC-MS/MS (ESI negative) m/z 273 → 194 (quantifier)	Cream	0.01	0.01	78	8.2	3
			Whey	0.01	0.01	95	9.0	3
			Milk	0.01	0.01	96	9.0	10
					0.05	113	9.7	3
			Muscle	0.01	0.01	84	11.3	5
					0.05	102	6.0	3
			Fat	0.01	0.01	81	14.0	5
					0.1	90	1.9	3
			Liver	0.01	0.01	86	16.9	3
					0.2	64	7.9	3
			Kidney	0.01	0.01	99	28.9	5
					1.5	101	4.4	3

B.5.1.2.2. *Section Toxicology*

No new mammalian toxicity studies (dietary/gavage non-radiolabelled studies or inhalation studies) with the active substance ethofumesate were submitted/evaluated for the purpose of renewal.

B.5.1.2.3. *Section Fate and Behaviour*

Following (non-radiolabelled) analytical methods used in risk assessment studies provided for AIR 3 procedure were evaluated in this chapter:

TASKFORCE

Reference:	AE C508493 (ethofumesate-2-hydroxy): Aerobic degradation in four European soils
Author(s), year:	Traub, M., 2011
Report/Doc. number:	S11-00957, M-431094-01
Guideline(s):	OECD Test Guideline 307, 2002
GLP:	yes

The metabolite was a major metabolite in soil photolysis. Therefore, the degradation rate was investigated.

Analytical Procedures :

The study was performed with non-labeled NC 8493. Duplicate test systems were taken per sampling interval. The entire soil per flask was processed three times at ambient temperature and once under hot conditions by microwave extraction. The combined extracts were analyzed for NC 8493 residues by reversed phase high performance liquid chromatography/mass spectrometry (HPLC-MS/MS) in multiple reaction monitoring (MRM) mode using NC 8493 standards in pure solvent for calibration curve.

Principle of method

The test item was extracted from the soil with 80 mL acetonitrile/water (4/1, v/v) under ambient conditions. The suspension was shaken for at least 30 min. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 1295g for 5 minutes. The extraction was repeated two times.

The additional hot extraction of the samples was done using a microwave. The soil samples were extracted with approx. 80 mL acetonitrile/water (4/1, v/v) for 15 minutes with a microwave at 60-70°C. After this the ambient and microwave extracts were combined for the final analysis. The total extract volume was detected by weighing. About 1 – 2 mL of the supernatant was filtered over 0.45 µm single-use RC filters and transferred into a glass vial for HPLC-MS/ MS analysis.

LC conditions: column: Phenomenex Synergi Fusion-RP 80-A, 50 x 2 mm, (No. 00B-4424-B0) + 4 mm guard column; gradient mobile phase: A: Water + 0.1 % acetic acid, B: Methanol + 0.1 % acetic acid

MS/MS conditions: ESI negative; two transitions: m/z 257→78.9 (quantifier), m/z 257→63.9 (qualifier)

Soil characteristics:

Soil Designation	Soil ID	Origin
Laacher Hof AXXa	AX	Monheim, North Rhine- Westphalia, Germany
Hoefchen Am Hohenseh 4a	HH	Burscheid, North Rhine- Westphalia, Germany
Dollendorf II	DD	Blankenheim, North Rhine- Westphalia, Germany
Laacher Hof Wurmweise	WW	Monheim, North Rhine- Westphalia, Germany

The soils were different with respect to texture and physicochemical properties. The texture was sandy loam, slit loam or clay loam.

Validation

Specificity

LC-MS/MS using two transitions is highly specific to the metabolite NC 8493. Analysis of control specimens of soil showed no significant residues of NC8493 above 20% of the LOQ and that no significant interferences were present.

Linearity

A calibration curve was constructed by injecting standard solutions within the range from 0.8 ng/mL to 120 ng/mL (8-point- calibration), $r = 0.9992$. Plot and equation of the calibration curve is available.

Accuracy

The mean recoveries at each fortification level at the LOQ level and with the 20 x LOQ level (application rate) are in the range of 78.6 – 92.7 %.

LOQ

LOQ based on the lowest fortification level is 0.012 mg/kg for NC8493.

Repeatability

RSD < 20%

Validation is summarized in Table B.5.1.2.3-1.

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	AE C509607: Aerobic degradation in four European soils
Author(s), year:	Traub, M.; 2012
Report/Doc. number:	S11-00958, M-431784-01-1
Guideline(s):	OECD Test Guideline No. 307, 2002
GLP:	yes

Principle of method

The test item was extracted from the soil with 80 mL acetonitrile/water (4/1, v/v) under ambient conditions. The suspension was shaken for at least 30 min. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 1295g for 5 minutes. The extraction was repeated two times.

The additional hot extraction of the samples was done using a microwave. The soil samples were extracted with approx. 80 mL acetonitrile/water (4/1, v/v) for 15 minutes with a microwave at 60-70°C. After this the ambient and microwave extracts were combined for the final analysis. The total extract volume was detected by weighing. About 1 – 2 mL of the supernatant was filtered over 0.45 µm single-use RC filters and transferred into a glass vial for HPLC-MS/ MS analysis.

LC conditions: column: Phenomenex Synergi Fusion-RP 80-A, 50 x 2 mm, (No. 00B-4424-B0) + 4 mm guard column; gradient mobile phase: A: Water + 0.1 % acetic acid, B: Methanol + 0.1 % acetic acid

MS/MS conditions: ESI negative; two transitions: m/z 255.2→177.0 (quantifier), m/z 255.2→63.9 (qualifier)

Soil characteristics:

Soil Designation	Soil ID	Origin
Laacher Hof AXXa	AX	Monheim, North Rhine- Westphalia, Germany
Hoefchen Am Hohenseh 4a	HH	Burscheid, North Rhine- Westphalia, Germany
Dollendorf II	DD	Blankenheim, North Rhine- Westphalia, Germany
Laacher Hof Wurmweise	WW	Monheim, North Rhine- Westphalia, Germany

The soils were different with respect to texture and physicochemical properties. The texture was sandy loam, slit loam or clay loam.

Validation

Specificity

LC-MS/MS using two transitions is highly specific to the metabolite NC 20645. Analysis of control specimens of soil showed no significant residues of NC 20645 above 20% of the LOQ and that no significant interferences were present.

Linearity

Matrix matched calibration curves were constructed by injecting standard solutions within the range from 1 ng/mL to 150 ng/mL (9-point- calibration), $r > 0.99$. Plot and equation of the calibration curve is available.

Accuracy

The mean recoveries at each fortification level at the LOQ level and with the 20xLOQ level (application rate) are in the range of 94.8 – 110.1%.

LOQ

LOQ based on the lowest fortification level is 0.016 mg/kg for NC8493.

Repeatability

RSD < 20%

Validation is summarized in Table B.5.1.2.3-1.

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Ethofumesate-carboxylic acid (as potassium salt: AE C639175): Aerobic degradation in four European soils
Author(s), year:	Traub, M.; 2012
Report/Doc. number:	S11-03264; M-432551-01-1
Guideline(s):	OECD Test Guideline No. 307, 2002
GLP:	yes

Principle of method

The test item was extracted from the soil with 80 mL acetonitrile/water (4/1, v/v). The suspension was shaken for at least 30 min. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 1295g for 5 minutes. The extraction was repeated two times. The additional extraction of the samples was done using a microwave. The soil samples were extracted with approx. 80 mL acetonitrile/water (4/1, v/v) for 15 minutes at 60-70°C.

After this the ambient and microwave extracts were combined for the final analysis.

About 1 – 2 mL of the supernatant was filtered over 0.45 µm single-use RC filters and transferred into a glass vial for HPLC-MS/MS analysis.

LC conditions: column: Phenomenex Synergi Fusion-RP 80-A, 50 x 2 mm, (No. 00B-4424-B0) + 4 mm guard column; gradient mobile phase: A: Water + 0.1 % acetic acid, B: Methanol + 0.1 % acetic acid

MS/MS conditions: ESI negative; two transitions: m/z 273.0 → 193.9 (quantifier), m/z 273.0 → 78.9 (qualifier)

Soil characteristics:

Soil Designation	Soil ID	Origin
Laacher Hof AXXa	AX	Monheim, North Rhine- Westphalia, Germany
Hoefchen Am Hohenseh 4a	HH	Burscheid, North Rhine- Westphalia, Germany
Dollendorf II	DD	Blankenheim, North Rhine- Westphalia, Germany
Laacher Hof Wurmweise	WW	Monheim, North Rhine- Westphalia, Germany

The soils were different with respect to texture and physicochemical properties. The texture was sandy loam, slit loam or clay loam.

Validation

Specificity

LC-MS/MS using two transitions is highly specific to the metabolite AE C639175. Analysis of control specimens of soil showed no significant residues of AE C639175 above 20% of the LOQ and that no significant interferences were present.

Linearity

Matrix matched calibration curves were constructed by injecting standard solutions within the range from 1 ng/mL to 150 ng/mL (9-point- calibration), $r > 0.99$. Plot and equation of the calibration curve is available.

Accuracy

The mean recoveries at each fortification level [at the LOQ level and with the 22xLOQ level (application rate)] are in the range of 80.2–103.1%.

LOQ

LOQ based on the lowest fortification level is 0.319mg/kg for AE C639175 (i.e. NC 20645-potassium).

Repeatability

RSD < 20%

Validation is summarized in Table B.5.1.2.3-1.

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

UPL

Reference:	NC8493 (A Metabolite of Ethofumesate) – Aerobic Rate of Degradation in Three Soils
Author(s), year:	Malekani, K. (2013)
Report/Doc. number:	13845.6134
Guideline(s):	OECD Guideline 307, OPPTS 835.4100
GLP:	yes

Principle of method

Soil extractions were carried out using 150 mL acetonitrile:water (4:1, v:v) and the soil and solvent mixture was shaken for 1 minute and then centrifuged for approximately 10 minutes. The procedure was repeated and combined extracts were brought to a final volume of 300 mL with the extraction solvent, mixed well and the aliquots filtered.

Aliquots of the 4-hour sampling post-extracted soil (PES) samples were submitted to reflux extraction. The PES was refluxed with acetonitrile:water (4:1, v:v) for 4 hours. The cooled mixture was transferred to centrifuge bottles using acetonitrile:water (4:1, v:v) as a rinse and centrifuged for 10 minutes. The supernatant was decanted and adjusted to 175 mL with 4:1 acetonitrile:water, filtered and analysed by LC-MS/MS.

LC conditions: column: Waters Atlantis T3 50 mm x 2.1 mm, 3µm, gradient mobile phase: A: 0.2% Formic acid, 0.01% Ammonium formate in water, B: Methanol

MS/MS conditions: ESI negative; two transitions: m/z 257.3→178 (quantifier), m/z 250→150 (qualifier)

Soil Property	Fislis	Horn	Sevelen
Soil type (USDA)	Silt Loam	Loam	Sandy Loam

Detailed characteristics of the soils are reported within the study.

ValidationSpecificity

LC-MS/MS using two transitions is highly specific to the metabolite NC 8493. No representative LC/MS/MS chromatogram control specimens are provided. Only chromatograms of Sevelen soil spiked with 0.5 mg/kg NC8493 at different times are available.

Linearity

No information on calibration (range, levels correlation coefficient) is reported. Only Representative LC/MS/MS chromatograms of a calibration standards of NC8493 10 ng/mL and 200 ng/mL in sample matrix are provided (transition m/z 257.3→178).

Accuracy

No details are reported.

LOQ

LOQ is reported in the text (0.03 mg/kg) but not supported by recovery and repeatability details.

Repeatability

No details are reported.

Conclusion

The analytical method cannot be evaluated since no details on validation parameters according to guidance document SANCO 3029/99 are reported within the study.

Table B.5.1.2.3-1: Validation results

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Traub, M., 2011 M-431094-01 Taskforce	NC 8493	LC-MS/MS	Soil 1 AX	0.012	0.012	92.3	0.5	5
					0.289	82.7	1.8	5
			Soil 2 HH	0.012	0.012	85.4	1.6	5
					0.289	80.8	1.8	5
			Soil 3 DD	0.012	0.012	77.2	2.6	5
					0.289	76.1	2.8	5
			Soil 4 WW	0.012	0.012	85.4	1.4	5
					0.289	76.3	1.2	5
Traub, M., 2012; M-431784-01 Taskforce	NC 8493	LC-MS/MS	Soil 1 AX	0.016	0.016	107.1	2.8	5
					0.268	100.0	1.8	5
			Soil 2 HH	0.016	0.016	106.9	2.2	5
					0.265	104.8	0.6	5
			Soil 3 DD	0.019	0.019	89.9	3.0	5
					0.296	81.5	7.0	5
			Soil 4 WW	0.016	0.016	106.9	1.5	5
					0.277	100.1	1.1	5
Traub, M., 2012; M-432551-01-1 Taskforce	AE C639175 (i.e. NC 20645-potassium)	LC-MS/MS	Soil 1 AX	0.319	0.319	99.6	11.9	5
					0.014	95.4	3.2	5
			Soil 2 HH	0.319	0.319	85.9	6.8	5
					0.014	98	12.3	5
			Soil 3 DD	0.319	0.319	84.1	1.1	5
					0.014	91	1.0	5
			Soil 4 WW	0.319	0.319	84.6	2.0	5
					0.014	93.9	1.7	5

B.5.1.2.4. *Section Ecotoxicology*

Following (non-radiolabelled) analytical methods used in risk assessment studies provided for AIR 3 procedure were evaluated in this chapter:

TASKFORCE

Reference:	Bobwhite quail dietary reproduction study,
Author(s), year:	██████████ 2001
Report/Doc. number:	C013708
Guideline(s):	US EPA 71-4, OECD 206
GLP:	yes

In this analytical study, the homogeneity, stability and content of the test substance Ethofumesate (code: AE B049913 00 1D97 0002) in the diet were investigated.

The diet samples were derived from the Bobwhite Quail reproduction study no. 99.0060 conducted at ██████████
██████████ for chemical analysis throughout the study.

The quantification of the test substance in the diet was performed by HPLC separations (reverse phase, UV-detection) of organic solvent (acetonitrile) extracts of the test substance-diet mixtures.

For this study five mixtures of each concentration were used. The content and the homogeneity of the test substance in each diet preparation was carried out. In addition, the stability of the test substance in the diet was verified for all concentrations at the beginning of the study (first mixture).

Principle of method

Approx. 10 g of the diet samples were stirred on a magnetic stirrer with 100 ml acetonitrile in an Erlenmeyer-flask. After a sedimentation period of one hour the supernatants were diluted with acetonitrile : water = 70:30 (v:v).

Each sample was individually extracted and afterwards injected into the HPLC.

Column: ODS-Hypersil 250 mm x 4 mm, 5 µm; detection at 280 nm.

Validation

Analysis of the diet preparations showed that they were homogeneous and the achieved mean concentrations analysed directly after preparation ranged between 80 and 120 % (3 samples per concentration) of the nominal concentration and were entirely acceptable.

No information on calibration, chromatograms demonstrating the lack of interferences are available.

Conclusion

No evaluation according to guidance document 3029/99 rev.4 is possible.

Reference:	The acute toxicity of ethofumesate technical to the sheepshead minnow, (<i>Cyprinodon variegatus</i>) in a static system	
Author(s), year:		1992
Report/Doc. number:	A83384	
Guideline(s):		
GLP:	yes	

Principle of method

The aqueous sample was diluted with deionized water to bring the concentrated sample within the working range of the calibration curve. After volume adjustment, the concentration of ethofumesate was quantified by high performance liquid chromatography (HPLC) using UV detection at 230 nm.

Column: Spherisorb ODSI 5 µm particle size 150 x 4.6 mm i.d. (Keystone Scientific); mobile phase: 3:1 methanol:deionized water (v:v)

Validation

Specificity

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

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 0.25 mg/L to 5.0 mg/L (6-point- calibration), $r = 0.999$. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 4 mg/L and 50 mg/L. Two recoveries per concentration are determined. The mean over all fortifications are (n=4) 92.3%. (No sample number is mandatory in guidance document 3029/99 rev.4 for accuracy).

LOQ

4 mg/L

Repeatability

RSD = 2.4% (calculated over both fortifications)

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Zebrafish (Danio rerio), Life cycle test flow through conditions
Author(s), year:	██████ 2013
Report/Doc. number:	M-464613-01-1
Guideline(s):	
GLP:	yes

The method for the 'Determination of ethofumesate in aqueous test medium using GC-EIMS/ MS' was developed in experiments, which were completed prior to this GLP study. The validation of the analytical method was performed in compliance with GLP and according to the guidelines SANCO/825/001, and is part of this report.

The method is applicable for matrix charged water samples in concentrations above the validated LOQ of 50 µg/L; the benzofuranyl alkylsulfonate herbicide benfuresate was used as an internal standard (IS).

Principle of method

Analytical sub-samples of 1.0 or 2.0 mL volume of the water samples were taken out of the EPA vials and were pipetted into 8mL centrifuge tubes using an Eppendorf 'research 1000' pipette. Analogous sub-samples of the stock solutions with volumes of 50.0 or 250 µL were pipetted again into 8mL centrifuge tubes using a M25 or M250 Microman pipette; these samples were filled up to a total volume of 1.0 mL with purified water. Afterwards 100 µL of the IS 'working solution' and 5 mL of toluene were added for extraction; the absolute spiked IS amount was 3.0 µg. The centrifuge tubes were closed with screw caps and were then agitated vigorously by hand for approx. 0.5 min and additionally for 10 min on a horizontal shaker at about 200 strokes/min. For a rapid phase separation the tubes were centrifuged at 1500 rpm for 2 minutes. Finally, 1 mL of the toluene phases were filled into 2mL auto sampler vials; 1 µL of the toluene extracts were measured directly with GC/EI-MS/MS.

GC-column: Varian factorFOUR™ capillary column, 30 m x 0.25 mm ID, 0.10 µm VF-5ms (Varian no. CP8943)

MS/MS-conditions: TIC (Total ion current)

Validation

Specificity

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

Linearity

Ten calibration standard solutions were prepared in the concentration range from 6.0 to 1200 µg/L. The concentration of the internal standard in all calibration solutions was 600 µg/L and met the benfuresate concentration in the prepared samples. $r^2 > 0.999$. Equation and plots are available.

Accuracy

Mean recoveries are between 70 and 110 %.

LOQ

0.05 mg/L

Repeatability

RSD < 20%

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Acute toxicity of BCS-BB94377 (tech.) to the waterflea <i>Daphnia magna</i> in a static laboratory test system
Author(s), year:	Riebschläger, T., 2012
Report/Doc. number:	M-434284-02-1
Guideline(s):	
GLP:	yes

Principle of method

The samples were analysed by direct injection into an HPLC instrument or injected after appropriate dilution with test water. Gradient HPLC detection at 230 nm; column: Phenomenex Luna 3 μ Phenyl-Hexyl; length 100 mm; i.d 2.0 mm; mobile phase A: deionized water (adjusted to pH 3 with o-phosphoric acid) mobile phase B: acetonitrile

Validation

Specificity

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

Linearity

The linearity of the HPLC-UV detection was determined for BCS-BB94377 in the concentration range from 0.017 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9993

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for BCS-BB94377 was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Acute toxicity of BCS-AV65501 (tech.) to the waterflea <i>Daphnia magna</i> in a static-renewal laboratory test system
Author(s), year:	Riebschläger, T., 2012
Report/Doc. number:	M-434289-02-1
Guideline(s):	
GLP:	yes

Principle of method

The samples were analysed by direct injection into an HPLC instrument or injected after appropriate dilution with test water. Gradient HPLC detection at 230 nm; column: Phenomenex Luna 3u Phenyl-Hexyl; length 100 mm; i.d 2.0 mm; mobile phase A: deionized water (adjusted to pH 3 with o-phosphoric acid) mobile phase B: acetonitrile

Validation

Specificity

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

Linearity

The linearity of the HPLC-UV detection was determined for BCS-AV65501 in the concentration range from 0.017 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9988

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for BCS-AV65501 was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Acute toxicity of ethofumesate acid to the waterflea <i>Daphnia magna</i> in a static laboratory test system
Author(s), year:	König, N., 2013
Report/Doc. number:	M-444843-01-1
Guideline(s):	
GLP:	

Principle of method

The water samples are adjusted to pH3 with o-phosphoric acid and analysed by direct injection into the HPLC instrument or injected after appropriate dilution with test water (adjusted to pH3 with o-phosphoric acid). Identification and quantitative determination are done by means of HPLC-UV detection at 275 nm.

Column: Aqua 5µ,C18 125A , 150 mm; i.d 2.0 mm 5µm; mobile phase: A: deionized water (adjusted to pH3 with o-phosphoric acid) B: acetonitrile; A/B isocratic 50 %

Validation

Specificity

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

Linearity

The linearity of the HPLC-UV detection was determined for ethofumesate acetic acid in the concentration range from 0.0167 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9999.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for ethofumesate acetic acid was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	The acute toxicity of ethofumesate technical to the mysid shrimp, <i>Mysidopsis bahia</i> in a static system
Author(s), year:	Schupner, J.K. & Stachura, B.J., 1992
Report/Doc. number:	M-155657-01-1
Guideline(s):	
GLP:	yes

Principle of method

The aqueous sample was diluted with deionized water to bring the concentrated sample within the working range of the calibration curve. After volume adjustment, the concentration of ethofumesate was quantified by high performance liquid chromatography (HPLC) using UV detection at 230 nm.

Column: Spherisorb ODSI 5 µm particle size 150 x 4.6 mm i.d. (Keystone Scientific); mobile phase: 3:1 methanol:deionized water (v:v)

Validation

Specificity

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

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 0.25 mg/L to 4.0 mg/L (6-point- calibration), $r = 0.999$. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 4 mg/L and 50 mg/L. Two recoveries per concentration are determined. The mean over both fortifications are (n=4) 100.5 %. (No sample number is mandatory in guidance document 3029/99 rev.4 for accuracy).

LOQ

4 mg/L

Repeatability

RSD = 2.4% (calculated over both fortifications)

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Pseudokirchneriella subcapitata growth inhibition test with ethofumesate (techn.)
Author(s), year:	Bruns, E., 2010
Report/Doc. number:	M-302092-03-1
Guideline(s):	
GLP:	yes

Analytical Method 00876, Method for the determination of ethofumesate in test water from aquatic toxicity tests by HPLC-UV, Dr. B. Brumhard, Report of Bayer CropScience AG, MR-079/04 dated June 17, 2004.

Principle of method

Due to the high nominal concentration, these samples had to be diluted with test water ETX to reach the tested linearity range of the method. These diluted samples were directly injected into the HPLC-UV instrument. The dilutions for these samples are given in the following table. The injection volume was 100 µL. Each sample was injected in duplicate. Detection at 200 nm. Column: Phenomenex Aqua C18 5µ; 150 x 2 mm. Mobile phase: A: Milli-Q-Water (adjusted to pH 4 with o-phosphonic acid; B: acetonitrile; A/B 50/50 isocratic

Specificity

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

Linearity

The linearity of the UV-detector was checked for ethofumesate in the range from 1.133 µg/L to 566.5 µg/L (n = 6) with an injection volume of 500 µL. The correlation coefficient was 1.000. Plot and equation of the calibration curve is available.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

1.133 µg/L.

Repeatability

The repeatability for ethofumesate was determined based on 10 injections of a standard solution of 1.133 µg/L and 9 injections of a standard solution of 11.33 µg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Pseudokirchneriella subcapitata growth inhibition test with ethofumesate-NC8493 (AE C508493)
Author(s), year:	Bruns, E., 2012
Report/Doc. number:	M-436372-01-1
Guideline(s):	
GLP:	yes

Principle of method

The samples were analysed by direct injection into an HPLC instrument or injected after appropriate dilution with test water. Gradient HPLC detection at 230 nm; column: Phenomenex Luna 3 μ Phenyl-Hexyl; length 100 mm; i.d 2.0 mm; mobile phase A: deionized water (adjusted to pH 3 with o-phosphoric acid) mobile phase B: acetonitrile

Validation

Specificity

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

Linearity

The linearity of the HPLC-UV detection was determined for BCS-BB94377 in the concentration range from 0.017 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9993. Plot and equation of the calibration curve is available.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for BCS-BB94377 was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Pseudokirchneriella subcapitata growth inhibition test with BCS-AV65501 - limit test
Author(s), year:	Bruns, E., 2012
Report/Doc. number:	M-437568-02-1
Guideline(s):	
GLP:	yes

Principle of method

The samples were analysed by direct injection into an HPLC instrument or injected after appropriate dilution with test water. Gradient HPLC detection at 230 nm; column: Phenomenex Luna 3u Phenyl-Hexyl; length 100 mm; i.d 2.0 mm; mobile phase A: deionized water (adjusted to pH 3 with o-phosphoric acid) mobile phase B: acetonitrile

Validation

Specificity

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

Linearity

The linearity of the HPLC-UV detection was determined for BCS-AV65501 in the concentration range from 0.017 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9988. Plot and equation of the calibration curve is available.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for BCS-AV65501 was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CW35117
Author(s), year:	Sobczyk, H., 2013
Report/Doc. number:	M-459906-01-1
Guideline(s):	
GLP:	yes

Principle of method

The water samples are adjusted to pH3 with o-phosphoric acid and analysed by direct injection into the HPLC instrument or injected after appropriate dilution with test water (adjusted to pH3 with o-phosphoric acid). Identification and quantitative determination are done by means of HPLC-UV detection at 275 nm. Column: Aqua 5µ,C18 125A , 150 mm; i.d 2.0 mm 5µm; mobile phase: A: deionized water (adjusted to pH3 with o-phosphoric acid) B: acetonitrile; A/B isocratic 50 %

Validation

Specificity

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

Linearity

The linearity was determined for ethofumesate acetic acid in the concentration range from 0.0167 mg/L to 10.0 mg/L (n= 6). The correlation coefficient was 0.9999. Plot and equation of the calibration curve is available.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

LOQ

0.05 mg/L

Repeatability

The repeatability for ethofumesate acetic acid was determined based on 10 injections of a standard solution of 0.05 mg/L and 10 injections of a standard solution of 0.5 mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Toxicity of ethofumesate technical to the blue green algae <i>Anabaena flos-aquae</i>
Author(s), year:	Banman, C.S., Daly, R.A. & Lam, C.V., 2009
Report/Doc. number:	M-349150-01-1
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: Phenomenex Luna 3 μ C8(2), 50 x 2.00 mm; mobile phase: A = 1.5% acetic acid in HPLC-grade water
C = 100 % acetonitrile; MS-transitions: Ethofumesate: 287/121, Ethofumesate-ethoxy-d5: 292/122.

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 0.004- and 1.09-mg/L standard concentrations. The correlation coefficient R^2 is > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The method was validated by spiking dilution water with Ethofumesate technical at 0.008-, 0.08-, 4.12-, and 20.6-mg/L concentrations.

LOQ

0.008 mg/L

Repeatability

Repeatability is calculated for spiked samples at 0.008-, 0.08-mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Toxicity of ethofumesate technical to the ssaltwater diatom <i>Skeletonema costatum</i>
Author(s), year:	Banman, C.S., Daly, R.A. & Lam, C.V., 2009
Report/Doc. number:	M-347965-01-1
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: Phenomenex Luna 3 μ C8(2), 50 x 2.00 mm; mobile phase: A = 1.5% acetic acid in HPLC-grade water
C = 100 % acetonitrile; MS-transitions: Ethofumesate: 287/121, Ethofumesate-ethoxy-d5: 292/122.

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 0.004- and 1.09-mg/L standard concentrations. The correlation coefficient R^2 is > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The method was validated by spiking dilution water with Ethofumesate technical at 0.12-, 1.24-, 4.93-, and 20.6-mg/L concentrations.

LOQ

0.12 mg/L

Repeatability

Repeatability is calculated for spiked samples at 0.12-, 1.24-mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Toxicity of ethofumesate technical to the aquatic macrophyte, <i>Myriophyllum spicatum</i>
Author(s), year:	Banman, C.S., 2013
Report/Doc. number:	M-41145-02-1
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: Phenomenex Luna 3 μ C8(2), 50 x 2.00 mm; Mobile Phase: A = 1:9 Methanol: 10 mM Ammonium bicarbonate in HPLC-grade water, B = 9:1 Methanol: 10 mM Ammonium bicarbonate in HPLC-grade water; MS-transitions: Ethofumesate: 287/259.6, Ethofumesate-ethoxy-d5: 292/260.6.

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 0.001- and 0.54-mg/L standard concentrations. The correlation coefficient R^2 is > 0.99 . Plot and equation of the calibration curve is available.

Accuracy

The method was validated by spiking dilution water with Ethofumesate technical at 0.005, 0.05, 0.50, and 5.01 mg/L concentrations.

LOQ

0.005 mg/L

Repeatability

Repeatability is calculated for spiked samples at 0.005-, 0.05-mg/L.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Effect of ethofumesate technical on new shell growth in the eastern oyster (<i>Crassostrea virginica</i>) under flow-through test conditions
Author(s), year:	Yurk, J.J. & Ache B.W., 1992
Report/Doc. number:	M-155654-01-1
Guideline(s):	
GLP:	yes

Principle of method

An aliquot of seawater containing Ethofumesate is directly injected into the High Performance Liquid Chromatograph (HPLC) equipped with an ultraviolet (UV) detector and a computer controlled data acquisition system. Ethofumesate is quantitated by comparing computer integrated response (as peak area) of the sample to the response of a set of HPLC calibration standards using a regression equation generated from the concentration and response of the calibration standards.

Column: Vydac Ultrasphere ODS 150 x 4.6 mm, 5 μ HPLC Mobile Phase:50:50 Methanol/HPLC grade water (volume:volume). No wavelength for detection is stated.

Validation

Specificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

The preparation of 8 concentrations in the range from 0.206 to 41.0 mg/L is reported. Further is stated: *Calibrations are considered acceptable if the correlation coefficient of the curve is greater than or equal to 0.995.* However, no graph / equation / correlation coefficient is available.

Accuracy

Two fortification levels are investigated. For level 0.504 mg/L the mean recovery is 98% and for level 36 mg/L the mean recovery is 103%

LOQ

0.504 mg/L (i.e. lowest sufficiently validated fortification)

Repeatability

For level 0.504 mg/L the RSD is 1.6% and for level 36 mg/L the RSD is 1.7%.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

No wavelength for detection is stated. No graph / equation / correlation coefficient is available. The notifier is of the opinion that according to the requirements in place in 1992 the study is adequately validated and sufficient for the purpose of the study. This cannot be evaluated since guidance document 3029/99 rev.4 is published in 11/07/00 and former releases are not found.

Reference:	Ethofumesate (tech.) - Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days continuous laboratory feeding limit test
Author(s), year:	Kling, A., 2013
Report/Doc. number:	M 469458-01-1
Guideline(s):	
GLP:	yes

Reference:	Determination of Ethofumesate in Feeding Solutions
Author(s), year:	R. Schoening and R. Masoudi, 2013
Report/Doc. number:	MR-13/127
Guideline(s):	
GLP:	yes

Attached as an integral part of the study above

Principle of method

The analytical method 01116 was developed for the determination of phenmedipham, phenmedipham-MHPC, desmedipham, desmedipham-EHPC, ethofumesate, AE C509607 and lenacil in/on plant materials. The test items were extracted from 10 g sample material two times with 25 mL ethyl acetate/methanol (80/20, v/v) using a microwave oven. After filtration, the extract, was made up to volume. An aliquot of the raw extract was concentrated to dryness using a TurboVap at a temperature of 50°C and re-dissolved in 5 mL acetonitrile/water (80/20 v/v, containing 1 mL/L formic acid), ultra-sonicated. **The** solution was filtered with a minisart RC 15 **Filter** and analysed by HPLC-MS/MS for phenmedipham, phenmedipham-MHPC, desmedipham, desmedipham-EHPC, ethofumesate and lenacil.

Due to the fact that the concentration in the feeding solutions of study S13-00144 were at a very high level the samples were only diluted and determined using HPLC-MS/MS for quantitation.

HPLC column, (PhenomenexLunaC18(2)-HST 50 x 2mm, 2.5 µm with pre-column); Mobile phase A (BIN Pump): Water/Methanol (9/1, v/v) + 10mMol/L Ammonium Formate + 120µL Formic Acid

Mobile phase B {BIN Pump): Water/M ethanol (1/9, v/v) + 10mMol/L Ammonium Formate + 120µL/L Formic Acid

Mobile phase C {ISO Pump): Water/Acetonitrile (4/1, v/v)+ 0.1 mL/L Formic Acid.

MS/MS: ESI pos., two transitions: m/z: 304→121, m/z 304→161

Validation

Specificity

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 0.063 µg/L to 10.0 µg/L (n= 5). The correlation coefficients were >0.999. Plots and equation of the calibration curves are available.

Accuracy

Mean recovery rates were between 70 to 110%

LOQ

0.01 mg/kg

Repeatability

The RSD were below 20%

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

UPL

Reference:	Acute toxicity of NC 20645 to Daphnia magna in a 48-hour static test
Author(s), year:	Juckeland, D., 2013
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method

NC 20645 was analysed by HPLC-MS. MS for quantification.

Column: Phenomenex Kintex C18, 100 x 2.1 mm; isocratic mobile phase 65% A Water with 1 mL/L formic acid, 35% B Methanol with 1 mL/L formic acid and 5 mmol/L ammonium formate. Detection 200-300 nm
MS-SIM ESI pos. m/z 292 [M⁺ NH₄]⁺

ValidationSpecificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

The linearity were determined for NC 20645 in the concentration range from 3.8 mg/L to 11.4 mg/L (n= 6). The correlation coefficients were >0.999. Plot and equation of the calibration curve is available.

Accuracy

Mean recovery rates were between 70 to 110%.

LOQ

38.07 mg/L

Repeatability

The RSD were below 20%.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Acute toxicity of NC 8493 to Daphnia magna in a 48-hour static test
Author(s), year:	Juckeland, D., 2013
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method

NC 8493 was analysed by HPLC-UV-DAD.

Column: Phenomenex Synergi MAX-RP 4 μ 80A. 150 x 2.1 mm; isocratic mobile phase 65% A Water with 1 mL/L phosphoric acid, 35% B acetonitrile. Detection 200-300 nm, 226 and 280 were chosen for evaluation.

ValidationSpecificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

The linearity were determined for NC 8493 in the concentration range from 40 mg/L to 120 mg/L (n= 6). The correlation coefficient is >0.999. Plot and equation of the calibration curve is available.

Accuracy

Mean recovery rates were between 70 to 110%.

LOQ

49.9 mg/L

Repeatability

The RSD were below 20%.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Assessment of side effects of ethofumesate technical on the larvae of the midge, <i>Chironomus riparius</i> with the laboratory test method
Author(s), year:	Stäbler, D., 2003
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method

After extraction with acetonitrile/water the samples were injected directly into the HPLC system. Detection at 226 nm, column: Nucleosil 120 C18 3µm, mobile phase: water + phosphoric acid (3 mL 85% age phosphoric acid ad 1 L)/acetonitrile

ValidationSpecificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

Low range calibration (1-10 ng/injection) (n>5) and high range (10-100 ng/injection) (n>5) R>0.99 for both.

Accuracy

Mean recovery rates were between 70 to 110%.

LOQ

1 mg/L for water and pore water

30 mg/L for sediment

Repeatability

The RSD were below 20%.

Validation is summarized in Table B.5.1.2.4-1.

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Effects of NC 20645 on <i>Desmodesmus subspicatus</i> in an algal growth inhibition test under static conditions
Author(s), year:	Juckeland, D., 2013
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method

NC 20645 was analysed by HPLC-MS. MS for quantification.

Column: Phenomenex Kintex C18, 100 x 2.1 mm; isocratic mobile phase 65% A Water with 1 mL/L formic acid, 35% B Methanol with 1 mL/L formic acid and 5 mmol/L ammonium formate. Detection 200-300 nm

MS-SIM ESI pos. m/z 292 [M⁺ NH₄]⁺

ValidationSpecificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

The linearity were determined for NC 20645 in the concentration range from 0.282 mg/L to 22.98 mg/L (n= 6).

The correlation coefficients were >0.999. Plot and equation of the calibration curve is available.

Accuracy

Mean recovery rates were between 70 to 110%.

LOQ

0.34 mg/L

Repeatability

The RSD were below 20%

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	Acute toxicity of NC 8493 to Daphnia magna in a 48-hour static test
Author(s), year:	Juckeland, D., 2013
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method

NC 8493 was analysed by HPLC-UV-DAD.

Column: Phenomenex Synergi MAX-RP 4 μ 80A. 150 x 2.1 mm; isocratic mobile phase 65% A Water with 1 mL/L phosphoric acid, 35% B acetonitrile. Detection 200-300 nm, 226 and 280 were chosen for evaluation.

ValidationSpecificity

The blank value of all control samples was below 0.3 x LOQ.

Linearity

The linearity were determined for NC 8493 in the concentration range from 0.60 mg/L to 24.05 mg/L (n= 6).

The correlation coefficient is >0.999. Plot and equation of the calibration curve is available.

Accuracy

Mean recovery rates were between 70 to 110%.

LOQ

0.764 mg/L

Repeatability

The RSD were below 20%.

Validation is summarized in Table B.5.1.2.4-1

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	A 7-day aquatic plant toxicity test using Lemna minor with ethofumesate
Author(s), year:	Bogers, M., 2001
Report/Doc. number:	
Guideline(s):	
GLP:	yes

Principle of method**Validation**Specificity

No chromatograms are reported.

Linearity

Two concentrations are used for calibration. Concentrations are not reported. No plot and equation of the calibration curve is available.

Accuracy

Not according to guidance document 3029/99 rev.4

LOQ

Not according to guidance document 3029/99 rev.4

Repeatability

Not according to guidance document 3029/99 rev.4

Conclusion

Validation is not according to guidance document 3029/99 rev.4.

Table B.5.1.2.4-1: Validation results regarding section ecotoxicology

References	Analyte	Detection method	Matrix	LOQ [mg/L] or [mg/kg]	Fortification level [mg/L] or [mg/kg]	Mean recovery [%]	RSD [%]	n
1992 A83384 Taskforce	Ethofumesate	HPLC	water	4	4	92.3	2.4	2
					50			2
2013 M-464613-01-1 Taskforce	Ethofumesate	GC-MS/MS	water	0.05	0.05	91.5	7.20	5
					0.1	95.2	4.28	5
					1	100.2	1.02	4
					5	96.5	2.88	5
Riebschläger, T., 2012 M-434284-02-1 Taskforce	BCS-BB94377, Ethofumesate-2- hydroxy, NC 8493	HPLC	water	0.05	0.05	9.552*	1.6	10
					0.5	94.834*	0.4	10
Riebschläger, T., 2012 M-434289-02-1 Taskforce	BCS-AV65501, Ethofumesate- carboxylic acid, NC 20645	HPLC	water	0.05	0.05	8.541*	1.7	10
					0.5	84.971*	0.5	10
König, N., 2013 M-444843-01-1 Taskforce	Ethofumesate- acetic acid, BCS-CW35117	HPLC	water	0.05	0.05	25.827*	1.2	10
					0.5	240.76*	0.3	10
Schupner J.K. & Stachura, B.J., 1992 M-155657-01-1 Taskforce	Ethofumesate	HPLC	water	4	4	100.5	2.4	2
					50			2
Bruns, E., 2010 M-302092-03-1 Taskforce	Ethofumesate	HPLC	water	0.001	0.001	17424*	3.02	10
					0.011	175354*	1.77	9
Bruns, E., 2012 M-436372-01-1 Taskforce	BCS-BB94377, Ethofumesate-2- hydroxy, NC 8493	HPLC	water	0.05	0.05	9.552*	1.6	10
					0.5	94.834*	0.4	10

References	Analyte	Detection method	Matrix	LOQ [mg/L] or [mg/kg]	Fortification level [mg/L] or [mg/kg]	Mean recovery [%]	RSD [%]	n
Bruns, E., 2012 M-437568-02-1 Taskforce	BCS-AV65501, Ethofumesate– carboxylic acid, NC 20645	HPLC	water	0.05	0.05	8.541*	1.7	10
					0.5	84.971*	0.5	10
Sobczyk, H., 2013 M-459906-01-1 Taskforce	Ethofumesate– acetic acid, BCS-CW35117	HPLC	water	0.05	0.05	25.827*	1.2	10
					0.5	240.76*	0.3	10
Banman, C.S., Daly, R.A. & Lam, C.V., 2009 M-349150-01-1 Taskforce	Ethofumesate	HPLC	water	0.008	0.008	96.7	3.9	3
					0.08	96	9.1	3
					4.12	89	-	2
					20.6	92	-	1
Banman, C.S., Daly, R.A. & Lam, C.V., 2009 M-347965-01-1 Taskforce	Ethofumesate	HPLC	water	0.12	0.12	91	4.6	3
					1.24	91	9.4	3
					4.93	90	-	2
					20.6	92	-	1
Banman, C.S., 2013 M-41145-02-1 Taskforce	Ethofumesate	HPLC	water	0.005	0.005	105	9.4	3
					0.05	102	1.5	3
					0.50	104	-	2
					5.01	104	-	1
Yurk, J.J. & Ache B.W., 1992 M-155654-01-1 Taskforce	Ethofumesate	HPLC	water	0.504	0.504	98	1.6	3
					36.0	103	1.7	3
Kling, A., 2013 M 469458-01-1 Taskforce	Ethofumesate	HPLC-MS/MS	aqueous sucrose solution	0.01	0.01	86	4.1	5
					0.10	102	1.6	5
					200	103	2.7	2
Juckeland, D., 2013 UPL	NC 20645	HPLC-MS	water	38.07	38.07	104	1.16	5
					95.89	101	1.60	5
Juckeland, D.,	NC 8493	HPLC-MS	water	49.9	49.9	102	0.04	5

References	Analyte	Detection method	Matrix	LOQ [mg/L] or [mg/kg]	Fortification level [mg/L] or [mg/kg]	Mean recovery [%]	RSD [%]	n
2013 UPL					99.9	102	0.09	5
Stäbler, D., 2003 UPL	Ethofumesate	HPLC	water and pore water	1	1	92.5	0.9	5
					10	99.2	0.6	5
					100	87.6	1.1	5
			sediment	30	30	95.0	6	5
					300	97.0	2.7	5
					600	102	2.5	5
Juckeland, D., 2013 UPL	NC 20645	HPLC-MS	water	0.341	0.341	95	1.29	5
					19.04	102	0.45	5
Juckeland, D., 2013 UPL	NC 8493	HPLC-MS	water	0.764	0.764	100	0.3	5
					19.98	100	0.1	5

* Peak area

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES (CA 4.2)

B.5.2.1. Plant matrices

TASKFORCE

For ethofumesate residues, the agreed residue definition for risk assessment and enforcement in target plants (primary crops) and rotational crops (succeeding crops) is the sum of *parent compound ethofumesate* and the *metabolite NC 9607*, expressed in ethofumesate equivalents (cf. Reg (EU) No 149/2008 and 524/2011, as well as EFSA Journal 2010; 8(11):1901). However, monitoring of the relevant ethofumesate residue is only possible if NC 9607 is included in the residue definition as *common moiety* which comprises the determination of the metabolite NC 20645 (free and conjugated form) and metabolite NC 9607 itself. Therefore the current residue definition is not sufficiently precise and should be changed accordingly.

Since the residue analytical methods for plants have to include an acidic hydrolysis step to cleave the conjugate of NC 20645 (main metabolite in aerial plant matrices) and to convert the exocon and the non-conjugated metabolite NC 20645 to the *common moiety product NC 9607*, a multi-method like the DFG S19 or the QuEChERS method is not applicable as enforcement method. Therefore some of the methods developed for data collection and risk assessment were also presented as enforcement methods. These analytical methods were successfully validated by independent laboratory validations.

For all studies submitted during the frame of the first Annex I inclusion please refer to the tables below in grey typeface and to the corresponding section in the Monograph and in the baseline dossier provided by the Task Force Ethofumesate. New studies are summarized below (in black typeface)).

Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:

Report:	KCA 4.2 /01;Specht;1988;M-155359-01
Title:	Verification of the applicability of the DFG multi- method S 19 for the quantitative determination of residues of ethofumesate in water and beet.
Report No:	A89831
Document No:	M-155359-01-2
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 4.2 /02;Wrede, A.;1997;M-165538-01
Title:	Ethofumesate AE B049913 (Hoe 082551, ZK 49913) Analytical method for the determination of residues of ethofumesate and its metabolite NC 9607 (AE C509607) in sugar beets and chickpeas by GC
Report No:	A89866
Document No(s):	Report includes Trial Nos.: CR 96/021 M-165538-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes

Report:	KCA 4.2 /03;Reichert, N.;2000;M-199578-01
Title:	Independent laboratory validation of the method of analysis for the determination of ethofumesate and metabolite NC 9607 (AE C0509607) in sugar beet and pea
Report No:	C009953
Document No:	M-199578-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /04;Wrede, A.;2000;M-199547-01
Title:	Validation of the method AL 081/96-0 in peas and sugar beet roots by GC-MSD - ethofumesate - Code: AE B049913
Report No:	C009934
Document No:	M-199547-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /05;Schneider, E.;2000;M-351870-01
Title:	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in sugar beet roots and sugar beet leaves
Report No:	OFC00004912
Document No:	M-351870-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /06;Witte, A.;2000;M-351894-01
Title:	Residue analysis of ethofumesate and ethofumesate-2-keto in sugar beet - Independent laboratory validation (ILV)
Report No:	OFC00004913
Document No:	M-351894-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /07;Tew, E. L.; Cole, M.;2001;M-237088-01
Title:	Analytical method for the determination of Ethofumesate and its metabolites NC 9607, NC 8493 and NC 20645 in sugar beet roots and tops
Report No:	C045437
Document No:	M-237088-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 4.2 /08;Eckert, J. A.;2001;M-240796-01
Title:	Independent laboratory Validation of Aventis CropScience Method-Analytical Method for the Determination of Ethofumesate and Its Metabolites, NC 9607, MC 8493 and NC 20645 in Sugar Beet Roots and Tops
Report No:	B003792
Document No(s):	Report includes Trial Nos.: AV-01-01 M-240796-01-1
Guidelines:	USEPA (=EPA): OPPTS 860.1340(C)(6);Deviation not specified
GLP/GEP:	no

New data for AIR (in black typeface):

Reference:	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in various plant commodities
Author(s), year:	Thom, M.; 2005;
Report/Doc. number:	OFC00004832, M-351876-01-1
Guideline(s):	Sanco/825/00, rev. 7 (17/03/2004)
GLP:	yes

Reference:	Independent laboratory validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in plant commodities
Author(s), year:	Klimmek, S.; 2005
Report/Doc. number:	OFC00004833, M-351896-01-1
Guideline(s):	SANCO: 825/00, rev.7 91/414/EEC
GLP:	yes

Principle of the method:

The presented residue analytical method is a modification of method PR00/001 (Schneider, E.; 2000; M-351870-01 provided for first Annex I inclusion) which was developed as monitoring/enforcement method for the determination of the relevant residues of ethofumesate (comprising parent compound and the metabolites NC 9607 (free form) and NC 20645 (free and conjugated form) in crop commodities with high starch and high water content. The modification is able to determine the relevant residues of ethofumesate in dry crop matrices, commodities with high acid and high oil content by GC-MS. The extraction techniques are essentially identical, as in method PR00/001, but an additional degreasing step was included for oily matrices, as well as an additional solid phase clean-up step for dry commodities and commodities with high oil content.

Ethofumesate and its metabolites were extracted with acetone from red currant (commodity with high acid content) and wheat samples (dry commodity) or acetonitrile/acetone (9:1, v/v) from sunflower seed samples (commodity with high oil content). The crude extract was filtered, and the extracted filter cake was saved for analysis of the metabolites NC 9607 and NC 20645. The extracts were diluted with water and evaporated to a remaining of water. After adding 3 N KOH solution, the remaining water was separated by liquid liquid extraction with n-hexane. The water layer was added to the filter cake and used for analysis of the metabolites as described below.

The n-hexane extract was filtered through sodium sulfate, evaporated to near dryness and stored until further analysis of ethofumesate.

For determination of the metabolites, the filter cake and the water layer from liquid liquid extraction were brought to a neutral pH value by adding hydrochloric acid. After addition of acetone, this mixture was boiled with reflux. The sample was cooled down to room temperature, centrifuged and/or filtered with suction on a Buechner funnel with glass fibre filter, and the clear solution was transferred to a Schott bottle. Hydrochloric acid was added, the bottle was closed and heated to 100 °C for approx. 90 minutes. Due to the acidic conditions metabolite NC 20645 (conjugated and free form) was converted into metabolite NC 9607, which was the analytical target. The resulting extract was filtered with suction on a Buchner-funnel with glass fibre filter and the clear solution was cleaned up by solid phase extraction with LiChrolutEN. Elution was performed with acetonitrile/methanol (1:1, v/v). The eluate was collected into the concentrated n-hexane extract containing

ethofumesate. The extracts were evaporated to a remaining of solvent, adjusted to a defined volume and used for GC/MS analysis.

Wheat grain and sunflower seed samples were diluted in acetonitrile/water (3:1) and cleaned up by solid phase extraction with ENVICarb. The eluate was combined with the concentrated n-hexane extract containing ethofumesate and the samples were evaporated to a remaining of solvent, brought to a defined volume and used for GC/MS analysis.

Recovery samples were prepared by spiking homogenized control samples with diluted standard solutions of ethofumesate and the common moiety NC 9607. The fortification levels were 0.02 mg/kg and 0.2 mg/kg per matrix and analyte. Matrix-matched standards were used for quantification.

GC (EI-SIM) conditions (red currant): column: J & W Scientific DB-5 MS, 30 m x 0.25 mm, 0.5µm film thickness; selected ions: Ethofumesate-2-keto(NC 9607: m/z = 256, 177, 150; Ethofumesate: m/z = 286, 179, 161

GC (EI-SIM) conditions (wheat corn and sunflower seed): column: Varian VF-5MS (DB-5 equivalent), 30 m x 0.25 mm, 0.5µm film thickness; selected ions: Ethofumesate-2-keto (NC 9607): m/z = 256 (quantification), 177, 150; (256, 177, 149)* Ethofumesate: m/z = 286 (quantification), 179, 161 (286, 207, 179)*

* Due to interferences on mass 179 and 177 for sunflower seeds extracts determination additional confirmatory mass fragments (underlined) were used in samples at LOQ, blanks and matrix matched standard solutions.

Validation:

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with ethofumesate and NC 9607 at two different concentrations. Mean recoveries per fortification level for both analytes were in a range of 73-102%. These values are within the guideline requirements of mean recovery (70 % - 110 %).

Specificity

Due to the use of a highly specific detection system (mass selective detection of three m/z ratios above m/z= 100), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for ethofumesate and the analytical target NC 9607.

Linearity

The detector response for ethofumesate and the common moiety NC 9607 was first or second order covering a range from 7 ng/mL to at least 400 ng/mL (n ≥ 6) with correlation coefficients of r > 0.9975. Plots of the graphs and parameter of the equations are available.

Limit of quantification

The limit of quantification (LOQ) was defined as the lowest fortification level with a mean recovery between 70% and 110%, with a relative standard deviation not exceeding 20% and blanks not exceeding 30% for quantification ions. These criteria are fulfilled for the 0.02 mg/kg fortification level for the observed matrices.

Since minor interference were observed for the determination of ethofumesate in the wheat matrix and for ethofumesate and NC 9607 in sunflower seeds, a limit of quantification (LOQ) of 0.02 mg/kg resulted for both analytes in all plant matrices.

Residues in the untreated sample used for recovery experiments were not detectable (< 30% of LOQ).

Precision (repeatability)

The relative standard deviation (RSD) over all fortification levels ranged from 2% to 18%. These values were within guideline requirements of RSD (<20%).

Reproducibility – independent laboratory

An independent laboratory validation was conducted for method PR00/001 (Witte, A.; 2000; M-351894-01, provided for first Annex I inclusion) in the beet matrices root (high starch content) and leaves (high water content) showing that the general extraction procedure and the analysis by GC-MS is reproducible. This ILV was already submitted for the first Annex I listing of ethofumesate. The modified steps, as needed for commodities with high oil content, were validated in a separate ILV (Klimmek, S.; 2005; M-351896-01-1).

Samples of sunflower seeds were fortified with parent compound ethofumesate and the common moiety NC 9607 at the nominal fortification levels of 0.02 and 0.20 mg/kg, i.e. the LOQ and the 10-fold LOQ.

Analysis of samples was performed and detected according to the primary method (Thom, M.; 2005; M-351876-01-1). The mean recoveries for each fortification level and each analyte were between 73% and 85%, with relative standard deviations of < 20%. Minor interfering signals in the blank control specimens were detected, but <30% of LOQ.

Extraction efficiency:

No residues (according to the residue definition; refer to chapter B.7) above the LOQ are present in food commodities (representative use: sugar beets). Therefore there is no need to address extraction efficiency. Reference to guidance document SANCO/825/00 rev.8.1.

A summary of the validation results is given in Table B.5.2.1-1.

Conclusion:

Method PR00/001 for commodities with high starch and high water content, as well as its modification for dry commodities, commodities with high oil and high acid content meet all necessary performance requirements to determine ethofumesate residues (comprising parent compound and metabolites NC 9607 and NC 20645 (free and conjugated form) in plant matrices, with an LOQ of 0.02 mg/kg for commodities with high oil content and 0.05 mg/kg for with high starch and high water content, as well as its modification for dry commodities, and commodities with high acid content. Results of an ILV showed that the method and its modification fulfil the reproducibility requirements and are therefore applicable as an enforcement method.

However, in none of the available monitoring methods, the conversion of NC 20645 to the common moiety NC 9607 was shown. The conversion was assumed based on the results described in the transformation experiment (see Schulte, G.; 2013; M-459805-01 –B.5.1.2.1 Section Residues).

To overcome this deficiency, a new enforcement method has been developed, covering representative commodities of all four matrix groups (dry commodities, commodities with high water content, high oil content and high acid content) and as well a matrix difficult to analyse (hops). Ethofumesate residues (comprising parent ethofumesate, metabolite NC 20645 in conjugated and free form and metabolite NC 9607) are analysed by HPLC-MS/MS. All metabolites are converted to the common moiety NC 20645 prior to analysis.

Enforcement method:

Reference:	Validation of the analytical method 01392 for the determination of the relevant ethofumesate metabolites in plant matrices by HPLC-MS/MS
Author(s), year:	Schulte, G.; Diehl, P.; 2014
Report/Doc. number:	MR-13/101, M-479926-01
Guideline(s):	SANCO/3029/99 (11/07/00), SANCO/825/00, rev. 8 (16/11/2010); no deviations specified
GLP:	yes
Reference:	Independent Laboratory Validation (ILV) of the analytical method 01392 for the determination of the relevant ethofumesate metabolites in plant matrices by HPLC-MS/MS
Author(s), year:	Betson, S.; 2014
Report/Doc. number:	RL/ SN/ 2014-001
Guideline(s):	Regulation (EC) No 1107/2009; SANCO/825/00, rev. 8.1 (16/11/2010); US EPA Residue Chemistry Test Guideline OPPTS 860.1340 (1996) no deviations specified
GLP:	yes
Reference:	Ethofumesate - Discussion on the usability of plant enforcement method 01392 for metabolite AE C520645 in matrices with high oil content
Author(s), year:	Spiegel, K., 2014
Report/Doc. number:	M-497717-01
Guideline(s):	--
GLP:	no

The common moiety NC 20645 is the analytical target for metabolite NC 20645 in free and conjugated form and as well for metabolite NC 9607. The conjugate of NC 20645 was cleaved at low pH to its exocon and due to the low pH immediately transformed to NC 9607 by an intramolecular condensation reaction. In a separate step, NC 9607 (formed from the free and the conjugated form of NC 20645 at low pH and from metabolite NC 9607 itself) was converted at high pH to the common moiety NC 20645, which is the analyte target beside parent ethofumesate.

Principle of the method:

The analytical method 01392 was developed to determine the relevant residues of ethofumesate, comprising parent ethofumesate, metabolite NC 9607 and metabolite NC 20645 (open-ring-2-keto ethofumesate, in free and conjugated form). Residues of these compounds were extracted from sugar beet leaf, wheat grain, rape seed, orange fruit and hop green cone. The metabolites were converted to the common moiety NC 20645 prior to analysis. Ethofumesate and the common moiety were quantified by reversed phase HPLC with electrospray MS/MS-detection.

Metabolites NC 9607 and NC 20645 are inter-convertible and depending on the pH value of the sample solution either one or the other metabolite will predominate. This fact was utilized during sample preparation and analysis. To cleave the conjugate of metabolite NC 20645, acidic conditions are needed. Due to the acidic conditions, the formed exocon, as well as extracted amounts of free metabolite NC 20645, are immediately converted to NC 9607. This common moiety cannot easily analysed by LC-MS/MS due to lacking sensitivity. In

contrast to NC 9607, LC-MS/MS analysis of NC 20645 is the preferred analytical method. Therefore the common moiety NC 9607 was quantitatively converted to NC 20645 for following LC-MS/MS analysis.

Ethofumesate and its metabolites (NC 9607 and NC 20645 in free form) were extracted with acetone. Ethofumesate was separated from the metabolites by liquid-liquid extraction with n-heptane after pH adjustment with an aqueous sodium hydroxide solution (in the alkaline environment metabolite NC 9607 is converted to the common moiety NC 20645 and remains in the aqueous layer whereas ethofumesate is extracted in the organic solvent). For rape seed an additional clean-up step was added (liquid-liquid partitioning with acetonitrile). The n-heptane phase was evaporated to dryness and the residues were dissolved in methanol, which contained ammonium formate. Analysis of ethofumesate was done by LC-MS/MS.

The aqueous phase of the liquid-liquid extraction - containing the common moiety NC 20645 - was combined with the filter cake and the aqueous remainder of the acetone extraction. The aqueous remainder of the acetone extraction contained metabolite NC 20645 in conjugated form. Concentrated hydrochloric acid was added to the aqueous phase and the sample was boiled under reflux. Due to the acidic pH value, the conjugate of NC 20645 was cleaved and the exocon, as well as NC 20645, already present, were converted to the common moiety NC 9607. The aqueous solution was separated from the solids by filtration and extracted with ethyl acetate. A sodium hydroxide solution was added until an alkaline pH value was reached. Due to the alkaline pH value, the common moiety NC 9607 was converted to the common moiety NC 20645. The ethyl acetate phase was evaporated to dryness and the residues were dissolved in methanol, which was kept alkaline with sodium hydroxide solution and ammonium hydrogen carbonate. For residues in rape seeds, the ethyl acetate phase has to be subjected to an additional clean-up step (degreasing) before analysis. Analysis of the common moiety was done by LC-MS/MS.

Recovery samples were prepared by spiking homogenized control samples with diluted standard solutions of ethofumesate, NC 9607 and NC 20645. The fortification levels were 0.01 mg/kg and 0.1 mg/kg per matrix and analyte. Matrix-matched standards were used for quantification.

HPLC-MS/MS conditions part A (ethofumesate): column: Luna C18 (2)-HST, 5.0 cm length, 2.0 mm i.d., 2.5 µm (Phenomenex) with precolumn (e.g. SecurityGuard Cartridges, C18 4x2.0mm ID, Phenomenex) gradient mobile phase A: water/methanol (90/10, v/v) + 10mmol/L ammonium formate + 120µL/L formic acid, mobile phase B: water/methanol (10/90, v/v) + 10mmol/L ammonium formate + 120µL/L formic acid

MS/MS: ESI pos. mode

ethofumesate NH ₄ -adduct	m/z 304 → 121 for quantification,
ethofumesate NH ₄ -adduct	m/z 304 → 259 for confirmation
ethofumesate	m/z 287 → 121 for confirmation

HPLC-MS/MS conditions part B (NC 20645): column: Luna C18 (2)-HST, 5.0 cm length, 2.0 mm i.d., 2.5 µm (Phenomenex) with precolumn (e.g. SecurityGuard Cartridges, C18 4x2.0mm ID, Phenomenex) gradient mobile phase A: water/methanol (90/10, v/v) + 10mmol/L ammonium formate + 120µL/L formic acid, mobile phase B: water/methanol (10/90, v/v) + 10mmol/L ammonium formate + 120µL/L formic acid

MS/MS: ESI neg. mode

NC 20645	m/z 273 → 194 for quantification
NC 20645	m/z 273 → 149 m/z and 273 → 135 for confirmation

Specificity

The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). Three MRM transitions were selected, one for quantification and two for confirmation. The corresponding product ions were monitored for each analytical target (ethofumesate and common moiety NC 20645) and each matrix tested therefore an additional confirmatory method is not necessary. Apparent residues in control samples were always below 30% of the LOQ.

Accuracy

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with ethofumesate, NC 20645 and NC 9607 at concentrations of 0.01 and 0.1 mg/kg. Mean recoveries per fortification level for ethofumesate, NC 20645 and NC 9607 were in a range of 78-105% when monitoring the product ion for quantification. These values are within the guideline requirements of mean recovery (70 % - 110 %). The recovery results for all analytes are summarised in Table B.5.2.1-2.

Linearity

The detector response for ethofumesate and the common moiety NC 20645 was first or second order covering a range from 0.125 µg/L to 50 µg/L (multiple detection at 6 concentration levels) with correlation coefficients of $r \geq 0.999$.

Limit of quantification

The limit of quantification (LOQ), expressed in parent equivalents, was defined as the lowest fortification level with a mean recovery between 70% and 110%, with a relative standard deviation not exceeding 20% and blanks not exceeding 30% for quantification ions. These criteria are fulfilled for the 0.01 mg/kg fortification level for each analyte (parent ethofumesate and common moiety NC 20645) in all matrices under investigation.

Residues in the untreated sample used for recovery experiments were not detectable (< 30% of LOQ).

Precision (repeatability)

As a measure of the precision, the intra-laboratory repeatability (n=5) is given as the relative standard deviation (% RSD) for different sample materials at two fortification levels (LOQ and 10x LOQ). Mean relative standard deviations per fortification level ranged between 3.0-15.5% for the primary method. These values were within guideline requirements of RSD (<20%). The results are summarized in Table B.5.2.1-2.

Extraction efficiency:

No residues (according to the residue definition; refer to chapter B.7) above the LOQ are present in food commodities (representative use: sugar beets). Therefore there is no need to address extraction efficiency. Reference to guidance document SANCO/825/00 rev.8.1.

Reproducibility (ILV)

An independent laboratory validation (ILV) for method 01392 is conducted at the moment (Betson, S.; 2014) using the same plant matrices and the same fortification levels as the original method. Results of the ILV showed

that the general extraction procedure for all three analytes, as well as the analysis of parent ethofumesate and the common moiety NC 20645 by LC-MS/MS are reproducible. The product ions monitored for quantification and for confirmation were as follows:

ethofumesate m/z 287 \rightarrow 121 for quantification; m/z 287 \rightarrow 161 and 287 \rightarrow 259 for confirmation

NC 20645 m/z 273 \rightarrow 79 for quantification; m/z 273 \rightarrow 149 m/z and 273 \rightarrow 194 for confirmation

Specificity

HPLC-MS/MS using three characteristic MS/MS transitions for quantitation and confirmation is highly specific, therefore an additional confirmatory method is not necessary. Residues in control samples were below $0.3 \times \text{LOQ}$ ($0.015 \mu\text{g/L}$).

Linearity

The correlation between the injected amount of substance and the detector response was shown to be linear (for all analytes except metabolite analysis in rape seed for which a quadratic response was observed over this range) for matrix matched standards, covering a range from 0.125 to $50.0 \mu\text{g/L}$ ($n=6$). A graph and equation parameters are available. The correlation coefficients ranged from 0.9953 to 0.9999 .

Accuracy

Mean recoveries are in the range of 70 - 120% for all matrices and analytes which is according to guidance document SANCO 825/00 rev. 8.1.

Limit of Quantitation

The limit of quantification (LOQ) (expressed as ethofumesate) is 0.01 mg/kg for each analyte in all matrices.

Repeatability

The relative standard deviations (RSD) were $\leq 20\%$ with the exception of AE C520645 (NC 20645) in rape seed at $10 \times \text{LOQ}$ where the values are slightly above. According to guidance document SANCO 825/00 rev. 8.1 the ILV, if identical to the primary method needs only two matrices for validation one of them with high water content. Therefore the matrix containing high oil content can be neglected.

Validation is summarized in Table B.5.2.1-1

Conclusion

Method 01392 is considered valid as enforcement method for the determination of residues of parent ethofumesate, metabolite AE C509607 (NC 9607) and open-ring-2-keto ethofumesate [AE C520645 (NC 20645)] in free and conjugated form. A letter of access by Bayer CropScience enables UPL to address this Annex point. Information is given in Volume 4.

UPL

Reference:	Validation of the Analytical Method for the Determination of Ethofumesate (free form), NC 9607 (free form) and NC 20645 (free and conjugated form) in High protein/starch content, High water content, High oil content, High acid content and Difficult commodities
Author(s), year:	Schlewitz, P. (2013a)
Report/Doc. number:	B3016
Guideline(s):	SANCO/3029/99 rev.4 of 11/07/00, SANCO/825/00 rev.8.1
GLP:	yes

The method describes the determination of residues of Ethofumesate, NC 9607 and NC 20645 (free and conjugated form) in rice (high protein/starch content = dry commodity), lettuce (high water content), oilseed rape seeds (high oil content), oranges (high acid content) and tea (difficult commodities). Matrix-matched standard solutions were used for calibrating the instrument and determining the detector response. Recovery tests were performed by untreated control samples spiked with Ethofumesate, NC 9607 and NC 20645 (free form) before extraction.

The conjugated form of NC 20645 will end up in the aqueous and solid fractions after the extraction step. These fractions will be subjected to an acid hydrolysis step. This step will release the conjugated NC 20645 and will convert it into NC 9607. This means that the conjugated NC 20645 will finally be detected as NC 9607 in the LC-MS/MS. The conjugated form of NC 20645 was not available for spiking and the validation for the conjugated form had therefore to be performed with the free form of NC 20645. Therefore, a second spiking with free NC 20645 took place before the acid hydrolysis step to show the efficacy of this step converting the conjugated form of NC20645 in NC 9607.

Principle of the method:

Free residues are extracted from crop samples with acetonitrile in the presence of citrate buffer and sodium chloride. Acetonitrile containing free residues is purified on a charcoal cartridge and is analysed by LC/MS/MS. Conjugated residues are extracted from the aqueous phase and the remains of the samples by acidic hydrolysis to yield free metabolite followed by partition with toluene and purification on a charcoal and florisil cartridge.

LC-MS/MS conditions primary method: column: BEH Phenyl, ANADIAG Number 207, Waters, Internal 100 mm x 2.1, 1.7 µm; gradient mobile phase: A: water + 0.1% formic acid, B: methanol + 0.1% formic acid; Ethofumesate (in tea), NC 9607 and NC 20645 (conjugated form) residues were analyzed by a second UPLC (BEH C18 50 mm x 2.1, 1.7 µm) column for confirmatory purposes. MS: ES pos. and neg. for Residues of Ethofumesate and NC 9607 and ES neg. for NC 20645 (free and conjugated).

Transitions: ethofumesate: m/z 287.1 > 121.1 and 287.1 > 259.1

NC 9607: m/z 257.0 > 177.0, 257.0 > 149.0** and 257.0 > 201.0**

NC 20645: m/z 273.1 > 194.1 and 273.1 > 79.0

** Transition not quantified due to a lack of sensitivity

Validation:Specificity

Residues of Ethofumesate, NC 9607 and NC 20645 (free and conjugated) in rice (high protein/starch content = dry commodity), lettuce (high water content), oilseed rape seeds (high oil content), oranges (high acid content) and tea (difficult commodities) were determined by LC-MS/MS using two transitions, except for ethofumesate in tea, and NC 9607 only one transition was detected. In these cases a column of a different stationary phase was used.

No interfering signal was present accounting for more than 30% of the LOQ.

Accuracy (recoveries)

All mean recoveries at the fortification levels are in the range of 70 - 110%.

Linearity

The linearity/calibration of the method was checked by matrix-matched calibration solutions of Ethofumesate, NC 9607, NC 20645 free form and conjugated form of NC 20645 (as NC 9607), at 7 concentration levels, over the range 1.5 ng/mL and 60 ng/mL (corresponding to 0.003 to 0.120 in mg/kg). The linear correlation coefficients were typically > 0.990, showing a good linearity. Plots and equations of the calibration curves are available.

Limit of Quantification

0.01 mg/kg

Repeatability (precision)

The relative standard deviations were <20 % per fortification level.

Reproducibility (ILV)

No ILV is available. An letter of access by BCS enables UPL to use enforcement method Schulte, G.; Diehl, P.; 2014 and the ILV Betson, S.; 2014 as well.

Extraction efficiency

A comparison between this method and Inveresk method (Agrichem BV Inveresk Project Number 803205) was done on samples containing Ethofumesate and its metabolites issued from Biotek agriculture trials with Ethofumesate on sugar beet in 2012. The results indicate comparable amount of extracted residues, thus proving the efficiency of the extraction step. For details on the comparison please refer to this report.

For analytical Inveresk method used in the residue section please refer to DOCUMENT M-CA, Section 6 Point CA 6.6.1.

A summary of all validation results is given in Table B.5.2.1-1.

Conclusion:

The method for the determination of Ethofumesate and its metabolites is acceptable and validated according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev.4. However, no ILV was submitted and therefore not suitable as enforcement method.

Table B.5.2.1-1: Summary of validation results –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Thom, M.; 2005; M- 351876-01-1 Taskforce	ethofumesate	PR00/001 GC-MS	Red currant	0.05*	0.02	80	7	5
					0.2	102	2	5
			Wheat grain	0.05*	0.02	74	13	5
					0.2	74	4	5
			Sunflower seed	0.02	0.02	96	10	5
					0.2	100	4	5
	NC 9607	PR00/001 GC-MS	Red currant	0.05*	0.02	90	17	5
					0.2	82	18	5
			Wheat grain	0.05*	0.02	73	9	4**
					0.2	76	13	5
			Sunflower seed	0.02	0.02	98	5	5
					0.2	100	8	5
Witte, A.; 2000; M-351894-01 ILV Taskforce	ethofumesate	PR00/001 GC-MS	Sugar beet root	0.05	0.05	100	4	5
					0.5	100	2	5
			Sugar beet leaves	0.05	0.05	98	3	5
					0.5	91	5	5
	NC 9607	PR00/001 GC-MS	Sugar beet root	0.05	0.05	87	9	5
					0.5	92	6	5
			Sugar beet leaves	0.05	0.05	82	2	5
					0.5	83	19	5
Klimmek, S.; 2005; M- 351896-01-1 ILV Taskforce	ethofumesate	PR00/001 GC-MS	Sunflower seed	0.02	0.02	85	11	5
					0.2	81	10	5
	NC 9607	PR00/001 GC-MS	Sunflower seed	0.02	0.02	75	12	5
					0.2	73	8	5

* LOQ based on ILV Witte, A.; 2000; M-351894-01 prepared for first Annex I inclusion

** statistical outlier excluded from calculation of mean recovery and RSD

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Schulte, G.; Diehl, P.; 2014; M-479926-01 Taskforce and UPL enforcement	ethofumesate (NH ₄ adduct) determined and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 304 → 121 (quantifier)	Sugar beet (leaf)	0.01	0.01	96	4.1	5
					0.1	103	3.2	5
			Wheat (grain)	0.01	0.01	97	10.8	5
					0.1	89	9.7	5
			Rape (seed)	0.01	0.01	86	5.9	5
					0.1	82	9.9	5
			Orange (fruit)	0.01	0.01	100	5.1	5
					0.1	99	6.9	5
			Hop (green cone)	0.01	0.01	107	4.8	5
					0.1	103	2.7	5
	NC 9607 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 194 (quantifier)	Sugar beet (leaf)	0.01	0.01	91	3.5	5
					0.1	95	8.4	5
			Wheat (grain)	0.01	0.01	91	15.5	5
					0.1	95	7.1	5
			Rape (seed)	0.01	0.01	76	4.8	5
					0.1	81	4.3	5
			Orange (fruit)	0.01	0.01	94	3.8	5
					0.1	88	8.8	5
			Hop (green cone)	0.01	0.01	82	4.0	5
					0.01	74	5.1	5
	NC 20645 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 194 (quantifier)	Sugar beet (leaf)	0.01	0.01	84	6.0	5
					0.1	89	4.4	5
			Wheat (grain)	0.01	0.01	74	9.9	5
					0.1	105	4.4	5
			Rape (seed)	0.01	0.01	95	2.9	5
					0.1	85	3.0	5
			Orange (fruit)	0.01	0.01	95	4.5	5
					0.1	84	3.0	5
			Hop (green cone)	0.01	0.01	73	5.8	5
					0.01	83	6.9	5
	ethofumesate (NH ₄ adduct)	01392 LC-MS/MS	Sugar beet (leaf)	0.01	0.01	101	3.4	5
					0.1	103	3.0	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
	determined and calculated as ethofumesate	MRM m/z 304 → 259 (qualifier)	Wheat (grain)	0.01	0.01	96	13.5	5
					0.1	91	9.3	5
			Rape (seed)	0.01	0.01	87	6.8	5
					0.1	80	9.8	5
			Orange (fruit)	0.01	0.01	103	6.8	5
					0.1	100	7.0	5
			Hop (green cone)	0.01	0.01	106	6.9	5
					0.1	102	3.6	5
	NC 9607 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 149 (qualifier)	Sugar beet (leaf)	0.01	0.01	90	3.7	5
					0.1	96	7.3	5
			Wheat (grain)	0.01	0.01	91	16.0	5
					0.1	96	6.9	5
			Rape (seed)	0.01	0.01	78	4.6	5
					0.1	83	4.8	5
			Orange (fruit)	0.01	0.01	93	5.8	5
					0.1	91	9.1	5
			Hop (green cone)	0.01	0.01	84	1.5	5
					0.01	74	5.1	5
	NC 20645 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 149 (qualifier)	Sugar beet (leaf)	0.01	0.01	85	6.1	5
					0.1	89	3.8	5
			Wheat (grain)	0.01	0.01	81	8.7	5
					0.1	103	5.6	5
			Rape (seed)	0.01	0.01	95	2.7	5
					0.1	84	2.9	5
			Orange (fruit)	0.01	0.01	97	2.7	5
					0.1	85	3.5	5
			Hop (green cone)	0.01	0.01	71	6.4	5
					0.01	82	5.7	5
Betson, S.; 2014 M-497682-01-1 ILV Taskforce and	ethofumesate determined and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 287 → 121 (quantifier)	Sugar beet (leaf)	0.01	0.01	83	6.0	5
					0.1	92	3.8	5
			Wheat (grain)	0.01	0.01	96	2.4	5
					0.1	87	5.2	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
UPL			Rape (seed)	0.01	0.01	99	8.2	5
					0.1	88	6.3	4
			Orange (fruit)	0.01	0.01	90	3.6	5
					0.1	96	1.5	5
			Hop (green cone)	0.01	0.01	91	8.0	5
					0.1	93	11.2	5
	NC 9607 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 79 (quantifier)	Sugar beet (leaf)	0.01	0.01	72	17.7	5
					0.1	78	7.5	5
			Wheat (grain)	0.01	0.01	81	5.5	5
					0.1	108	3.5	5
			Rape (seed)	0.01	0.01	109	2.4	4
					0.1	101	2.4	4
			Orange (fruit)	0.01	0.01	87	19.0	5
					0.1	87	6.6	5
			Hop (green cone)	0.01	0.01	85	6.5	5
					0.01	91	7.9	4
	NC 20645 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 79 (quantifier)	Sugar beet (leaf)	0.01	0.01	74	4.6	4
					0.1	77	19.1	5
			Wheat (grain)	0.01	0.01	104	15.7	5
					0.1	103	4.2	5
			Rape (seed)	0.01	0.01	107	5.7	5
					0.1	101	22.4	4
			Orange (fruit)	0.01	0.01	70	11.7	4
					0.1	80	8.6	5
			Hop (green cone)	0.01	0.01	92	14.9	5
					0.01	90	8.0	5
	ethofumesate determined and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 287 → 161 (qualifier)	Sugar beet (leaf)	0.01	0.01	82	7.6	5
					0.1	92	3.0	5
			Wheat (grain)	0.01	0.01	95	2.2	5
					0.1	87	5.0	5
			Rape (seed)	0.01	0.01	96	10.7	5
					0.1	89	5.4	4

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Orange (fruit)	0.01	0.01	92	3.4	5
					0.1	95	2.4	5
			Hop (green cone)	0.01	0.01	95	14.7	4
					0.1	94	5.8	5
	NC 9607 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 149 (qualifier)	Sugar beet (leaf)	0.01	0.01	73	13	5
					0.1	78	4.9	5
			Wheat (grain)	0.01	0.01	87	8.9	5
					0.1	108	5.7	5
			Rape (seed)	0.01	0.01	112	4.2	4
					0.1	102	2.5	4
			Orange (fruit)	0.01	0.01	88	14.6	5
					0.1	85	6.9	5
	NC 20645 determined as NC 20645 and calculated as ethofumesate	01392 LC-MS/MS MRM m/z 273 → 149 (qualifier)	Hop (green cone)	0.01	0.01	84	10.6	5
					0.01	89	4.6	4
			Sugar beet (leaf)	0.01	0.01	82	19.6	5
					0.1	77	20.0	5
			Wheat (grain)	0.01	0.01	107	15.1	5
					0.1	105	3.0	5
			Rape (seed)	0.01	0.01	109	11.1	3
					0.1	101	23.3	4
			Orange (fruit)	0.01	0.01	71	18.4	4
					0.1	81	7.6	5
			Hop (green cone)	0.01	0.01	77	8.9	5
					0.01	85	7.2	5
Schlewitz, P. (2013a) UPL	ethofumesate	LC-MS/MS MRM m/z 287.1 > 121.1 (quantifier)	Rice	0.01	0.01	100.3	8.1	5
					0.1	96.9	5.0	5
			Lettuce	0.01	0.01	101.3	4.7	5
					0.1	102.3	7.3	5
			Oilseed rape seeds	0.01	0.01	84.5	8.1	5
					0.1	81.0	3.3	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
		m/z 287.1 > 259.1	Orange	0.01	0.01	92.1	6.2	5
					0.1	79.5	5.1	5
			Tea	0.01	0.01	91.1	14.3	5
					0.1	82.4	7.2	5
	NC 9607	LC-MS/MS MRM m/z 257.0 > 177.0 (quantifier)	Rice	0.01	0.01	101.1	6.7	5
					0.1	94.7	4.2	5
			Lettuce	0.01	0.01	102.1	5.9	5
					0.1	97.8	7.1	5
			Oilseed rape seeds	0.01	0.01	94.5	5.4	5
					0.1	91.3	5.7	5
			Orange	0.01	0.01	83.7	5.9	5
					0.1	83.2	6.4	5
			Tea	0.01	0.01	88.4	15.8	5
					0.1	83.7	7.1	5
	NC 20645 (free form)	LC-MS/MS MRM m/z 273.1 > 79.0 (quantifier)	Rice	0.01	0.01	89.3	15.6	5
					0.1	83.8	3.4	5
			Lettuce	0.01	0.01	92.2	17.6	5
					0.1	86.7	10.9	5
			Oilseed rape seeds	0.01	0.01	81.4	14.5	5
					0.1	95.8	9.7	5
			Orange	0.01	0.01	100.1	5.2	5
					0.1	76.4	8.6	5
			Tea	0.01	0.01	78.0	8.3	5
					0.1	73.8	3.7	5
	NC 20645	LC-MS/MS	Rice	0.01	0.01	88.6	9.2	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
	(conjugated form measured as NC9607)	MRM m/z 257.0 > 177.0 (quantifier)			0.1	80.5	5.2	5
			Lettuce	0.01	0.01	77.3	9.3	5
					0.1	72.6	3.1	5
			Oilseed rape seeds	0.01	0.01	96.6	12.7	5
					0.1	102.9	8.0	5
			Orange	0.01	0.01	93.3	9.2	5
					0.1	99.7	4.4	5
			Tea	0.01	0.01	89.1	13.6	5
					0.1	89.2	8.9	5
	ethofumesate	confirmatory	Rice	0.01	0.01	101.6	10.2	5
			Lettuce	0.01	0.01	109.0	0.5	5
			Oilseed rape seeds	0.01	0.01	100.8	5.7	5
			Orange	0.01	0.01	94.9	4.0	5
			Tea	0.01	0.01	84.6	13.7	5
	NC 9607	confirmatory	Rice	0.01	0.01	104.0	8.2	5
			Lettuce	0.01	0.01	103.9	6.0	5
			Oilseed rape seeds	0.01	0.01	87	5.9	5
			Orange	0.01	0.01	84.6	5.3	5
			Tea	0.01	0.01	80.8	10.0	5
	NC 20645 (free form)	confirmatory	Rice	0.01	0.01	86.9	16.4	5
			Lettuce	0.01	0.01	96.5	3.6	5
			Oilseed rape seeds	0.01	0.01	86.1	16.1	5
			Orange	0.01	0.01	84.5	11.2	5
			Tea	0.01	0.01	75.2	6.5	5
	NC 20645	confirmatory	Rice	0.01	0.01	87.6	14.2	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
	(conjugated form measured as NC9607)		Lettuce	0.01	0.01	88.1	5.5	5
			Oilseed rape seeds	0.01	0.01	83.9	9.3	5
			Orange	0.01	0.01	93.1	14.1	5
			Tea	0.01	0.01	80.6	12.9	5

B.5.2.2. Animal matrices

TASKFORCE

For ruminant and pig matrices the sum of *parent compound ethofumesate* and the *metabolite NC 9607*, expressed as ethofumesate equivalents, is defined as residue definition for enforcement (cf. Reg (EU) No 149/2008 and 524/2011). However, monitoring of the relevant ethofumesate residue is only possible if NC 9607 is included in the residue definition as *common moiety* which comprises the determination of the metabolites NC 20645 and NC 9607. Therefore the current residue definition is not sufficiently precise and should be changed accordingly.

A residue definition for poultry matrices was not yet considered necessary since only a negligible dietary exposure of poultry is expected. However, also the dietary burden of ruminants is rather low and no feeding studies are triggered (cf. MCA 6.4), neither for ruminants nor for poultry by the use of ethofumesate and thus, technically, **no MRLs need to be set and no enforcement method needs to be presented.**

Therefore no specific enforcement methods were submitted for first annex I inclusion.

For all studies submitted during the frame of the first Annex I inclusion please refer to the tables below in grey typeface plus to the corresponding section in the Monograph and in the baseline dossier (D-008920-01) provided by the Task Force Ethofumesate.

Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:

Report:	KCA 4.2 /09;Luettgens, U.;1994;M-351945-01
Title:	Ethofumesate - Validation of an analytical method for determination of ethofumesate residues in meat
Report No:	OFC00004920
Document No:	M-351945-01-1
Guidelines:	EEC directive 98/46/EC, Directive 91/414/EEC; L214/18. 1996;not specified
GLP/GEP:	yes

Report:	KCA 4.2 /10;Peatman, M. H.;1999;M-185949-01
Title:	Review of analytical methodology for residues in edible animal products (dairy, tissues, fat and offal) Ethofumesate AE B049913
Report No:	C003328
Document No:	M-185949-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

After the Annex I inclusion of ethofumesate, the multi-residue method DFG S19 was submitted to several national authorities for approval of ethofumesate containing products. However, this multi- method is not applicable to analyse for all constituents of the residue definition since the method does not include the acidic conversion of metabolite NC 20645 to the *common moiety product NC 9607*, or the separate analysis of the metabolites NC 9607 and NC 20645. However, notwithstanding of the current residue definition, it has to be discussed if a restriction of the residue definition for monitoring/enforcement to parent compound would be

more appropriate/workable. No ethofumesate related residues are expected in edible matrices of livestock (<0.01 mg/kg), taking into account the residue levels in potential feeding stuffs, obtained at the 1x dose rate (calculated on the dry weight basis) and the transfer factors estimated for the total radioactive residue in the livestock metabolism studies. Based on these data it becomes obvious that the monitoring/enforcement method is only needed to show misuse of ethofumesate containing products. In addition, official surveillance laboratories prefer multi-residue methods, which can include a large number of analytes. The DFG S19 method is such a method and therefore its validation and the respective ILVs are summarized in this AIR dossier.

Different modules of the DFG S19 method were validated for parent ethofumesate and the metabolite NC 9607 in the presented methods. However, the determination of the metabolite NC 9607 without prior conversion of metabolite NC 20645 to NC 9607 is not reasonable and therefore only the validation data for the parent compound in the different livestock matrices is summarized.

Report: KCA 4.2 /11; Rzepka, S.; 2004; M-234011-01
Title: Independent laboratory validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in food of animal origin
Report No: OFC00004922
Document No(s): Report includes Trial Nos.:
 AZ.G04-0038
 M-234011-01-1
Guidelines: SANCO: 825/00, rev.6;not specified
GLP/GEP: yes

Report: KCA 4.2 /12; Dorn, U.; 2006; M-271716-01
Title: Independent Laboratory Validation of an Analytical Method for the Determination of Ethofumesate and Ethofumesate-2-keto in Meat
Report No: OFC00012323
Document No: M-271716-01-1
Guidelines: Council Directive 91/414/EEC Annex II (Part A, Section 4.2), Annex III (Part A, Section 5.2)
 as amended by Commission Directive 96/46/EC
 EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7
 17/03/04;not specified
GLP/GEP: yes

New data for AIR (in black typeface):

Reference:	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in food of animal origin
Author(s), year:	Thom, M.; 2004
Report/Doc. number:	OFC00004921, M-351880-01-1
Guideline(s):	Sanco/825/00, rev. 6 (20/06/2000)
GLP:	yes

Reference:	Validation of an analytical method for the determination of ethofumesate and ethofumesate-2- keto in liver, kidney and fat
Author(s), year:	Mende, P.; 2009
Report/Doc. number:	S09-00540, M-358951-01-1
Guideline(s):	EC Guidance document on residue analytical methods, Sanco/825/00 rev.7 (17/03/2004)
GLP:	yes

Reference:	Independent laboratory validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in food of animal origin
Author(s), year:	Rzepka, S., 2004
Report/Doc. number:	OFC00004922, Report includes Trial Nos.: AZ.G04-0038, M-234011-01-1
Guideline(s):	SANCO: 825/00, rev.6
GLP:	yes

Reference:	Independent Laboratory Validation of an Analytical Method for the Determination of Ethofumesate and Ethofumesate-2-keto in Meat
Author(s), year:	Dorn, U., 2006
Report/Doc. number:	OFC00012323, M-271716-01-1
Guideline(s):	Council Directive 91/414/EEC Annex II (Part A, Section 4.2), Annex III (Part A, Section 5.2) as amended by Commission Directive 96/46/EC EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7 17/03/04
GLP:	yes

Principle of the method:

The DFG S19 multi-residue method was used as basis for the determination of ethofumesate residues in livestock matrices in the present GAB/IFU Method 20031394/01-RVAT.

Milk and egg samples were extracted with acetone/water (2/1, v/v) and partitioned using ethyl acetate/cyclohexane (1/1, v/v). Liver and kidney samples were extracted accordingly. For meat samples the DFG clean-up module 5 was used. For this purpose, the samples were extracted with acetonitrile/acetone (9:1) after addition of Calflo E (to adsorb fat contained in the sample) and Celite. Fat samples were extracted according to the DFG module E 6 by dissolving the fat sample in a mixture of ethyl acetate/cyclohexane (1/1, v/v).

The extracts obtained were further purified by gel permeation and silica gel chromatography prior to GC/MS analysis. Residues were quantified against standard in solvent.

GC-MS conditions: column: equivalent to DB-5 30 m x 0.25 i.d. 0.25µm; SIM: m/z 286, 161, 137

Mende, P.; 2009: column: equivalent to DB-5 30 m x 0.25 i.d. 0.25µm; SIM: m/z 207, 161, 137

The validation data for ethofumesate in muscle, milk and eggs were reported in Thom, M.; 2004, and the data for kidney, liver and fat in Mende, P.; 2009.

Validation:

Specificity

A specific detector (MS-EI) was used for analysis. Ethofumesate was identified by the retention time and by its specific mass fragment ratios (3 characteristic ions with m/z > 100 were monitored). Using this procedure, the GC-MS method is highly specific, thus an additional confirmatory method based on another principle is not

necessary. The acceptable tolerance of the mass-ratios from the qualifier ions to target ions was 20%. Minor interferences were only observed for ethofumesate mass fragment $m/z = 161$ at a calibration level of 0.01 mg/kg.

Accuracy (recoveries)

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with ethofumesate at concentrations of 0.02 and 0.20 mg/kg. Mean recoveries per fortification level for the quantifier ion were in a range of 77-109% for all livestock matrices.

Linearity

The correlation between the injected amount of substance and the detector response was linear for standards in matrix in the range from 10 to 600 ng/mL for analysis of meat, milk and eggs using six different concentration levels. The calibration graphs were of second order with the correlation coefficients of at least $r^2 = 0.9995$. For each matrix the effect of matrix matched standards vs. standards in toluene was examined at high and low concentration levels. Milk and egg samples had no significant matrix effects ($< +10\%$). For meat samples a significant matrix effect was observed (up to $+40\%$).

The correlation between the injected amount of substance and the detector response was also linear for standards in solvent in the range from 10 to 500 ng/mL for analysis of liver and kidney and from 1 to 50 ng/mL for analysis of fat using seven different concentration levels. The correlation coefficients of the $1/x$ weighted linear regression were at least $r^2 = 0.997$.

Limit of Quantification

The limit of quantification was defined as the lowest fortification level with mean recoveries ranging between 70% and 110% at a relative standard deviation not exceeding 20%. These conditions are fulfilled at the 0.02 mg/kg fortification level for minced meat, egg and milk and as well for kidney, liver and fat. The calculated limit of detection (LOD) was estimated to be at least 3 times lower than the respective LOQ, based on the linearity response data and matrix interference observed in control samples, i.e. 0.006 mg/kg.

Repeatability (precision)

The relative standard deviations (RSD) per fortification level ranged from 2% to 18% for the quantifier mass transition in all types of samples. These values are within the guideline requirements ($< 20\%$ RSD per fortification level). The overall RSD of ethofumesate in the analysed matrices is in a range from 3% to 14%.

Reproducibility (ILV)

Two independent laboratory validations were performed [Rzepka, S.; 2004 (samples of milk, meat and egg) and Dorn, U.; 2006 (samples of meat only)] for the methods described by Thom, M.; 2004 and Mende, P.; 2009. The samples were fortified with ethofumesate at the nominal fortification levels of 0.02 and 0.20 mg/kg, i.e. the LOQ and the 10-fold LOQ. Two replicate specimens per animal material were kept untreated, serving as blank controls.

Determination with GC/MS used the characteristic ions 286 m/z (Rzepka, S.; 2004; M-234011-01) or 137 m/z (Dorn, U.; 2006; M-271716-01) for quantification and calculation of residues and recoveries. For all matrices tested, and for both fortification levels, the mean recoveries were between 74% and 90%, with relative standard deviations of < 10%. Since significant interfering signals in the blank control specimens were detected when using the 161 m/z fragment ion (confirmatory ion) to trace ethofumesate in meat samples, GC-MS/MS was additionally used to demonstrate the specificity of the method. Using GC-MS/MS the molecular ion (m/z 286) was used as precursor ion and the daughter ion m/z 207 was monitored. The determined recoveries (fortification range 0.02 – 0.20 mg/kg) for ethofumesate were within 70 – 110%; the RSD was <20% in all cases.

Extraction efficiency:

No residues (according to the residue definition; refer to chapter B.7) above the LOQ are expected in food of animal commodities regarding the representative use: sugar beets. Therefore there is no need to address extraction efficiency. Reference to guidance document SANCO/825/00 rev.8.1.

A summary of all validation results is given in Tables B.5.2.2-1.

Conclusion

The data presented demonstrate that using GAB/IFU Method 20031394/01-RVAT [2] permits the determination of residues of ethofumesate in food of animal origin with satisfactory accuracy, precision and repeatability. The method is therefore considered to be valid as monitoring/ enforcement method if the residue definition is restricted to parent compound, only. The LOQ for the analytical target ethofumesate in all animal matrices is 0.02 mg/kg.

Since the multi-residue method for animal matrices is only applicable to analyse for parent compound ethofumesate – and since no clear decision on the residue definition for monitoring/enforcement has been taken yet - a new enforcement method for analysing ethofumesate and its relevant metabolites NC 9607 and NC 20645 has been developed by United Phosphorus Ltd (UPL) and is provided for milk, eggs, meat, fat and liver.

Ethofumesate related residues are analysed by HPLC-MS/MS. The LOQ is 0.01 mg/kg for each analyte. A data sharing agreement (letter of access) between UPL and the Task Force Ethofumesate allows the Task Force Ethofumesate to refer to this method and its independent laboratory validation (ILV), for details please see Volume 4.

UPL + TASKFORCENew residue definition for animal foodstuffs.

In the evaluation for MRL harmonisation (EFSA Journal of 2012)¹ EFSA proposed to set the residue definition in commodities of animal origin as 2-keto-ethofumesate and open-ring-2-keto-ethofumesate, expressed as Ethofumesate.

Based on the uncertainty of a new residue definition for Ethofumesate and its two metabolites NC 9607 and NC 20645 in Foodstuffs of Animal Origin, the dossier includes the additional information for monitoring purposes of residues in foodstuff of animal origin: A new HPLC-MS/MS method is provided for Ethofumesate and its metabolites NC 9607 and NC 20645 (Jooß, 2012, KCA 4.2/03) in foodstuff of animal origin for milk, egg, meat, liver and fat.

In addition an independent laboratory validation of the analytical HPLC-MS/MS method for Ethofumesate and its two metabolites NC 9607 and NC 20645 in foodstuffs of animal origin (Schlewitz, 2013b; KCA, 4.2/04) was carried out. The method was validated at 0.01 mg/kg in bovine muscle, liver, fat, cow's milk and hen eggs. It complies with the requirements of SANCO 825/00 rev. 8.1 and SANCO/3029/99 rev. 4.

Reference:	Ethofumesate - Validation of an Analytical Method for the Determination of the Ethofumesate and its two Metabolites NC 9607 and NC 20645 in Foodstuffs of Animal Origin
Author(s), year:	Jooß, S. (2012)
Report/Doc. number:	P 2371 G
Guideline(s):	SANCO/825/00, rev.8.1., SANCO/3029/99 rev. 4 and OECD ENV/JM/MONO(2007)17
GLP:	yes

Reference:	Independent laboratory validation of an analytical method for the analysis of Ethofumesate and its two metabolites NC 9607 and NC 20645 in foodstuffs of animal origin
Author(s), year:	Schlewitz, P. (2013b)
Report/Doc. number:	R B1218
Guideline(s):	SANCO/825/00, rev.8.1., SANCO/3029/99 rev. 4 and OECD ENV/JM/MONO(2007)17
GLP:	yes

The objective of this study was to develop and to validate an analytical method for the determination of Ethofumesate and its two metabolites NC 9607 (2-ketoethofumesate) and NC 20645 (2-methylpropionic acid ethofumesate) in various animal matrices to achieve a limit of quantitation (LOQ) of 0.01 mg/kg per analyte, always expressed as Ethofumesate.

¹ Reasoned opinion on the review of the existing maximum residue levels (MRLs) for Ethofumesate according to Article 12 of Regulation (EC) No 396/2005 – EFSA Journal 2012; 10(11): 2959

Principle of the method:

Residues of Ethofumesate and its metabolites NC 9607 and NC 20645 in animal matrices were extracted with acetonitrile/water and determined by LC-MS/MS in the positive and negative ion mode (for NC 20645), using 2 transition ions for quantitation and confirmation except for NC 9607 (2-ketoethofumesate) where the 1st transition was analyzed by a 2nd HPLC column for confirmatory purposes.

For method validation the animal specimens were fortified (5 replicates per fortification level) at 0.01 mg/kg (LOQ) and at 0.10 mg/kg (10 × LOQ) with Ethofumesate and its two metabolites NC 9607 and NC 20645. Additionally two specimens were kept untreated as blank controls per matrix type. One reagent blank was also analyzed.

LC- conditions: columns: Thermo, Aquasil C₁₈, 3.0 µm particle size, 150 mm length, 3.0 mm i.d. Pre-column: Phenomenex C₁₈, 4 mm length, 3.0 mm i.d. Phenomenex, Luna Phenyl hexyl, 3.0 µm particle size, 50 mm length, 2.0 mm i.d. Pre-column: Phenomenex C₁₈, 4 mm length, 3.0 mm i.d.; gradient mobile phase: A: water + 0.1% formic acid, B: methanol + 0.1% formic acid;

2nd LC column: Phenomenex Luna, Phenylhexyl column: Length: 50 mm, i.d.: 2.0 mm, particle size: 3.0 µm (confirmation of NC 9607)

MS/MS-conditions: Ethofumesate (ESI positive ion mode)

Transitions 287 m/z → 121.1 m/z (quantification), 287 m/z → 259.2 m/z (confirmation)

NC 20645 (ESI negative ion mode)

Transitions 273 m/z → 78.9 m/z (quantification), 273 m/z → 193.9 m/z (confirmation)

NC 9607 (ESI positive ion mode)

Transition 257 m/z → 176.9 m/z (quantification), 2nd LC column: (confirmation)

Validation:Specificity

LC-MS/MS two transitions and in the case of NC 9607 a 2nd column with a different stationary phase was used for confirmation. No interfering signal was present accounting for more than 30% of the LOQ.

Accuracy (recoveries)

All mean recoveries at the fortification levels are in the range of 70 - 110%.

Linearity

Calibration functions obtained from injections of five or six matrix matched standards, ranging from 1.0 to 75 ng/mL for milk, egg, meat and liver (all analytes) and from 0.50 to 75 ng/mL for fat (all analytes), were used to evaluate the specimens. Linear calibration functions were calculated and plotted by regression analysis. Correlation coefficients (r) were ≥ 0.99. Plots and equations of the calibration curve are available.

Limit of Quantification

0.01 mg/kg for all matrices and analytes.

Repeatability (precision)

The relative standard deviations were <20 % per fortification level.

Reproducibility (ILV)

An independent laboratory validation (ILV) is conducted (Schlewitz, P. (2013b) using the same conditions with minor deviations which have no adverse impact on the study, same animal matrices, and the same fortification levels as the original method

Specificity

The highly specific LC-MS/MS method uses two mass transitions for Ethofumesate (287 m/z → 121 m/z and 287 m/z → 259 m/z) and for NC 20645 (2-methylpropionic acid Ethofumesate; 273 m/z → 79 m/z and 273 m/z → 194 m/z) for quantitation and quantitative confirmation. NC 9607 (2-ketoethofumesate) uses one mass transition (257 m/z → 177 m/z) for quantitation. Confirmation is performed by a 2nd HPLC column using the same mass transition.

Linearity

The linearity of the detector response for Ethofumesate and its two metabolites NC 9607 and NC 20645 was studied in the range of 1.5 ng/mL to 60 ng/mL (0.7 ng/mL to 30 ng/mL for fat) in matrix-matched calibration solutions. The correlation coefficients were typically > 0.990, showing a good linearity.

The linearity of the detector response for the analytical method was demonstrated by injection of matrix-matched calibration solutions of Ethofumesate and its two metabolites NC 9607 and NC 20645, at 7 concentration levels, over the range 1.5 ng/mL to 60 ng/mL (0.7 ng/mL to 30 ng/mL for fat). Calibration curves were calculated for each analytical sequence.

The correlation coefficients for these linear regressions were typically > 0.990, showing a good linearity of the detector response. A graph and equation parameter of the calibration curve is available.

Accuracy

Mean recoveries are in the range of 70-120% for all matrices and analytes which is according to guidance document SANCO 825/00 rev. 8.1.

Limit of Quantitation

The limit of quantification (LOQ) (expressed as ethofumesate) is 0.01 mg/kg for each analyte in all matrices.

Repeatability

The relative standard deviations (RSD) were ≤ 20%

Validation is summarized in Table B.5.2.1-1

Extraction efficiency:

No residues (according to the residue definition; refer to chapter B.7) above the LOQ are expected in food of animal commodities regarding the representative use: sugar beets. Therefore there is no need to address extraction efficiency. Reference to guidance document SANCO/825/00 rev.8.1.

Conclusion

The analytical method for the determination of ethofumesate and the metabolites NC 9607 and NC 20645 is sufficiently validated and is considered as enforcement method, due to a letter of access by UPL the notifier Taskforce addresses this Annex point as well.

A summary of all validation results is given in Tables B.5.2.2-1.

Table B.5.2.2-1: Validation results animal matrices –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Thom, M.; 2004 M-351880-01 Taskforce	ethofumesate	DFG S19 GC-MS m/z 286	Milk	0.02	0.02	81	4	5
					0.2	82	3	5
			Meat	0.02	0.02	80	6	5
					0.2	85	9	5
			Eggs	0.02	0.02	82	7	5
					0.2	82	2	5
Mende, P.; 2009 M-358951-01-1 Taskforce	ethofumesate	DFG S19 GC-MS m/z 207	Liver	0.02	0.02	109	5	5
					0.2	105	18	5
			Kidney	0.02	0.02	102	18	5
					0.2	107	11	5
			Fat	0.02	0.02	77	6	5
					0.2	92	4	5
Rzepka, S.; 2004 M-234011-01 ILV Taskforce	ethofumesate	DFG S19 GC-MS m/z 286	Milk	0.02	0.02	86	8.8	5
					0.2	91	3.2	5
			Meat	0.02	0.02	70	7.4	5
					0.2	79	5.3	5
			Eggs	0.02	0.02	81	7.5	5
					0.2	92	4.5	5
Dorn, U.; 2006; M-271716-01 ILV Taskforce	ethofumesate	DFG S19 GC-MS m/z 137	Meat	0.02	0.02	80	10	5
					0.2	75	8	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Jooß S., 2012 UPL + Taskforce enforcement	ethofumesate	LC-MS/MS m/z 287 → 121 (quantifier)	Milk	0.01	0.01	86	5	5
					0.1	88	2	5
			Eggs	0.01	0.01	109	2	5
					0.1	104	3	5
			Meat	0.01	0.01	101	2	5
					0.1	99	2	5
			Liver	0.01	0.01	92	9	5
					0.1	91	4	5
			Fat	0.01	0.01	101	3	5
					0.1	99	2	5
	NC 20645	LC-MS/MS m/z 273 → 79 (quantifier)	Milk	0.01	0.01	89	2	5
					0.1	92	2	5
			Eggs	0.01	0.01	105	2	5
					0.1	79	2	5
			Meat	0.01	0.01	110	4	5
					0.1	108	2	5
			Liver	0.01	0.01	91	5	5
					0.1	85	3	5
			Fat	0.01	0.01	105	2	5
					0.1	106	1	5
	NC 9607	LC-MS/MS m/z 257 → 177 (quantifier)	Milk	0.01	0.01	93	2	5
					0.1	94	1	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Eggs	0.01	0.01	80	1	5
					0.1	96	3	5
			Meat	0.01	0.01	91	3	5
					0.1	94	1	5
			Liver	0.01	0.01	89	7	5
					0.1	85	3	5
			Fat	0.01	0.01	96	3	5
					0.1	96	1	5
	ethofumesate	LC-MS/MS m/z 287 → 259 (qualifier)	Milk	0.01	0.01	86	4	5
					0.1	87	3	5
			Eggs	0.01	0.01	105	3	5
					0.1	102	2	5
			Meat	0.01	0.01	98	1	5
					0.1	100	3	5
			Liver	0.01	0.01	93	10	5
					0.1	85	4	5
			Fat	0.01	0.01	95	3	5
					0.1	95	3	5
	NC 20645	LC-MS/MS m/z 273 → 194 (qualifier)	Milk	0.01	0.01	87	2	5
					0.1	93	2	5
			Eggs	0.01	0.01	110	3	5
					0.1	89	2	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Meat	0.01	0.01	110	5	5
					0.1	108	2	5
			Liver	0.01	0.01	93	4	5
					0.1	87	2	5
			Fat	0.01	0.01	107	3	5
					0.1	106	3	5
	NC 9607	LC-MS/MS confirmation m/z 257 → 177 + different stationary phase	Milk	0.01	0.01	93	3	5
					0.1	95	1	5
			Eggs	0.01	0.01	96	1	5
					0.1	98	1	5
			Meat	0.01	0.01	92	1	5
					0.1	96	1	5
			Liver	0.01	0.01	93	6	5
					0.1	88	3	5
			Fat	0.01	0.01	96	6	5
					0.1	98	1	5
Schlewitz, P. 2013b ILV UPL + Taskforce	ethofumesate	LC-MS/MS m/z 287 → 121 (quantifier)	Milk	0.01	0.01	101.7	9.9	5
					0.1	97.8	2.4	5
			Eggs	0.01	0.01	105.3	4.2	5
					0.1	105.4	1.2	5
			Muscle	0.01	0.01	109.0	2.3	5
					0.1	105.9	1.0	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Liver	0.01	0.01	111.8	3.4	5
					0.1	105.5	1.1	5
			Fat	0.01	0.01	107.3	4.3	5
					0.1	113.2	4.5	5
	NC 20645	LC-MS/MS m/z 273 → 79 (quantifier)	Milk	0.01	0.01	100.0	8.0	5
					0.1	98.5	6.4	5
			Eggs	0.01	0.01	105.5	4.2	5
					0.1	105.0	2.4	5
			Muscle	0.01	0.01	114.8	3.9	5
					0.1	107.2	4.5	5
			Liver	0.01	0.01	108.7	5.5	5
					0.1	102.9	2.3	5
			Fat	0.01	0.01	113.4	7.1	5
					0.1	113.4	3.3	5
	NC 9607	LC-MS/MS m/z 257 → 177 (quantifier)	Milk	0.01	0.01	101.1	9.2	5
					0.1	102.9	2.5	5
			Eggs	0.01	0.01	111.5	3.4	5
					0.1	107.6	2.1	5
			Muscle	0.01	0.01	105.0	2.6	5
					0.1	105.1	2.3	5
			Liver	0.01	0.01	96.7	9.7	5
					0.1	102.8	2.6	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
			Fat	0.01	0.01	94.9	13.2	5
					0.1	112.0	4.5	5

Additional studies are provided but not considered as enforcement methods or were not according to guidance document SANCO 825/00 rev. 8.1

Plant matrices

UPL

Reference:	Validation of an Analytical Method for the Determination of Residues of Ethofumesate and its Metabolites NC 8493, NC 9607 and NC 20645 in Sugar Beet Matrices.
Author(s), year:	Hamberger, R. (2012a)
Report/Doc. number:	
Guideline(s):	SANCO/3029/99 rev.4 of 11/07/00, SANCO/825/00 rev.8.1
GLP:	yes

Justification: Matrices investigated are not complete according to guidance document SANCO 825/00 rev.8.1.
No ILV is provided.

B.5.2.3. Soil**Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:**

Report:	KCA 4.2 /13;Schneider, E.;1999;M-193143-01
Title:	Validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in surface water (determination limit of 0.1 mcg/L; monitoring method)
Report No:	C006011
Document No:	M-193143-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /14;Schneider, E.;1995;M-351950-01
Title:	Ethofumesate - Determination of ethofumesate in soil with a determination limit of 50ug/kg - Monitoring method
Report No:	OFC00004916
Document No:	M-351950-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /15;Schneider, E.;2000;M-351953-01
Title:	PR00/003 - Confirmation method for the determination of residues of ethofumesate in soil
Report No:	OFC00004917
Document No:	M-351953-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /16;Schneider, E.;1999;M-193143-01
Title:	Validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in surface water (determination limit of 0.1 mcg/L; monitoring method)
Report No:	C006011
Document No:	M-193143-01-1
Guidelines:	not specified; Deviation not specified
GLP/GEP:	yes

Report:	KCA 4.2 /17;Fuchsbichler, G.; Frank, C.;1992;M-468443-01
Title:	Eine Methode zur Bestimmung von ethofumesat in Trinkwasser mittels Gaschromatographie
Report No:	M-468443-01-1
Document No:	M-468443-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /18;Schneider, E.;1992;M-468435-01
Title:	Determination of ethofumesate in the soil of rotating crops
Report No:	M-468435-01-1
Document No:	M-468435-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /19;Schneider, E.;1992;M-468439-01
Title:	Determination of ethofumesate and phenmedipham in soil high pressure liquid chromatography
Report No:	M-468439-01-1
Document No:	M-468439-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /20;Schneider, E.;1994;M-468448-01
Title:	PR93/016 - Validation of analytical method DrK078 - Determination of ethofumesate in air
Report No:	M-468448-01-1
Document No:	M-468448-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 4.2 /21;Schneider, E.;1991;M-468557-01
Title:	Determination of ethofumesate in water and leaching water high pressure liquid chromatography
Report No:	M-468557-01-1
Document No:	M-468557-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

TASKFORCE

Additional methods were performed, which were not submitted during the first Annex I inclusion process. These studies will be summarized below. For all studies submitted during the frame of the first Annex I inclusion please refer to the corresponding section in the Monograph and in the baseline dossier (D-008920) provided by the Task Force Ethofumesate (tables in grey typeface).

The soil analytical methods were modified in order to follow the state of the art in conduct of analytical methods in this field. These methods have not been taken into account for a corresponding update of the Monograph and thus a detailed description is included here.

The present method validation was performed for the determination of ethofumesate in soil by HPLC-MS/MS using the Multiple Reaction Monitoring (MRM) mode.

Reference:	Method 00806 for the determination of residues of Ethofumesate in soil by HPLC-MS/MS
Author(s), year:	Brumhard, B., 2003
Report/Doc. number:	00806, M-122176-01-1
Guideline(s):	EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.6 BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes Commission Directive 96/46/EC amending Council Directive 91/414/EEC Concerning the Placing of Plant Protection Compounds on the Market of 16 July 1996
GLP:	yes

Reference:	PR00/003 - Confirmation method for the determination of residues of ethofumesate in soil
Author(s), year:	Schneider, E., 2000
Report/Doc. number:	OFC00004917, M-351953-01-1
Guideline(s):	
GLP:	

Principle of method 00806

Soil samples of 20 g were extracted in a microwave with 40 mL of a mixture of water/acetonitrile. After extraction, a subsample of each soil extract is centrifuged to remove fine particles of soil. Identification and quantitation of ethofumesate was done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode.

LC conditions: column: LiChroCART 75-4 Superspher 100 RP 75 mm x 4.0 mm

gradient mobile phases: A: water/acetonitrile/acetic acid (900/100/0.1; v/v/v); B: acetonitrile/acetic acid (1000/0.1; v/v);

MS/MS: MRM transition was used: $m/z = 287 \rightarrow m/z = 121$ (Ethofumesate)

Soil characteristics:

Soil Höfchen, am Hohenseh 4a:		0 – 20 cm soil layer		Soil Laacher Hof, AXXa:		0 – 30 cm soil layer	
pH (in 0.01 M CaCl ₂ solution)		6.7		pH (in 0.01 M CaCl ₂ solution)		6.3	
Organic Carbon [%]		2.11		Organic Carbon [%]		1.02	
Organic Matter [%]		3.63		Organic Matter [%]		1.75	
Cation Exchange Capacity [meq / 100 g dry soil]		15		Cation Exchange Capacity [meq / 100 g dry soil]		8	
max. Water Holding Capacity [g / 100 g dry soil]		63.1		max. Water Holding Capacity [g / 100 g dry soil]		34.4	
Nitrogen [mg N / 100 g dry soil]		200		Nitrogen [mg N / 100g dry soil]		90	
				Fe [mg / kg dry soil]		5090	
Textural Description according to DIN				Textural Description according to USDA			
Fraction	[%]	Fraction	[%]	Fraction	[%]	Fraction	[%]
Clay	10.3	Clay	10.2	not determined		clay	5.0
Silt	81.5	Silt	81.3			silt	22.6
Sand	8.2	Sand	8.5			sand	72.4
Soil type: loamy silt		Soil type: silt				Soil type: sandy loam	

Validation

Specificity

LC/MS/MS using MS/MS transition for detection and quantification of ethofumesate ensures a high level of specificity. The blank values in all control samples were below 1/3 x LOQ.

Linearity

The correlation between the injected amount of substance and the detector response was linear, ranging from 1 µg/L to 100 µg/L (n=7); graph and equation parameter available. The correlation coefficient was > 0.9983.

Accuracy

The mean recoveries at each fortification level are in the range of 70 to 110%.

LOQ

The limit of quantification (LOQ) for ethofumesate in study 00806 is 5 µg/kg in soil. The fortification level of the confirmatory method PR00/003 is 50 µg/kg. Therefore, the limit of quantification (LOQ) for ethofumesate assayed with method 00806 is set at 50 µg/kg in soil.

Repeatability

The procedure was shown to yield repeatable results. During method validation, the relative standard deviations at the individual fortification levels and the overall relative standard deviations ranged between 1.7% and 8.9%.

Confirmatory method

Since only one transition is reported for method 00806, an additional GC-MS method (PR00/003) provided for first Annex I inclusion is submitted for confirmation.

Principle of method PR00/003 for confirmation

The method works according to the following principle: Extraction of ethofumesate from the wet soil with acetone, evaporation of the extract to the aqueous residue, extraction of the aqueous phase with dichloromethane, evaporation of the dichloromethane extract to dryness, re-solution in hexane and performance of silica gel clean up. Finally measurement is performed by means of GC-MS technique in the SIM mode using three specific ions.

GC-MS measurement was used with selected ion monitoring (SIM):

Ethofumesate: m/z = 161 (used for evaluation), m/z = 179, m/z = 286

Specificity

GC-MS using for detection and quantification of ethofumesate ensures a high level of specificity. The blank value was below 10 µg/kg.

Linearity

The correlation between the injected amount of substance and the detector response was linear, ranging from 0.495 µg/ml to 2.97 µg/mL. The correlation coefficient was 0.9898.

LOQ

The fortification level of the confirmatory method PR00/003 is 50 µg/kg. Therefore, the limit of quantification (LOQ) for ethofumesate assayed with method 00806 is set at 50 µg/kg in soil.

Repeatability

The mean recovery (n=3) was 95% (± 2.9%).

According to guidance document 825/00 rev.8.1 3 replicates are in line for confirmation by an independent analytical technique.

Validation summarized in Table B.5.2.3-1.

Conclusion

The LC-MS/MS method 00806 has been sufficiently validated in soil (LOQ = 50 µg/kg or 0.05 mg/kg).

UPL

Based on the uncertainty of a new residue definition for Ethofumesate in soil² the following additional information for monitoring purposes of residues in soil is provided: A new validation of an analytical method for the determination of residues of Ethofumesate and its metabolite NC8493 in soil. The method applies analysis by GC with MS detection. It complies with the requirements of SANCO 825/00 rev. 8.1.

Reference:	Ethofumesate - Validation of an Analytical Method for the Determination of Residues of Ethofumesate and its metabolite NC 8493 in Soil
Author(s), year:	Hamberger, R. (2012b)
Report/Doc. number:	12A04042-01-VMS
Guideline(s):	SANCO/825/00, rev.8.1
GLP:	yes

Principle of method

10 g of the soil sample (BBA 2.3 soil with batch No. F232706), 5 mL water and 20 mL acetonitrile were combined and shaken for at least 12 hours. Thereafter, 1 g sodium chloride and 4 g magnesia sulphate were added and shaken again for approx. 1 min to separate the phases and drying the acetonitrile extract. An aliquot of 10 mL was evaporated to dryness. The dry residue was reconstituted in 2.5 mL n-hexane / ethyl acetate 6:4 (v/v). The extract was applied to a solid-phase column with 0.5 g activated carbon (EnviCarb™, Supelco Na. 57094, equilibrated with 5 mL of hexane/ethyl acetate (6:4, v/v)). The analytes were eluted with 2 × 4 mL hexane/ethyl acetate (6:4, v/v).

All eluates were collected in a pear shaped flask, the solvent evaporated to dryness at 40°C and reconstituted in 1 mL toluene for GC/MS analysis

GC-MS (EI-SIM) conditions: column: Phenomenex ZB-35, 30 m × 0.25 mm i.d., 0.25 µm

Ethofumesate parent compound with three characteristic fragment Ions with m/z = 286 (quantification), m/z = 207 and 161 (confirmation).

NC8493 metabolite with three characteristic fragment ions with m/z = 179 (quantification), m/z = 229 and 258 (confirmation).

Soil (BBA 2.3) characteristics:

Parameter		
pH	(Calcium chloride)	6.5
TOC	[%]	1.14
CEC	[mval/100g]	9.27
Soil Density	(Volumetric method)	[g/L] 1300
Particle sizes according to German DIN		
(2000 to > 630 µm)	[%]	3.5
(630 to ≥ 200 µm)	[%]	25.9
(200 to ≥ 63 µm)	[%]	29.0
(63 to ≥ 20 µm)	[%]	23.2
(20 to ≥ 6.3 µm)	[%]	7.4
(6.3 to ≥ 2 µm)	[%]	2.8
(< 2 µm)	[%]	8.2
Soil type (DIN 4220)		Loamy sand (Si)
Particle sizes according to USDA		
Sand	(2000 to ≥ 50 µm)	[%] 63.9
Silt	(50 to ≥ 2 µm)	[%] 27.1
Clay	(< 2 µm)	[%] 9.0
Soil type (USDA)		Loamy sand

²

According to Sanco 221/2000 rev. 7b metabolites found in soil metabolism studies are to be considered as residues if they have been found in relevant soil degradation and soil metabolism studies at concentrations of 5% of the applied amount (radioactivity) in 2 subsequent samplings.

ValidationSpecificity

GC-MS is highly specific for Ethofumesate and its metabolite NC 8493. Analysis as well of control specimens of soil with GC/MS using three characteristic fragment ions with $m/z > 100$ per analyte yielded no significant residues of Ethofumesate and its metabolite NC8493 above 30% of the LOQ indicating that no significant interferences were present.

Linearity

The linearity was proven by injecting matrix-matched standard solutions in the range from 7.5 µg/L to 500 µg/L (five points, single injection each) for Ethofumesate and in the range from 30 µg/L to 2000 µg/L (five points, single injection each) for metabolite NC8493. Injections of samples were interspersed with injections of matrix-matched standards to provide a continuous check of the instrument calibration.

The calibration graphs for Ethofumesate were linear within the range from 7.5 µg/L to 500 µg/L (corresponding to 0.0015 mg/kg to 0.1 mg/kg in soil) with correlation coefficients of $r^2 \geq 0.9995$ (respectively $r \geq 0.9997$).

The calibration graphs for metabolite NC8493 were linear within the range from 30 µg/L to 2000 µg/L (corresponding to 0.006 mg/kg to 0.4 mg/kg in soil) with correlation coefficients of $r^2 \geq 0.9991$ (respectively $r \geq 0.9995$). Equations are available for each ion fragment.

Accuracy

The mean recoveries at each fortification level are in the range of 70 to 110%.

LOQ

Limit of quantification: LOQ was 0.005 mg/kg for Ethofumesate and 0.02 mg/kg for NC8493.

Repeatability

During method validation, the relative standard deviations at the individual fortification levels relative standard deviations ranged between 1.5% to 4.3% .

Validation is summarized in Table B.5.2.3-1.

Conclusion

The analytical method is sufficiently validated for the determination of ethofumesate and the metabolite NC8493.

Table B.5.2.3-1: Validation results for soil –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Brumhard, B; (2003) M-122476-01-1 Taskforce	ethofumesate	00806 LC-MS/MS m/z 287 →121	Soil Höfchen	0.05*	0.005	104	5.5	5
			Soil Laacher Hof		0.05	92	6.5	5
			Soil Höfchen	0.05*	0.005	90	8.9	5
			Soil Laacher Hof		0.05	90	1.7	5
Schneider, E.; 2000 M-351953-01 confirmatory Taskforce	ethofumesate	PR00/003 GC-MS m/z = 161	Standard soil 2.2	0.05	0.05	95	2.9	3
Hamberger, R. (2012b) UPL	ethofumesate	GC-MS m/z = 286 (quantification)	Standard soil BBA 2.3	0.005	0.005	83	2.6	5
					0.05	80	1.5	5
	ethofumesate	GC-MS m/z = 207 (qualification)		0.005	0.005	84	2.3	5
					0.05	80	1.5	5
	ethofumesate	GC-MS m/z = 161 (qualification)		0.005	0.005	84	0.7	5
					0.05	80	1.5	5
	NC 8493	GC-MS m/z = 179 (quantification)		0.02	0.02	80	3.3	5
					0.20	78	4.3	5
	NC 8493	GC-MS m/z = 258 (qualification)		0.02	0.02	81	2.9	5
					0.20	78	3.3	5
	NC 8493	GC-MS m/z = 229 (qualification)		0.02	0.02	81	2.2	5
					0.20	79	3.3	5

* The confirmatory method PR00/003 (Schneider, E. 2000) is spiked lowest at fortification level 0.05 mg/kg to confirm the LOQ of the primary method 00806. Therefore the LOQ is set at 0.05 mg/kg.

B.5.2.4. Water

TASKFORCE

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

According to Regulation 283/2013 an ILV for drinking water is required. For this reason a new primary method was validated.

Reference:	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS
Author(s), year:	Krebber, R.; Braune, M.; 2013
Report/Doc. number:	MR-13/085, M-466732-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000;
GLP:	yes

Reference:	Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS
Author(s), year:	Stanislawski, T., 2013
Report/Doc. number:	P3117 G
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on Pesticide Residue Analytical Methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 Commission Regulation (EU) No 283/2013 (section 4.2) of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.;
GLP:	yes

Principle of method 01387

The analytical method 01387 was developed for the determination of ethofumesate in drinking and surface water by direct injection into the HPLC-MS/MS instrument without further clean-up. Identification and quantitation of ethofumesate was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation. The method was validated using surface water from the river Rhine.

LC conditions: column: Ascentis Express 2.7 µm C18, 50 mm x 2.1 mm, Supelco (or equivalent)

gradient mobile phases:

A: Deionized water/formic acid (1000/0.12, v/v) + 10 mM ammonium formate;

B: Methanol/formic acid (1000/0.12, v/v) + 10 mM ammonium formate

C: Deionized water/methanol/formic acid 500/500/0.12, v/v/v)

MS/MS: 2 MRM transitions were used: $m/z = 304 \rightarrow m/z = 121$ (quantitation)

$m/z = 304 \rightarrow m/z = 241$ (confirmation)

Surface water characteristics (River Rhine sampled in Leverkusen-Hitdorf):

Parameter	Value
Total organic carbon (TOC)	2 mg/L
Dissolved organic carbon (DOC)	2 mg/L
Conductivity	448 μ S/cm
pH	7.3
Water hardness	9.9 dH
Filterable solids	14 mg/L
Dry residue after filtration	290 mg/L

Validation

Specificity

HPLC-MS/MS using two characteristic MS/MS transitions for quantitation and confirmation is highly specific, therefore an additional confirmatory method is not necessary. Residues in control samples were below 0.3 x LOQ.

Linearity

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for ethofumesate, covering a range from 0.015 to 5.0 μ g/L (n=6) graph and equation parameter are available. The correlation coefficients were 0.9998 for both MRM transitions.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

Limit of Quantitation

The limit of quantification (LOQ) for ethofumesate is 0.05 μ g/L in drinking and surface water.

Repeatability

The relative standard deviations (RSD) during method validation at the individual fortification levels ranged from 1.7 to 2.9% for both MRM transitions.

Reproducibility (ILV)

The independent laboratory validation (ILV) (Stanislowski, T., 2013) uses the same conditions and the same fortification levels as the primary method.

Surface water characteristics (River Danube at Ulm, Germany):

Parameter	Value
Total organic carbon (TOC) (EN 1484:1997)	1.90 mg/L
Dissolved organic carbon (DOC) (EN 1484:1997)	1.7 mg/L
Conductivity (EN 27888:1993)	584 µS/cm (25°C)
pH (DIN 38 404-C 5)	8.16
Water hardness (calculated)	3.20 mmol/L (17.9°d)
Filterable solids (EN 872 Whatman GF 6)	3.1 mg/L

Specificity

HPLC-MS/MS using two characteristic MS/MS transitions for quantitation and confirmation is highly specific, therefore an additional confirmatory method is not necessary. Residues in control samples were below 0.3 x LOQ (0.015 µg/L).

Linearity

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for ethofumesate, covering a range from 0.015 to 1.0 µg/L (n=5) graph and equation parameter are available. The correlation coefficients for both MRM transitions were 0.9995 and 0.9992, respectively.

Accuracy

Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

Limit of Quantitation

The limit of quantification (LOQ) for ethofumesate is 0.05 µg/L in drinking and surface water.

Repeatability

The relative standard deviations (RSD) during method validation at the individual fortification levels ranged from 1.4 to 5.5% for both MRM transitions

Conclusion

The method meets all guideline criteria to determine residues of ethofumesate in drinking and surface water at a limit of quantitation (LOQ) of 0.05 µg/L. A validation for drinking water was not required because the validation of surface water is sufficient.

Validation is summarized in Table B.5.2.4-1

UPL

Based on the uncertainty of a new residue definition for Ethofumesate in surface water and according to latest requirements for a post registration monitoring method for drinking water, the following additional information for monitoring purposes of residues in surface and drinking water is included:

1. A new validation of an analytical method for the determination of residues of Ethofumesate in drinking and surface water by Jooß (2011). The method applies analysis by LC with MS/MS detection using 2 transition ions for quantitation and confirmation. It complies with the requirements of SANCO 825/00 rev. 8.1. The target limit of quantification is 0.05 µg/L.
2. A new validation of an analytical method for the determination of residues of Ethofumesate and its metabolites NC9607 and NC20645 in surface water by Hamberger (2012c). Analysis is carried out by means of GC with MS detection. It complies with the requirements of SANCO 825/00 rev. 8.1.
3. An independent laboratory validation of the method in surface water by Hamberger (2012c).

Reference:	Ethofumesate - Validation of an Analytical Method for the Determination of Ethofumesate in Water
Author(s), year:	Jooß, S. (2011)
Report/Doc. number:	P 2368 G
Guideline(s):	SANCO/825/00 rev. 8.1, 16/11/2010 and SANCO/3029/99 rev. 4
GLP:	yes

Principle of the method

Residues of Ethofumesate were determined in drinking and surface water by direct injection with LC-MS/MS in the positive ion mode, using 2 transition ions for quantitation and confirmation.

LC conditions: column: Supelco, Ascentis Express C₁₈, 2.7 µm particle size, 50 mm length, 2.1 mm i.d. Pre-column: Phenomenex C₁₈, 4 mm length, 3.0 mm i.d. gradient mobile phases:

A: H₂O/MeOH 8/2, v/v with 0.1% formic acid and 5 mM NH₄ acetate ; B: H₂O/MeOH 1/9, v/v with 0.1% formic acid and 5 mM NH₄ acetate

MS/MS: 2 MRM transitions were used: 287 m/z → 121.1 m/z (quantitation)
287 m/z → 259.2 m/z (confirmation)

Water characteristics:

Drinking water (local tap water supply):

pH 7.64, total water hardness: 2.24 mmol/L corresponding to 12.6 °dH. The water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following:

Conductivity at 25°C:	523 µS/cm
Magnesium (EN ISO 11885:1997):	13.1 mg/L
Calcium (EN ISO 11885:1997):	68.3 mg/L

Surface water (River Brenz (Germany):

pH 7.92, total water hardness: 2.59 mmol/L corresponding to 14.5 °dH. The water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following:

Conductivity at 25°C:	569 µS/cm
Magnesium (EN ISO 11885:1997):	4.0 mg/L
Calcium (EN ISO 11885:1997):	97.3 mg/L
DOC (dissolved organic carbon, EN 1484:1997):	0.87 mg/L
Silt content (EN 872 Whatman GF 6):	<0.5 mg/L

Validation

Specificity

HPLC-MS/MS using two characteristic transitions for quantitation and confirmation is highly specific, therefore an additional confirmatory method is not necessary. Residues in control samples were below 20% of the LOQ.

Linearity

The calibration graphs for Ethofumesate were linear within the range from 0.01 µg/L to 0.75 µg/L (n=6). Correlation coefficients (r) were ≥ 0.997 . Plots and equations of the graphs are available.

Accuracy

For both MS/MS transitions monitored, average recoveries for Ethofumesate were in the acceptable range of 70% and 110%,

Limit of Quantitation

The limit of quantification (LOQ) for ethofumesate is 0.05 µg/L in drinking and surface water.

Repeatability

The relative standard deviations (RSD) is $< 20\%$

Reproducibility (ILV)

No independent laboratory validation (ILV) is available.

Conclusion

The analytical method is sufficiently validated; however, an ILV is missing, for the determination of ethofumesate in surface and drinking water.

Validation is summarized in Table B.5.2.4-1

Reference:	Validation of an Analytical Method for the Determination of Residues of Ethofumesate and its Metabolites NC9607 and NC20645 in Surface Water
Author(s), year:	Hamberger, R. (2012c)
Report/Doc. number:	12A04042-01-VMWA
Guideline(s):	SANCO/825/00, rev.8.1.
GLP:	yes
Reference:	Ethofumesate-Independent Laboratory Validation of an analytical method for the determination of Ethofumesate and its metabolites NC 20645 and NC 9607 in surface water
Author(s), year:	Brown D. (2014)
Report/Doc. number:	S13-04250
Guideline(s):	SANCO/825/00, rev.8.1
GLP:	yes

The used method is performed for the determination of residues of Ethofumesate and its metabolites NC 9607 and NC 20645 in surface water.

Principle of the method

Surface water was acidified and the analytes were enriched by solid phase extraction. The solid phase was washed with a water/methanol mixture and pure water. Ethofumesate and its metabolites were extracted with acetonitrile. After evaporation, the eluate was brought to a defined volume with acetonitrile and analyzed using capillary gas chromatography with mass selective detection (GC-MSD).

GC conditions: column: Phenomenex ZB-35, 30 m x 0.25 mm i.d., 0.25 µm Part No. 7HG-G003-11

MS (EI-SIM): Ethofumesate: m/z 286.0 (quantifier), m/z 161.0, 207.0 (qualifiers);

NC 9607: m/z 256.0 (quantifier), m/z 149.0, 150.0 (qualifiers)

NC 20645 is converted to NC 9607 under GC/MS conditions

Surface water characteristics:

Origin	River Enz, Neuenbürg/Germany
pH	7.77
conductivity	130 µS/cm
DOC	45 mg/L
Total hardness	0.37 mmol/L (2.1 dGH)

Validation

Specificity

No significant interferences from the specimen matrix were detected at the retention times corresponding to the analytes in any of the control specimens used for recovery experiments.

Analysis of control specimens of surface water with GC/MS using three characteristic fragment ions with m/z > 100 per analyte yielded no significant residues of Ethofumesate and its metabolites NC 9607 and NC 20645 above 30% of the LOQ indicating that no significant interferences were present.

Linearity

The linearity of the detector response was confirmed by injecting five matrix-matched standard solutions covering the working range of 5 µg/L to 500 µg/L (corresponding to 0.02 µg/L to 2.0 µg/L in surface water) (n=5) for Ethofumesate, metabolite NC 9607 and for metabolite NC 20645.

Correlation coefficients $r^2 \geq 0.997$ for each analyte. Plots and equations of the graphs are available.

Accuracy

The average recoveries for Ethofumesate and the metabolites NC 9607 and NC 20645 were in the acceptable range of 60% and 120%.

Limit of Quantitation

The limits of quantification (LOQs) for ethofumesate and the metabolites NC 9607 and NC 20645 are 0.1 µg/L each.

Repeatability

The relative standard deviations (RSD) is < 30%

Reproducibility (ILV)

The independent laboratory validation (ILV) Brown D. (2014) uses the same method principles and the same fortification levels as the primary method.

Surface water characteristics (Ramsley Brook at Wilson, UK;):

Determinand	Result	Units
Dissolved Organic Carbon	35.4	mg/L
Total Hardness (EDTA titration)	555	mg/L as CaCO ₃
Total Alkalinity	308	mg/L as CaCO ₃
Bicarbonate	376	mg/L
Carbonate	<0.1	mg/L
Electrical Conductivity	1332	µS/cm
pH	7.6	

Specificity

No significant interferences were detected in any of the reagent blank and control specimens. Three ion fragments > m/z 100 were detected for each analyte. Residues detected in the control samples were < 30% LOQ.

Linearity

Linearity was checked in the range of 0.005 µg/mL (corresponding to 0.02µg/L) to 0.5 µg/mL (n=5).

Correlation coefficients are >0.999. Plots and equation parameters of the calibration curve are available.

Accuracy

Recoveries were found between 70 and 120% for each analyte.

Limit of Quantitation

The limit of quantification (LOQ) for ethofumesate is 0.05 µg/L in drinking and surface water.

Repeatability

The relative standard deviations (RSD) was < 20%.

Validation is summarized in Table B.5.2.4-1

Conclusion

The analytical method is sufficiently validated for ethofumesate and the metabolites NC 9607 and NC 20645,

Table B.5.2.4-1: Method validation for ethofumesate in water –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Mean Area counts	RSD [%]	n
Krebber, R.; Braune, M., 2013, M-466732-01 Taskforce enforcement	ethofumesate	01387 LC-MS/MS m/z 304 →121 (quantification)	Surface water	0.05	0.05	7841	2.5	5
		0.5			62499	2.9	5	
		01387 LC-MS/MS m/z 304 → 241 (qualification)		0.05	0.05	15172	2.9	5
					0.5	113583	1.7	5
Stanislawski, T., 2013, M-470714- 02 ILV Taskforce	ethofumesate	LC-MS/MS m/z 304 → 121 (quantification)		0.05	0.05	36871	5.5	5
		0.5			298009	1.4	5	
		LC-MS/MS m/z 304 → 241 (qualification)		0.05	0.05	103425	2.7	5
					0.5	996780	1.0	5

References	Analyte	Detection method	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n	
Jooß, S. (2011) UPL	ethofumesate	LC-MS/MS 287 m/z → 121 m/z (quantification)	Drinking water	0.05	0.05	105	4	5	
		0.5			81	3	5		
		LC-MS/MS 287 m/z → 259 m/z (qualification)		0.05	0.05	101	5	5	
					0.5	81	4	5	
		LC-MS/MS 287 m/z → 121 m/z (quantification)	Surface water	0.05	0.05	95	2	5	
					0.5	108	1	5	
				LC-MS/MS 287 m/z → 259 m/z (qualification)	0.05	0.05	95	3	5
						0.5	108	3	5

References	Analyte	Detection method	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Hamberger, R. (2012c) UPL enforcement	ethofumesate	GC-MS m/z 286 (quantifier)	Surface water	0.1	0.1	102	2.3	5
					1.0	100	3.0	5
	NC 9607	GC-MS m/z 256 (quantifier)		0.1	0.1	97	3.9	5
					1.0	83	2.5	5
	NC 20645 determined as NC 9606	GC-MS m/z256 (quantifier)		0.1	0.1	64	5	5
					1.0	73	6.8	5
	ethofumesate	GC-MS m/z 207 (qualifier)		0.1	0.1	101	3.8	5
					1.0	100	3.0	5
	NC 9607	GC-MS m/z 149 (qualifier)		0.1	0.1	96	3.2	5
					1.0	83	2.5	5
	NC 20645 determined as NC 9606	GC-MS m/z 150 (qualifier)		0.1	0.1	64	5	5
					1.0	73	6.8	5
	ethofumesate	GC-MS m/z 161 (qualifier)		0.1	0.1	101	3.8	5
					1.0	99	3.0	5
	NC 9607	GC-MS m/z 149 (qualifier)		0.1	0.1	96	2.9	5
					1.0	84	2.0	5
	NC 20645 determined as NC 9606	GC-MS m/z 150 (qualifier)		0.1	0.1	63	3.6	5
					1.0	74	8.0	5
Brown D. (2014) ILV UPL	ethofumesate	GC-MS SIM 3 fragment ions	Surface water	0.1	0.1	100	2.0	5
					1.0	98	3.0	5
	NC 9607			0.1	0.1	96	2.0	5
					1.0	99	1.5	5
	NC 20645 determined as NC 9606			0.1	0.1	99	2.7	5
					1.0	98	2.4	5

B.5.2.5. Air

This issue is addressed in the DAR for first inclusion. No confirmatory is required for matrix air if the analyte is sufficiently validated in the matrices soil and water.

B.5.2.6. Body fluids and tissues

According to guidance document SANCO/825/00 rev. 8.1 no method is required since ethofumesate is not classified. However, to be in line with Regulation 1107/99 analytical methods are available for animal matrices including tissues (meat) and fluids (milk) in this DRAR (Jooß S., 2012) and is as well addressed for dog plasma (McKenzie 1994) in the original DAR (1998).

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	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1 /08	Fuchsichler, G.	1989	Ethofumesat und 2-oxo-2,3-dihydro-3,3-dimethyl-benzofurane-5-yl-methansulfonat (ethofumesat-2-keto), bestimmung in zuckerrueben Universität München; Freising; Germany Feinchemie Schwebda, Report No.: HVA 10/89, Edition Number: <u>M-463306-01-1</u> Date: 1989-07-12 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /19	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.1.1 /09	Fuchsichler, G.	1992	Ethofumesate; SC500; sugar beet; Germany; BBA Technische Universitaet Muenchen, Weihenstephan, Freising, Germany Feinchemie Schwebda , Report No.: HVA 14/91, Report includes Trial Nos.: FSG 3189-H-RI-A FSG 3189-H-RI-B FSG 3189-H-RIV-A FSG 3189-H-RIV-B Edition Number: <u>M-357851-01-2</u> Date: 1992-09-09 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /20	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.1.1 /10	Schneider, E.	1991	Bestimmung von Ethofumesat und Ethofumesat-2-keto in Spinat und Mais - Gaschromatographische Methode mit massenselektivem Detektor - not applicable - Feinchemie Schwebda, Report No.: <u>M-463329-01-1</u> . Edition Number: <u>M-463329-01-1</u> Date: 1991-02-02 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /21	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.1.1 /11	Krebs, G.; Schneider	1992	Determination of ethofumesate and phenmedipham in sugar beet plants high-pressure liquid chromatography - not applicable - Feinchemie Schwebda, Report No.: DrK063, Edition Number: <u>M-463318-01-1</u> Date: 1992-07-07 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /22	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.1.1 /12	Eichelmann, C.; Krautstrunk, G.	2011	Analytical method - Determination of AE B049913 (Ethofumesate) in technical grade and pure active substance by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: AM022208FP2, Edition Number: <u>M-411668-01-1</u> Date: 2011-08-03 GLP/GEP: no, unpublished	N	Y	Revised analytical method	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 4.1.1 /13	Eichelmann, C.; Krautstrunk, G.	2011	Amendment no 1 to study report PA11/058 - Validation of HPLC-analytical method AM022208FP2 - Determination of AE B049913 (Ethofumesate) in technical grade and pure active substance by high performance liquid chromatography (HPLC) Bayer CropScience, Report No.: PA11/058 A1, Edition Number: <u>M-411672-02-1</u>	N	Y	2011-02-17 to 2011-05-24 Validation according to SANCO/3	Bayer CropScience	Submitted for the purpose of renewal (2014)

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
			Date: 2011-08-03 ...Amended: 2012-06-01 GLP/GEP: yes, unpublished			030		
KCA 4.1 1 /22	Bittner, P.; Grimmig, B.	2006	Water determination according to Karl Fischer (CIPAC MT 30.5) Bayer CropScience, Report No.: PM000903MF3, Edition Number: <u>M-106281-03-2</u> Date: 2006-09-18 ...Amended: 2007-07-24 GLP/GEP: no, unpublished	N	Y		Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 4.2 /01	Specht	1988	Verification of the applicability of the DFG multi- method S 19 for the quantitative determination of residues of ethofumesate in water and beet. Dr. Specht & Partner, Chemische Laboratorien GmbH, Germany Bayer CropScience, Report No.: A89831, Edition Number: <u>M-155359-01-2</u> Date: 1988-09-06 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /02	Wrede, A.	1997	Ethofumesate AE B049913 (Hoe 082551, ZK 49913) Analytical method for the determination of residues of ethofumesate and its metabolite NC 9607 (AE C509607) in sugar beets and chickpeas by GC Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A89866, Report includes Trial Nos.: CR 96/021 Edition Number: <u>M-165538-01-1</u> Date: 1997-01-31 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /17	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /03	Reichert, N.	2000	Independent laboratory validation of the method of analysis for the determination of ethofumesate and metabolite NC 9607 (AE C0509607) in sugar beet and pea Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Bayer CropScience, Report No.: C009953, Edition Number: <u>M-199578-01-1</u> Date: 2000-10-10 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /04	Wrede, A.	2000	Validation of the method AL 081/96-0 in peas and sugar beet roots by GC-MSD - ethofumesate - Code: AE B049913 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C009934, Edition Number: <u>M-199547-01-1</u> Date: 2000-09-28 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /18	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /05	Schneider, E.	2000	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in sugar beet roots and sugar beet leafs UCL GmbH, Koeln, Germany Feinchemie Schwebda , Report No.: OFC00004912, Edition Number: <u>M-351870-01-1</u> Date: 2000-02-23	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)

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
KCA 4.2 /06	Witte, A.	2000	GLP/GEP: yes, unpublished Residue analysis of ethofumesate and ethofumesate-2-keto in sugar beet - Independent laboratory validation (ILV) GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Feinchemie Schwebda, Report No.: OFC00004913, Edition Number: <u>M-351894-01-1</u> Date: 2000-06-29 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /07	Tew, E. L.; Cole, M.	2001	Analytical method for the determination of Ethofumesate and its metabolites NC 9607, NC 8493 and NC 20645 in sugar beet roots and tops Aventis CropScience; Bayer CropScience, Report No.: C045437, Edition Number: <u>M-237088-01-1</u> Date: 2001-10-31 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /23	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /08	Eckert, J. A.	2001	Independent laboratory Validation of Aventis CropScience Method-Analytical Method for the Determination of Ethofumesate and Its Metabolites, NC 9607, MC 8493 and NC 20645 in Sugar Beet Roots and Tops Enviro-Bio-Tech, Ltd., (EBT), Bernville, PA, USA Bayer CropScience, Report No.: B003792, Report includes Trial Nos.: AV-01-01 Edition Number: <u>M-240796-01-1</u> EPA MRID No.: 45818104 Date: 2001-12-13 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /09	Luettgens, U.	1994	Ethofumesate - Validation of an analytical method for determination of ethofumesate residues in meat Dr.Krebs Analytik, Koeln, Germany Feinchemie Schwebda, Report No.: OFC00004920, Edition Number: <u>M-351945-01-1</u> Date: 1994-12-20 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /10	Peatman, M. H.	1999	Review of analytical methodology for residues in edible animal products (dairy, tissues, fat and offal) Ethofumesate AE B049913 AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report No.: C003328, Edition Number: <u>M-185949-01-1</u> GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /28	N	N	-	Bayer CropScience	In DAR (1998)
KCA 4.2 /11	Rzepka, S.	2004	Independent laboratory validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in food of animal origin Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Feinchemie Schwebda, Report No.: OFC00004922, Report includes Trial Nos.:	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)

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
			AZ.G04-0038 Edition Number: <u>M-234011-01-1</u> Date: 2004-06-29 GLP/GEP: yes, unpublished					
KCA 4.2 /12	Dorn, U.	2006	Independent Laboratory Validation of an Analytical Method for the Determination of Ethofumesate and Ethofumesate-2-keto in Meat PTRL Europe GmbH, Ulm, Germany Feinchemie Schwebda , Report No.: OFC00012323, Edition Number: <u>M-271716-01-1</u> Date: 2006-02-13 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /13	Schneider, E.	1999	Validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in surface water (determination limit of 0.1 mcg/L; monitoring method) Dr. Krebs Analytik GmbH, Koeln, Germany Feinchemie Schwebda , Report No.: C006011, Edition Number: <u>M-193143-01-1</u> Date: 1999-11-02 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /08	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /14	Schneider, E.	1995	Ethofumesate - Determination of ethofumesate in soil with a determination limit of 50ug/kg - Monitoring method Dr.Krebs Analytik, Koeln, Germany Feinchemie Schwebda , Report No.: OFC00004916, Edition Number: <u>M-351950-01-1</u> Date: 1995-03-03 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /15	Schneider, E.	2000	PR00/003 - Confirmation method for the determination of residues of ethofumesate in soil Dr.Krebs Analytik, Koeln, Germany Feinchemie Schwebda , Report No.: OFC00004917, Edition Number: <u>M-351953-01-1</u> Date: 2000-02-24 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /16	Schneider, E.	2000	PR00/002 - Validation of an analytical method for the determination of residues of ethofumesate in air - Monitoring method UCL GmbH, Koeln, Germany Feinchemie Schwebda , Report No.: OFC00004919, Edition Number: <u>M-351963-01-1</u> Date: 2000-02-24 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /17	Fuchsbichler, G.; Frank, C.	1990	Eine Methode zur Bestimmung von ethofumesat in Trinkwasser mittels Gaschromatographie Bayerische Hauptversuchsanstalt fuer Landwirtschaft der Technischen Universitaet Muenchen-Weihenstephan, Germany Feinchemie Schwebda, Report No.: <u>M-468443-01-1</u> , Edition Number: <u>M-468443-01-1</u> Date: 1990-01-02 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /18	Schneider, E.	1992	Determination of ethofumesate in the soil of rotating crops Dr. Krebs Analytik, Köln, Germany	N	N	-	Adama (formerly Feinchemie	In DAR (1998)

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
			Feinchemie Schwebda, Report No.: <u>M-468435-01-1</u> , Edition Number: <u>M-468435-01-1</u> Date: 1992-06-22 GLP/GEP: yes, unpublished				Schwebda)	
KCA 4.2 /19	Schneider, E.	1992	Determination of ethofumesate and phenmedipham in soil high pressure liquid chromatography Dr. Krebs Analytik, Köln, Germany Feinchemie Schwebda, Report No.: <u>M-468439-01-1</u> , Edition Number: <u>M-468439-01-1</u> Date: 1992-07-07 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /20	Schneider, E.	1994	PR93/016 – Validation of analytical method DrK078 – Determination of ethofumesate in air Dr. Krebs Analytik, Köln, Germany Feinchemie Schwebda, Report No.: <u>M-468448-01-1</u> , Edition Number: <u>M-468448-01-1</u> Date: 1994-02-16 GLP/GEP: yes, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /21	Schneider, E.	1991	Determination of ethofumesate in water and leaching water high pressure liquid chromatography Dr. Krebs Analytik, Köln, Germany Feinchemie Schwebda, Report No.: <u>M-468557-01-1</u> , Edition Number: <u>M-468557-01-1</u> Date: 1991-04-03 GLP/GEP: no, unpublished	N	N	-	Adama (formerly Feinchemie Schwebda)	In DAR (1998)
KCA 4.2 /22	Thom, M.	2005	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in various plant commodities GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Feinchemie Schwebda , Report No.: OFC00004832, Edition Number: <u>M-351876-01-1</u> Date: 2005-01-17 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment.	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 4.2 /23	Klimmek, S.	2005	Independent laboratory validation of an analytical method for the determination of ethofumesate and ethofumesate-2-keto in plant commodities Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Feinchemie Schwebda , Report No.: OFC00004833, Edition Number: <u>M-351896-01-1</u> Date: 2005-04-05 GLP/GEP: yes, unpublished	N	Y	Needed for risk assessment.	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 4.2 /24	Thom, M.	2004	Validation of an analytical method for the determination of residues of ethofumesate and ethofumesate-2-keto in food of animal origin GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Feinchemie Schwebda , Report No.: OFC00004921, Edition Number: <u>M-351880-01-1</u> Date: 2004-03-15 GLP/GEP: yes, unpublished	N	Y	Needed for dietary risk assessment.	Adama (formerly Feinchemie Schwebda)	Submitted for the purpose of renewal (2014)
KCA 4.2	Mende, P.	2009	Validation of an analytical method for the	N	Y	Needed	Adama	Submitte

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
/25			determination of ethofumesate and ethofumesate-2- keto in liver, kidney and fat Eurofins-GAB GmbH, Niefem-Oeschelbronn, Germany Feinchemie Schwebda , Report No.: S09-00540, Edition Number: <u>M-358951-01-1</u> Date: 2009-09-11 GLP/GEP: yes, unpublished			for risk assessment t.	(formerly Feinchemie Schwebda)	d for the purpose of renewal (2014)
KCA 4.2 /26	Brumhard, B.	2003	Method 00806 for the determination of residues of Ethofumesate in soil by HPLC-MS/MS Bayer CropScience, Report No.: 00806, Edition Number: <u>M-122176-01-1</u> <u>Method Report No.: MR-038/03</u> Date: 2003-02-05 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /30	N	Y	Needed for risk assessment t.	Bayer CropScience	Submitte d for the purpose of renewal (2014)
KCA 4.2 /27	Krebber, R.; Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Bayer CropScience, Report No.: MR-13/085, Edition Number: <u>M-466732-01-1</u> <u>Method Report No.: MR-13/085</u> Date: 2013-10-09 GLP/GEP: yes, unpublished	N	Y	Enforcem ent analytical method for water meeting all guideline criteria	Task Force Ethofumesate	Submitte d for the purpose of renewal (2014)
KCA 4.2 /28	Class, T.	2013	Independent laboratory validation of the BCS method 01333 and 01387 for the determination of various pesticides in surface water by DI-HPLC-MS/MS PTRL Europe GmbH, Ulm, Germany Report No.: P3117G, Edition Number: <u>M-468318-01-1</u> <u>Method Report No.: P3117G</u> Date: 2013-10-15 GLP/GEP: yes, unpublished	N	Y	Validatio n of the Enforcem ent analytical method for water meeting all guideline criteria	Task Force Ethofumesate	Submitte d for the purpose of renewal (2014)
KCA 4 2/01	Schlewitz, P.	2013a	VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE (FREE FORM), NC 9607 (FREE FORM) AND NC 20645 (FREE AND CONJUGATED FORM) IN HIGH PROTEIN/STARCH CONTENT, HIGH WATER CONTENT, HIGH OIL CONTENT, HIGH ACID CONTENT AND DIFFICULT COMMODITIES United Phosphorus Ltd., R B3016 Anadiag S.A., Haguenau, France GLP: yes Published: no	N	Y	New data for active ingredie nt, not previous ly submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA 4 2/03	Jooß, S.	2012	ETHOFUMESATE - VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF THE ETHOFUMESATE AND ITS TWO METABOLITES NC 9607 AND NC 20645 IN FOODSTUFFS OF ANIMAL ORIGIN United Phosphorus Ltd., P 2371 G PTRL Europe, Ulm, Germany GLP: yes Published: no	N	Y	New data for active ingredie nt, not previous ly submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA 4 2/04	Schlewitz, P.	2013b	INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL	N	Y	New data for	UPL	Submitte d for the

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			METHOD FOR THE ANALYSIS OF ETHOFUMESATE AND ITS TWO METABOLITES NC 9607 AND NC 20645 IN FOODSTUFFS OF ANIMAL ORIGIN United Phosphorus Ltd., R B1218 Anadiag S.A., Haguenau, France GLP: yes Published: no			active ingredie nt, not previously submitte d nor evaluate d		purpose of renewal (2014)
KCA 4 2/05	Schlewitz, P.	2013c	STATEMENT TO DEVIATION NO. 121214 OF STUDY B1218 United Phosphorus Ltd., B1218 Anadiag S.A., Haguenau, France GLP: yes Published: no	N	Y	New data for active ingredie nt, not previously submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA 4 2/06	Hamberger, R.	2012b	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES NC8493 IN SOIL AgriChem B.V., 12A04042-01-VMS CIP Chemisches Institut Pforzheim GmbH, Germany GLP: yes Published: no	N	Y	New data for active ingredie nt, not previously submitte d nor evaluate d	ACM*	Submitte d for the purpose of renewal (2014)
KCA 4 2/07	Jooß, S.	2011	ETHOFUMESATE - VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE IN WATER United Phosphorus Ltd., P 2368 G PTRL Europa, Ulm, Germany GLP: yes Published: no	N	Y	New data for active ingredie nt, not previously submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA 4 2/08	Hamberger, R.	2012c	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF ETHOFUMESATE AND ITS METABOLITES NC9607 AND NC20645 IN SURFACE WATER AgriChem B.V., 12A04042-01-VMWA CIP Chemisches Institut Pforzheim GmbH, Germany GLP: yes Published: no	N	Y	New data for active ingredie nt, not previously submitte d nor evaluate d	ACM*	Submitte d for the purpose of renewal (2014)
KCA 4 2/09	Brown, D.	2014a	ETHOFUMESATE - INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE AND ITS METABOLITES NC 20645 AND NC 9607 IN SURFACE WATER United Phosphorus Ltd., S13-04250 Eurofins Agrosience Service GmbH GLP: yes Published: no	N	Y	New data for active ingredie nt, not previously submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA	Heintze, A.	2003a	VALIDATION OF AN ANALYTICAL	N	Y	New	UPL	Submitte d for the

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
4 2/10			METHOD FOR THE DETERMINATION OF ETHOFUMESATE IN AIR. United Phosphorus Ltd., 20021050/01- CMLU GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no			data for active ingredie nt, not previous ly submitte d nor evaluate d		purpose of renewal (2014)
KCA 4 2/11	Heintze, A.	2003b	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE-2-KETO IN AIR. United Phosphorus Ltd., 20021049/01- CMLU GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany GLP: yes Published: no	N	Y	New data for active ingredie nt, not previous ly submitte d nor evaluate d	UPL	Submitte d for the purpose of renewal (2014)
KCA 4 2/12	Rooseboom -Reimers, A.	2003	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE AND 2- KETOETHOFUMESATE IN AIR AgriChem B.V., V4799/02 TNO Nutrition and Food Res., Zeist, The Netherlands GLP: yes Published: no	N	Y	New data for active ingredie nt, not previous ly submitte d nor evaluate d	ACM*	Submitte d for the purpose of renewal (2014)

* 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.