

# ***European Commission***



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

**ETHOFUMESATE**

**Volume 3 – B.7 (AS)**

Rapporteur Member State: Austria

Co-Rapporteur Member State: Denmark

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## Version History

When	What
1998	Initial DAR
May 2000	Addendum: Supplemental Evaluation and Assessment
21 December 2000	Addendum to the Monograph, Rev. 2
2015/01	DRAR

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## **B.7. RESIDUE DATA**

*For B.7 Residue data, all individual studies, whether performed on the active substance or on formulated products, are evaluated in this Volume, i.e. Volume 3 (AS). The main reason to handle residue data different than data for the other sections is that the revised data requirements do not specify requirements for residue data on the formulated products. Additionally (and in contrast to other sections of the evaluation) there is no expected benefit for the product authorisation step to have residue data presented as per product.*

### **INTRODUCTION**

Ethofumesate is an herbicidal active substance and was included into Annex I of Directive 91/414 in 2002. Directive 91/414/EEC has been repealed by Regulation (EC) No 1107/2009 of 21 October 2009 concerning the placing of plant protection products on the market. Accordingly ethofumesate is deemed to be approved under Regulation (EC) No 1107/2009, as set out in Part A of the Annex of Commission Implementing Regulation (EC) No 540/2011 as regards the list of approved substances (entry No. 29).

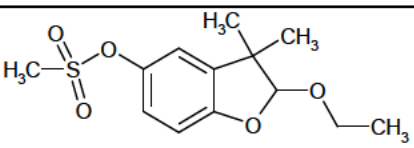
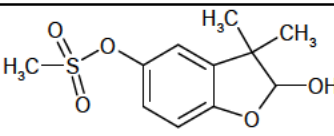
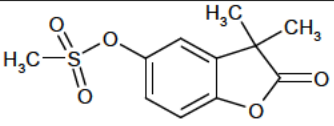
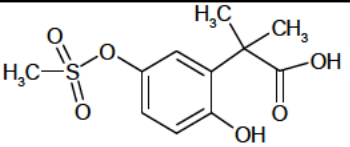
This renewal assessment report (DRAR) contains summaries of studies on ethofumesate, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. All studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC, are contained in the Monograph (DAR Ethofumesate, 2000) 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 this report. However to increase the readability and comprehensibility of the present renewal assessment report, main data and results of some previously evaluated studies are briefly summarized.

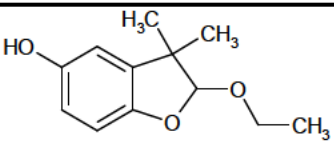
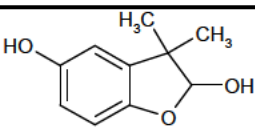
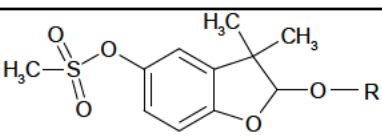
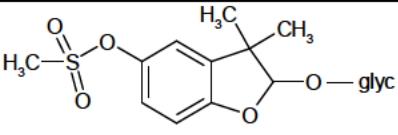
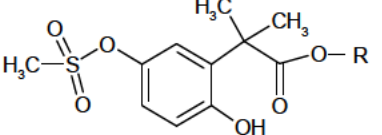
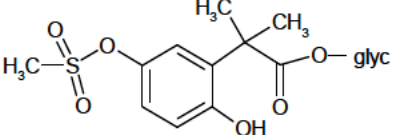
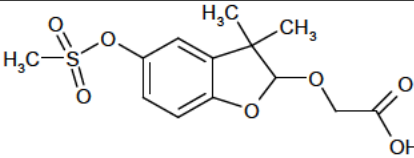
Ethofumesate is a racemic mixture of (two) enantiomers. The synthesis process for ethofumesate does not involve chiral auxiliaries and therefore the resulting isomeric mixture has always been a racemic one (1:1). The herbicidal activity of the two enantiomers has been shown to be equivalent and not different 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.

In the scope of the submission of the original Annex II dossier by the Task Force Ethofumesate in 1996, several uses were supported with residue trial data, e.g. in strawberries, legume vegetables, pulses, beetroot (red beet), sugar beet, fodder beet, tobacco, and grasses (ryegrass, pasture, grassland).

In the ethofumesate renewal dossiers ("AIR3") the present notifiers have included only one "representative use" on sugar beet (beetroot and fodder beet) in northern and southern Europe.

The presented studies used different synonyms and codes for the active substance ethofumesate, its metabolites and the reference compounds used. In order to enhance a common basis for the evaluation a list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound is presented below. The matrices in which the metabolites were identified are also included in this list. In the present dossier section generally the name or the corresponding "NC-code" (e.g. ethofumesate-2-hydroxy and NC 8493) were used.

Code Number (Synonyms)	Description	Compound found in:	Structure
<b>Ethofumesate</b>  Synonym: a.s. <b>NC 8438,</b> AE B049913	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate (IUPAC in addendum 8) 5-Benzofuranol, 2-ethoxy-2,3-dihydro-3,3-dimethyl-, methanesulfonate (CAS) [CAS No.: 26225-79-6]	All matrices	
<b>Ethofumesate-2-hydroxy</b>  Synonym: <b>NC 8493,</b> AE C508493, BCS-BB94377, hydroxy-derivative, 2-hydroxy-ethofumesate, Fumesate	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate (IUPAC in addendum 8)	Animals Rat Lactating cow Laying hen  Plants Sugar beet Ryegrass CRC  Soil Soil aerobic Soil anaerobic  Water Photolysis in water	
<b>Ethofumesate-lactone</b>  Synonym: <b>NC 9607,</b> AE C509607, 2-keto- <b>Ethofumesate,</b> Ethofumesate-2-keto, Oxo-derivative, Fumesate lactone	2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulfonate (IUPAC in addendum 8)	Animals Rat Lactating cow Laying hen  Plants Sugar beet Ryegrass CRC  Soil Soil aerobic Soil anaerobic	
<b>Ethofumesate-carboxylic acid</b>  Synonym: <b>NC 20645,</b> 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 in addendum 8)	Animals Rat Lactating cow Laying hen  Plants Sugar beet Onion Tobacco Ryegrass CRC  Soil Soil aerobic Soil anaerobic  Water Water/sediment Aerobic mineralization in surface water	

Code Number (Synonyms)	Description	Compound found in:	Structure
Ethofumesate–5- hydroxy  Synonym: NC 10458 AE C510458	2-ethoxy-3,3-dimethyl-2,3- dihydro-1-benzofuran-5-ol (IUPAC)	Plants CRC (in traces following acidic extraction in radish foliage)  Soil (in traces)  Water (in traces in natural water photolysis)	
Ethofumesate–2,5- dihydroxy  Synonyms: NC 17900 AE C517900	3,3-dimethyl-2,3-dihydro-1- benzofuran-2,5-diol (IUPAC)	Plants CRC (in traces following acidic extraction in radish foliage)  Soil (only proposed)  Water (in traces in natural water photolysis)	
<b>Ethofumesate–2- hydroxy-conjugate</b> :		Plants Sugar beet Tobacco Ryegrass CRC	 the conjugate R in crops was not identified
Ethofumesate–2- hydroxy-glycoside		Soil Preparative soil aerobic study for identification of unknown metabolites in lysimeter leachate	 aglycon ethofumesate–2-hydroxy identified, hexose not specified
<b>Ethofumesate– carboxylic acid conjugate</b>		Plants Sugar beet Onion Tobacco Ryegrass	 the conjugate R in crops was not identified
Ethofumesate– carboxylic acid glycoside		Soil: Preparative soil aerobic study for identification of unknown metabolites in lysimeter leachate	 aglycon ethofumesate–carboxylic acid identified, hexose not specified
Ethofumesate-acetic acid  Synonym: BCS-CW35117	((3,3-dimethyl-5- [(methylsulfonyl)oxy]-2,3- dihydro-1-benzofuran-2- yl)oxy)acetic acid (IUPAC)	Water aerobic mineralization in surface water	

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Code Number (Synonyms)	Description	Compound found in:	Structure
Methanesulfonic acid  Synonym: MSA, Methansulphonic acid	Methanesulfonic acid	Plants excluded in sugar beet as representative matrix for plants  All other matrices: not significant in all other matrices (but not analyzed in soil, water)	CH <sub>3</sub> -SO <sub>3</sub> H
Carbon dioxide  Synonym: CO <sub>2</sub>		Soil	CO <sub>2</sub>

### **B.7.1. STORAGE STABILITY OF RESIDUES**

As residue samples in trials with ethofumesate were routinely stored frozen for longer periods of time prior to their analysis, the stability of residues during storage of samples and effects of frozen storage on the residue levels were investigated.

The storage stability of ethofumesate residues in sample extracts is routinely tested during method development. Since the validity of the methods is based on and confirmed by factors such as reproducibility for interruption during the work-up process, it can be concluded that the stability of residues in extracts is always guaranteed. In addition, when conducting analyses of "normal" samples, the entire analytical procedure is monitored by conducting concurrent recoveries with each sample set.

#### **B.7.1.1. Plant Matrices**

In the Monograph prepared in the framework of Directive 91/414/EEC the storage stability of ethofumesate and its metabolite ethofumesate-lactone (NC 9607) was assessed for sugar beets. The results of the respective studies indicated that ethofumesate and its metabolite are stable in deep-frozen samples ( $\leq -18\text{ }^{\circ}\text{C}$ ) of the tested plant commodities (roots and leaves) for at least 1 or 2 years, respectively.

An additional study on the storage stability of ethofumesate and its metabolites NC 9607 and NC 8493 in grass was submitted in 2005. The results indicated that ethofumesate and its metabolites NC 9607 and NC 8493 exhibited good stability during frozen storage in grass over a storage period of at least 18 months.

##### *New data for AIR:*

The storage period from the original studies sufficiently covers the longest period of time for which samples from new field residue trials presented in this DRAR were stored before analysis. Hence, the results of the storage stability studies validate the residue values obtained from these trials (chapter B.7.3) with respect to the stability of ethofumesate and its metabolites NC 9607 and NC 8493 in deep-frozen samples with high water and high starch content.

Although metabolite NC 20645 and its conjugates form the major residues in plant matrices, metabolite NC 20645 was never included in one of the submitted storage stability studies.

Therefore, additional data has been generated to investigate the storage stability of ethofumesate and its metabolites in different plant matrices.

##### **B.7.1.1.1. Storage stability of NC 20645 and conjugated NC20645 in plant matrices**

An interim report of a new storage stability study has been submitted to prove the storage stability of NC 20645 for at least 6 months.

The structure of NC 20645 conjugate (endocon from the conjugated metabolite) was not elucidated. Therefore NC 20645 as the exocon from the conjugated metabolite was chosen as representative compound to prove the stability in deep-frozen samples. During analysis, the conjugate and its exocon are converted to the common moiety NC 9607, which is the analytical target.



Report:	KCA 6.1 /04;Schulte, G.; 2013; M-459806-01
Title:	STORAGE STABILITY OF OPEN-RING-2-KETO ETHOFUMESATE (AE C520645) IN PLANT MATRICES FOR 24 MONTHS - PHASE REPORT AFTER 6 MONTHS
Report No:	MR-13/086
Document No:	M-459806-01-1
Guidelines:	- Regulation (EC) No 1107/2009 -OECD Guidelines for the Testing of Chemicals. Stability of Pesticide Residues in Stored Commodities. 506. 2007-10-16 -US EPA OCSPP 860.1380, Storage Stability Data; Deviations not specified
GLP/GEP:	yes

## I. Materials and Methods

To determine the freezer stability of the analyte NC 20645 in plant materials, individual 10g control samples of sugar beet leaf (high water content), sugar beet body (high starch content), rape seed (high oil content), dry bean seed (high protein content) and orange fruit (high acid content) were spiked with 1 µg NC 20645, resulting in a fortification level of 0.1 mg/kg.

Except for the day-0 analysis, samples were stored in glass containers in a freezer at  $\leq -18^{\circ}\text{C}$  for later use. For day-0 analysis, five treated samples of each material were chosen, as well as two control sample of each. In addition, two recoveries spiked at the respective LOQ level and two recoveries spiked at the 10-fold LOQ level were analysed. After 30, 90 and 180 days three fortified and three control samples of each plant material were removed from the deep-freezer and allowed to reach room temperature. Subsequently, two of the control samples of each plant material were fortified with the test items to determine the concurrent recoveries (the fortification level was at 0.01 mg/kg as the spiked storage samples). The samples were extracted and analyzed concurrently with the third control sample and the spiked storage samples.

NC 20645 was determined using analytical method 01343 (cf. study MR-12/056, Schulte, G.; 2013; M-448288-01; KCA 4.1.2/36), which was validated prior to and parallel to the analysis of the stored samples.

Method 01343 was developed for the determination of open-ring-2-keto-ethofumesate (NC 20645) in/on plant materials. Open-ring-2-keto-ethofumesate (NC 20645) was extracted from sugar beet leaf, sugar beet body, rape seed, dry bean and orange fruit, with acetonitrile/water (4/1, v/v) using a shaker. After filtration of the extract, the stable isotopically labelled internal standard was added. The internal standard is hydrolysed during analysis to the corresponding phenyl- $^{13}\text{C}_6$  open-ring-2-keto-ethofumesate. 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 internal stable labeled standards.

The LOQ was 0.01 mg/kg for NC 20645 (expressed in analyte equivalents).

## II. Findings

The mean values of the concurrent recovery rates per sample material, and spiking level were in the range of 86-101%, with relative standard deviations clearly below 20%. Details of recovery data are shown in Table 7.1.1-6. At day 0, average residue recoveries of NC 20645 ranged from 91-101% of nominal. In samples analysed after approximately 6 months of frozen storage (185-187 days), storage stability recoveries, corrected to day 0, ranged from 84-100% for NC 20645. The non-corrected recoveries ranged from 82-94%. At all sampling dates and in all sample materials, NC 20645 was recovered above 80%. All recovery samples are summarized in the following tables. There was no evidence of any continued degradation of the analyte in any of the sample

materials. Thus, NC 20645 can be considered stable in all relevant plant matrix types for a period of at least six month.

Analytical investigations of samples stored up to 24 months will follow.

**Table 7.1.1-1 Storage stability data and concurrent recovery data for NC 20645 in sugar beet leaf**

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Sugar beet leaf	NC 20645						
	0	0.0966 0.0918 0.0881 0.0869 0.0911	97 92 88 87 91	91	100	87	105
	30	0.0952 0.0962 0.0937	95 96 94	95	104	95	101
	89	0.0898 0.0900 0.0897	90 90 90	90	99	89	102
	187	0.0781 0.0830 0.0851	78 83 85	82	90	90	92
	360	Ongoing					
	540	Ongoing					
	720	Ongoing					

**Table 7.1.1-2 Storage stability data and concurrent recovery data for NC 20645 in sugar beet body**

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Sugar beet body	NC 20645						
	0	0.0922 0.0910 0.0940 0.0951 0.0966	92 91 94 95 97	94	100	102	92
	30	0.0873 0.0846 0.0879	87 85 88	87	92	86	101
	89	0.0952 0.0976 0.0955	95 98 95	96	102	92	104
	187	0.0933 0.0927 0.0956	93 93 96	94	100	97	97
	360	Ongoing					
	540	Ongoing					
	720	Ongoing					

**Table 7.1.1-3 Storage stability data and concurrent recovery data for NC 20645 in rape seed**

Table 7.1.1-5 Storage stability data and concurrent recovery data for NC 20645 in Rape seed							
Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Rape seed	NC 20645						
	0	0.0977 0.0952 0.0964 0.0978 0.0987	98 95 96 98 99	97	100	92	106
	31	0.0922 0.0911 0.0887	92 91 89	91	93	98	93
	89	0.0747 0.0757 0.0702	75 76 70	74	76	97	76
	185	0.0826 0.0781 0.0849	83 78 85	82	84	97	85
	360	Ongoing					
	540	Ongoing					
	720	Ongoing					

**Table 7.1.1-4 Storage stability data and concurrent recovery data for NC 20645 in dry bean seeds**

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Dry bean	NC 20645						
	0	0.1014 0.1046 0.0965 0.0948 0.0991	101 105 97 95 99	99	100	94	106
	31	0.0979 0.0929 0.0915	98 93 92	94	95	99	95
	89	0.0832 0.0865 0.0851	83 86 85	85	85	95	90
	185	0.0890 0.0868 0.0860	89 87 86	87	88	93	94
	360	Ongoing					
	540	Ongoing					
	720	Ongoing					

**Table 7.1.1-5 Storage stability data and concurrent recovery data for NC 20645 in orange fruit**

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange fruit	NC 20645						
	0	0.0980 0.1009 0.0953 0.1112 0.0993	98 101 95 111 99	101	100	99	102
	31	0.0979 0.0948 0.0940	98 95 94	96	95	97	99
	89	0.0997 0.0992 0.0953	100 99 95	98	97	99	99
	185	0.0878 0.0897 0.0867	88 90 87	88	88	89	99
	360	Ongoing					
	540	Ongoing					
	720	Ongoing					

a Normalized Recovery = (average recovery / average recovery at day 0) x 100%

b Corrected percent recovery = (average % recovery (stored) / Average of fresh concurrent recoveries) x 100%

Mean values were calculated with unrounded values. Therefore minor deviations may occur when the values given in the table are used.

**Table 7.1.1-6 Concurrent recoveries for NC 20645**

Sample Material	Storage Interval [d]		Concurrent Recoveries [%]					
			0.01 mg/kg fort. level			0.10 mg/kg fort. level		
	nominal	actual	Single Values	Mean	RSD	Single Values	Mean	RSD
Sugar Beet Leaf	0	0	92. 79	86	--	89. 88	89	--
	30	30	--	--	--	94. 95	95	--
	90	89	--	--	--	88. 89	89	--
	180	187	--	--	--	89. 90	90	--
overall				86	--		90	3.0
Sugar Beet Body	0	0	100. 116	108	--	95. 95	95	--
	30	30	--	--	--	87. 85	86	--
	90	89	--	--	--	92. 92	92	--
	180	187	--	--	--	96. 97	97	--
overall				108	--		92	4.7
Rape Seed	0	0	89. 93	91	--	93. 93	93	--
	30	31	--	--	--	98. 98	98	--
	90	89	--	--	--	98. 96	97	--
	180	185	--	--	--	97. 97	97	--
overall				91	--		96	2.2
Dry Bean	0	0	94. 92	93	--	93. 95	94	--
	30	31	--	--	--	100. 98	99	--
	90	89	--	--	--	97. 92	95	--
	180	185	--	--	--	90. 95	93	--
overall					--		95	3.5

Sample Material	Storage Interval [d]		Concurrent Recoveries [%]					
			0.01 mg/kg fort. level			0.10 mg/kg fort. level		
	nominal	actual	Single Values	Mean	RSD	Single Values	Mean	RSD
Orange Fruit	0	0	92. 102	97	--	101. 100	101	--
	30	31	--	--	--	99. 94	97	--
	90	89	--	--	--	99. 99	99	--
	180	185	--	--	--	87. 91	89	--
overall				97	--		96	5.2

### III. Conclusions

After a deep freezer storage period at -18°C of about 6 months, the mean recovery rates of metabolite NC 20645 in stored samples of sugar beet leaf, sugar beet body, rape seed, dry bean and orange fruit, representing a wide array of plant-based sample materials (i.e. high water, high oil, high starch, high protein, high acid containing commodities), ranged between 82% and 94%.

Furthermore the concurrent recoveries determined from freshly fortified samples were in a range of 89% to 97% (mean values). Altogether, the study results demonstrate that the residues of NC 9607 and NC 20645 are stable in the tested plant commodities for at least 6 months under deep-freezer storage conditions.

The storage stability study is still ongoing up to 24 months.

#### B.7.1.1.2. Storage stability of ethofumesate, NC 20645, conjugated NC 20645 in sugar beet

A new storage stability study was submitted, which investigates the stability of ethofumesate and its metabolite NC 20645 including its conjugates in sugar beet matrices (leaves and roots) during storage at  $\leq -18^{\circ}\text{C}$  for a period of 12 months. This study is summarised below.

Report:	KCA 6.1/01, Hamberger, R. (2013)
Title:	DETERMINATION OF THE STORAGE STABILITY OF ETHOFUMESATE AND ITS METABOLITE NC20645 IN SUGAR BEET MATRICES DURING STORAGE AT $< \text{OR} = \text{TO} - 18^{\circ}\text{C}$ FOR A PERIOD OF 12 MONTHS
Document No:	12A04042-01-SSSB
Guidelines:	7032/VI/95 rev. 5
GLP:	Yes

### I. Materials and Methods

Organic sugar beet specimens (sugar beet whole plant with roots (early growth stage), sugar beet leaves with tops and sugar beet roots) were fortified at 0.1 mg/kg (10 fold LOQ) with ethofumesate and its metabolite NC 20645 (free form), respectively. The specimens were stored deep frozen at  $\leq -18^{\circ}\text{C}$  and analysed 0, 3, 6 and 12 months after fortification. At the day 0, two freshly fortified specimens per analyte and per matrix were analysed together with one control specimen per matrix. For the time points from 3 to 12 months two stored and two freshly fortified specimens per analyte and per matrix were analysed together with one control specimen per matrix. Two replicate fortified specimens for each analyte and matrix were prepared for each sampling time point.

Ethofumesate and its metabolite NC 20645 were analysed using the method described in Hamberger, R. (2012a), report 11A04042-01-VMSB, which has been validated before according to SANCO 3029/99 rev. 4 and SANCO 825/00 rev. 8.1 as presented in this DRAR Section B5.

Within the storage stability study, procedural recoveries were done which verified the validity of the used method. Sugar beet specimens (whole young plants with roots, leaves with tops and roots) were extracted with acetone. Water was added and the solvent was evaporated. The aqueous phase was made alkaline resulting in conversion of NC 9607 into its open form, i.e. NC 20645. After liquid-liquid partitioning with hexane, ethofumesate partitioned into the hexane phase whereas the metabolite NC 20645 remained in the aqueous phase.

The remaining plant material and the alkaline aqueous phase were combined and adjusted to neutral pH. After adding 10% acetone the sample was cooked under reflux. After filtration the filtrate was subjected to an acid hydrolysis step, which converted the conjugated bound residues into their open form followed by SPE. The ethofumesate metabolites were eluted with methanol/acetonitrile. A solvent exchange to ethyl acetate was carried out.

The extract containing ethofumesate and the extract containing ethofumesate metabolites were combined and cleaned up by SPE and analysed by GC-MSD.

By this method, the metabolite NC 20645 is converted to NC 9607 and finally detected as NC 9607. This method will extract conjugated residues as well as it includes an acid hydrolysis step. Although no conjugated NC 20645 was available for spiking, stability of the conjugated form is covered by the previous studies on conjugated 2-keto ethofumesate (= conjugated NC 20645) and by the stability of the free form of NC 20645.

## II. Results and Discussion

A degradation of Ethofumesate and its metabolite NC 20645 was not observed during storage at  $\leq -18^{\circ}\text{C}$  within 12 months. Results are given in the tables below.

**Table 7.1.1-7 Recovery of Ethofumesate in samples of sugar beets after frozen storage**

Commodity	Storage periods	Residues and recoveries in specimens stored frozen (not corrected for procedural recoveries)				Procedural recoveries for freshly fortified samples	
	[months]	Individual results (mg/kg)	Mean (mg/kg)	Individual results (%)	Mean (%)	Individual results (%)	Mean (%)
Sugar beet whole plant with roots (early growth stage)	0	0.0766 0.0745	0.0756	77 75	76	77 75	76
	3	0.0847 0.0864	0.0856	85 86	86	73 84	79
	6	0.0772 0.0810	0.0791	77 81	79	95 93	94
	12	0.100 0.0945	0.0973	100 95	97	94 97	96
Sugar beet leaves with tops (mature)	0	0.0722 0.0723	0.0723	72 72	72	72 72	72
	3	0.0861 0.0735	0.0798	86 74	80	68 88	78
	6	0.0883 0.0898	0.0891	88 90	89	88 78	83
	12	0.101 0.0968	0.0989	101 97	99	99 99	99

Sugar beet roots (mature)	0	0.0873 0.0868	0.0871	87 87	87	87 87	87
	3	0.0909 0.0643	0.0776	91 64	78	94 90	92
	6	0.0740 0.0775	0.0758	74 78	76	73 77	75
	12	0.0964 0.0935	0.0950	96 94	95	96 97	97

**Table 7.1.1-8 Recovery of NC 20645 in samples of sugar beets after frozen storage**

Commodity	Storage periods	Residues and recoveries in specimens stored frozen (not corrected for procedural recoveries)				Procedural recoveries for freshly fortified samples	
	[months]	Individual results (mg/kg)	Mean (mg/kg)	Individual results (%)	Mean (%)	Individual results (%)	Mean (%)
Sugar beet whole plant with roots (early growth stage)	0	0.0745 0.0751	0.0748	75 75	75	75 75	75
	3	0.0705 0.0718	0.0712	71 72	71	66 76	71
	6	0.0768 0.0760	0.0764	77 76	76	91 99	95
	12	0.0832 0.0767	0.0800	83 77	80	88 86	87
Sugar beet leaves with tops (mature)	0	0.0821 0.0785	0.0803	82 79	80	82 79	81
	3	0.0735 0.0613	0.0674	74 61	67	60 77	69
	6	0.0740 0.0775	0.0758	74 78	76	78 67	73
	12	0.0740 0.0655	0.0698	74 66	70	89 85	87
Sugar beet roots (mature)	0	0.0759 0.0755	0.0757	76 76	76	76 76	76
	3	0.0791 0.0615	0.0703	79 62	70	67 81	74
	6	0.0607 0.0635	0.0621	61 64	62	59 68	64
	12	0.0819 0.0800	0.0810	82 80	81	84 82	83

### III. Conclusions

After a deep freezer storage period at -18°C of about 12 months, the mean recovery rates in stored samples of sugar beet leaves, sugar beet roots (i.e. high water and high starch containing commodities), were 99% (sugar beet leaves) and 95% (sugar beet root) for ethofumesate and 70% (sugar beet leaves) and 81% (sugar beet roots) for metabolite NC 20645.

Procedural recoveries determined from freshly fortified samples were 99% (sugar beet leaves) and 97% (sugar beet root) for ethofumesate and 87% (sugar beet leaves) and 83% (sugar beet roots) for metabolite NC 20645.

Altogether, the study results demonstrate that the residues of ethofumesate and its metabolites NC9607 and NC 20645 (including its conjugates) are stable in the tested plant commodities for 12 months under deep-freezer storage conditions.

**B.7.1.1.3. Storage stability of NC 20645 and conjugated NC 20645 in sugar beet matrices**

A new storage stability study was submitted, which investigates the stability of metabolite NC 20645 including its conjugates (= conjugated NC 20645) in sugar beet matrices (leaves and roots) during storage at  $\leq -18^{\circ}\text{C}$  for a period of 2 years. This study is summarised below.

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Report:	KCA 6.1/5, Schlewitz P. (2014)
Title:	Frozen storage stability of residues of ethofumesate metabolite NC 20645 in sugar beet (roots and tops with leaves).
Document No:	R B1312
Guidelines:	Regulation (EC) No. 1107/2009 OECD 506
GLP:	Yes

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**I. Materials and Methods**

The objective of the study was to generate data about the stability of residues of NC 20645 in sugar beet roots and sugar beet tops with leaves upon deep freezing storage conditions (below  $-18^{\circ}\text{C}$ ). Sugar beet roots and sugar beet tops with leaves supplemented with NC 20645 were analysed after 0, 91/92, 180/181, 362 and 729 days of frozen storage.

All individual samples were cut into small pieces and were homogenized. Samples were then blended using dry ice and stored frozen at  $< -18^{\circ}\text{C}$  for at least 12 hours. The amount required for analysis was weighed into individual plastic bottle.

NC 20645, dissolved in methanol, was fortified to each individual storage samples at a target concentration of approximately 0.5 mg/kg. The fortified aliquots were stored frozen at a temperature below  $-18^{\circ}\text{C}$  until analysis. Some aliquots were not fortified and were stored frozen to be used as control samples and procedural recovery samples.

Samples were analysed according to the method validated by ANADIAG under study No A0019 entitled “Validation of the Method of Analysis of the residues of ethofumesate and its metabolite 2-keto ethofumesate (free and conjugated form) in Sugar Beets”.

The applicability of this analytical method for residue analysis of NC 20645 in wheat and sugar beet was confirmed by Eurofins study No S11-03715 entitled “Validation of the analytical method A0019 to confirm the conversion of NC 20645 to NC 9607 in sugar beet roots and tops and wheat grain and straw”.

Following the analytical method A0019 and its confirmation S11-03715, the metabolite NC 20645 is normally fortified not immediately before the initial extraction step but at a later stage when the method is ramifying into a method fork for the extraction of conjugated residues. This is a usual practice when a metabolite is predominately present as a conjugated residue as it is the case for NC 20645.

Since this approach is not possible within a storage stability study where the fortification has to be done before the initial extraction step, the behaviour of the metabolite NC 20645 following the whole method was investigated by a set of pre-tests. These pre-tests were not performed according to GLP and have therefore to be excluded from the statement of compliance. The findings from these pre-tests were later confirmed by acceptable procedural recoveries that were performed under GLP.

The findings of the pre-tests are included as extra information to the method that was used during the storage stability study as described below.



### **Principle of the method**

#### **A) Extraction of non-conjugated residues**

The pre-homogenised sample was transferred into a 250 mL flask and a methanol/dichloromethane mixture (10:90, v/v, 100 mL) was added. The sample was extracted using a high speed homogeniser for 1 minute followed by shaking on a lab-shaker after addition of Celite (10 g). The suspension was filtered on a Buchner funnel containing a filter paper. Extraction of the residue was repeated twice with extraction solvent (70 mL) using a magnetic stirrer for approximately 15 minutes. The filter cake was finally rinsed with extraction solvent (20 mL) and water (25 mL) and the solid residue was retained for extraction of water soluble conjugates (see point C). All filtrates and rinsings were combined in a 250 mL separatory funnel.

#### **B) Liquid-liquid partition of primary extracts**

After shaking the separatory funnel the emulsion was allowed to separate into a lower dichloromethane phase and an upper aqueous phase. The lower phase was filtered into a round bottom flask. The aqueous phase was re-extracted with additional 25 ml of dichloromethane. After phase separation, the organic phases were discarded because this organic fraction is containing the parent ethofumesate that is not a target analyte in this storage stability study. The aqueous phase was retained for extraction of water soluble conjugates (see point C).

*Pre-tests demonstrate that NC 20645 remains in the aqueous phase (pH about 7 – 8). This aqueous phase containing NC 20645 is added to the extraction of conjugated residues (see next step of the method).*

#### **C) Extraction of conjugated residues**

The filter cake from point A) and the aqueous phase from point B) were combined in a 500 mL flask and a water/methanol mixture (90:10, v/v, 100 mL) and hexane (20 mL) were added. The sample was heated by reflux on a gently boiling water bath for 1 hour. After cooling to room temperature, the sample was centrifuged at approximately 1500 rpm for 5 min. and the supernatant was decanted into a 500 ml round bottom flask. The residue was rinsed with water/methanol (90:10, v/v, 50 ml), centrifuged and the supernatants were combined.

*Pre-tests demonstrate that the free NC 20645 is included in the supernatant (extract).*

#### **D) Hydrolysis of conjugates**

Concentrated hydrochloric acid (100 mL) was added to the combined supernatants from point C) and the solution was heated to a gentle reflux for approximately 75 minutes. After cooling to room temperature, acetone (100 mL) was added and the hydrolysate was transferred into a 500 mL separatory funnel. Liquid-liquid partition was performed two times with 125 and 100 mL of dichloromethane. The dichloromethane extracts were combined and re-extracted twice with water (100 mL). All aqueous phases were discarded. The dichloromethane phase was filtered into a round bottomed flask through anhydrous sodium sulfate. The filter was rinsed with three portions of dichloromethane (15 mL) and the combined organic extracts were evaporated to dryness by rotary evaporation followed by a gentle stream of nitrogen. Following findings of the metabolism studies this strong acidic treatment converts NC 20645 into NC 9607.

#### **E) Florisil Clean-up**

A laboratory prepared chromatographic column containing a glass wool plug followed by a slurry of Florisil (5 g) in dichloromethane (50 mL) was used for purification of free and conjugate extracts. Residues of point D) were dissolved in dichloromethane (2 mL).

Samples were transferred onto the top of the column and drained until the solution has penetrated into the adsorbent layer. Residues were eluted with a dichloromethane/ethyl acetate mixture (97:3, v/v, 80 mL). The

eluates were reduced to dryness by rotary evaporation followed by a gentle stream of nitrogen. Residues were dissolved with hexane/acetone 50:50 mixture (5 mL) and analysed by gas chromatography with mass spectrometric detector.

The limit of quantification (LOQ) is 0.05 mg/kg for sugar beet roots and sugar beet tops with leaves.

At each storage interval 1 untreated control sample was analysed together with 1 fresh fortified procedural recovery sample and 2 stored fortified samples. For the time zero interval, 1 untreated control sample was analysed together with 3 fresh fortified samples.

## II. Results and Discussion

The residues in all control samples were below the limit of quantification of the analytical method. At each date of analysis, 1 control sample for each matrix was fortified with NC 20645 to determine the level of procedural recovery. The analytical results for storage stability are summarised in the tables below.

**Table 7.1.1-9 Recovery of NC 20645 in samples of sugar beets after frozen storage (Fortification level before storage: 0.516 mg/kg)**

Commodity	Storage periods	Residues and recoveries in specimens stored frozen (not corrected for procedural recoveries)				Procedural recoveries for freshly fortified samples (0.50-0.53 mg/kg added)	
	[months]	Individual results (mg/kg)	Mean (mg/kg)	Individual results (%)	Mean (%)	Amount recovered (mg/kg)	% Recovery
Sugar beet roots	0	0.534 0.380 0.573	0.496	103 74 111	96	0.56	109.2
	92	0.501 0.526	0.514	97 102	100	0.38	72.8
	180	0.434 0.426	0.430	84 83	83	0.43	86.1
	362	0.497 0.479	0.488	96 93	95	0.48	91.2
	729	0.369 0.391	0.380	72 76	74	0.55	109.4
Sugar beet leaves	0	0.374 0.427 0.381	0.394	72 83 74	76	0.39	75.2
	92	0.413 0.406	0.410	80 79	79	0.55	106.1
	180	0.471 0.473	0.472	91 92	91	0.49	98.6
	362	0.438 0.569	0.504	85 110	98	0.56	104.5
	729	0.376 0.368	0.372	73 71	72	0.49	97.6

## III. Conclusions

After a storage period at -18°C of up to 24 months (729 days), the recovery rates for metabolite NC 20645 in stored samples of sugar beet leaves, sugar beet roots (i.e. high water and high starch containing commodities), were between 72-102% (sugar beet roots) and between 71-110% (sugar beet leaves).

Procedural recoveries for metabolite NC 20645 determined from freshly fortified samples ranged between 73-109% (sugar beet roots) and between 75-106% (sugar beet leaves).

Altogether, the study results show a good stability of NC 20645 in sugar beet (roots and tops with leaves) for up to 24 months when stored frozen at -18°C.

### B.7.1.2. Animal Matrices

Storage stability of residues of ethofumesate and its metabolites in animal matrices have not been assessed in the Monograph prepared in the framework of Directive 91/414/EEC.

In the study (Report No A82968) carried out in 1992 total radioactive residues were determined within 1 week and analysis of metabolites in urine, liver and kidney was carried out approximately 1 - 6 months after sample collection.

In the study on cows (Report No C003362, 1999) initial analysis was complete within 6 weeks (profile of metabolites in each extract obtained from TLC). All analysis was complete within seven months of necropsy. The storage period in the feeding studies was – where reported – up to 5 months.

#### B.7.1.2.1. Storage stability of ethofumesate, NC 8493, NC 9607 and NC 20645 in animal matrices

An interim report of a new storage stability study has been submitted to prove the storage stability of ethofumesate, NC 8493, NC 9607 and NC 20645 in animal material samples for at least 3 months. This interim report is summarised below.

Report:	<u>KCA 6.1 /05;Perez, R.; Schmitt, J. L.; Patel, D.:2013;M-467206-01</u>
Title:	FREEZER STORAGE STABILITY OF ETHOFUMESATE IN ANIMAL MATRIX SAMPLES - INTERIM REPORT
Report No:	RAADP031
Document No:	M-467206-02-1
Guidelines:	Residue Chemistry Test Guidelines: OPPTS 860.1380 Storage Stability Data Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160; Deviation not specified
GLP/GEP:	yes

### I. Materials and Methods

The purpose of the study was to determine the freezer stability of ethofumesate, NC 8493 (which is not a constituent of the residue definition), NC 9607 and NC 20645 in animal material samples (muscle, liver, kidney, fat and milk) over a period of 24- months of freezer storage (0, 1, 3, 6, 12, 18 and 24 months). This interim report concerns the analytical data collected through 6 months of freezer stability.

For each storage interval (0, 1, 3 and 6) and sample matrix, recovery experiments were performed by fortifying control samples with the reference items at a nominal concentration of 0.10 mg/kg to demonstrate the performance of the method. The freshly fortified samples were extracted and analysed concurrently with the control and stored spiked samples at each storage interval.

Samples were stored in a freezer at an average temperature of approximately -21°C. For day-0 analysis, three treated samples of each material were chosen, as well as one control sample of each.

Samples were then analysed after one month (28 to 43 days), 3 months (92 to 112 days) and additionally after 6 months (188 to 196 days). Analysis of the samples after 6 months of storage will cover the longest storage period (kidney) in the feeding study.

At each sampling interval, two treated samples of each material were removed from storage and analysed, as well as a control sample and two samples for concurrent recovery. Samples used for concurrent recoveries were prepared at the same time and stored in the same fashion as the control samples, and spiked on the day of analysis.

Ethofumesate related residues (parent ethofumesate, NC 9607 and NC 20645, as well as NC 8493) were analytically determined by LC-MS/MS according to method AD-001-A10-02 (Section 5; cf. report RAADP014, [REDACTED] 2010; M-388797-01; KCA 4.1.2/37), which was validated prior to and parallel to the residue analysis of the samples.

This LC-MS/MS method converted metabolite NC 9607 to the common moiety NC 20645, which was the analytical target.

**Milk** samples were extracted in an aqueous mixture of water/brine/HCl and ethyl acetate. The samples were vortexed, centrifuged, and the ethyl acetate layer was drawn off to a glass vial. The remaining sample and aqueous extraction mixture was extracted two more times with ethyl acetate.

Samples of **muscle**, **kidney**, and **fat** were extracted directly with ethyl acetate.

**Liver** samples were extracted directly with methanol. A mixture of isotopic internal standards was added to the extract solution and 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  $\text{NH}_4\text{HCO}_3$  solution/acetonitrile (9:1, v/v). The resultant samples were analyzed by LC-MS/MS.

The LOQ of the method was 0.01 mg/kg for each of the analytes. The stored samples were always analysed for all four components, although each sample was spiked with one compound, only.

## II. Findings

At each storage interval, one stored control sample was analysed concurrently with the stored spiked samples. No residue above the LOQ was found in any of the control samples with an exception of two samples. Parent ethofumesate was detected at 0.0147 mg/kg in liver at the 3 months storage interval and at 0.0111 mg/kg in fat at the 0-day time interval. Residue results of the corresponding samples were corrected for the residues found in control samples.

The following tables summarize residues of ethofumesate, NC 8493, NC 9607 and NC 20645 recovered in muscle, liver, kidney, fat and milk stored spiked samples, as well as the concurrent recovery data.

In **muscle**, there was no evidence of any continued degradation of these compounds when stored deep-frozen over the tested period of 6 months. For metabolite NC 20645 a slight degradation was observed at the 3 months storage interval, this can be explained by the low recovery results at this sampling date.

**Table 7.1.2-1 Summary of stability data for deep-frozen muscle samples fortified with ethofumesate, NC 9607, NC 20645 and NC 8493 – samples fortified at 0.10 mg/kg**

Matrix	Analyte	Storage period months	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
			Individual results (mg/kg)			Individual results (%)			mean (%)	Individual results (%)			mean (%)
muscle	a.s.	0	0.103	0.101	0.103	103	101	103	103	103	101	103	102
		1	0.097	0.098	-	97	98	-	97	96	98	-	97
		3	0.105	0.105	-	105	105	-	105	97	97	-	97
		6	0.104	0.107	-	104	106	-	106	98	100	-	99
	NC 9607	0	0.098	0.098	0.094	98	98	94	97	98	98	94	97

	NC 20645	1	0.099	0.092	-	99	92	-	95	90	96	97	93
		3	0.072	0.076	-	82	76	-	74	83	82	-	83
		6	0.079	0.089	-	79	89	-	84	98	99	-	99
		0	0.076	0.083	0.082	76	83	82	80	76	83	82	80
		1	0.083	0.082	-	83	82	-	83	86	84	97	85
		3	0.056 (0.053)	0.061 (0.052)	-	56 (53)	61 (52)	-	<b>58</b> <b>(52)</b>	<b>69</b> <b>(60)</b>	<b>64</b> <b>(68)</b>	-	<b>66</b> <b>(64)</b>
	NC 8493	6	0.064	0.061	-	64	61	-	62	59	60	-	60
		0	0.097	0.096	0.094	97	96	94	96	97	96	94	96
		1	0.098	0.090	-	98	90	-	94	96	100	97	98
		3	0.087	0.086	-	87	86	-	87	86	87		87
		6	0.095	0.097	-	95	97	-	96	95	100	-	97

Values in brackets were determined for verification

In **liver**, there was no evidence of any continued degradation of ethofumesate, NC 8493, NC 9607 and NC 20645 when stored deep-frozen over the tested period of 6 months, except for metabolite NC 8493. At day 0, average residue recoveries of NC 8493 amounted to 84%. After a storage interval of 1 month, the metabolite was completely degraded and the storage stability recovery amounted to < 1%.

NC 8493 was found to readily convert to NC 9607 or NC 20645 (ca. 4h), both of which are detected and measured by the analytical method as NC 20645. Since NC 9607 and NC 20645 are included in the data collection method for every analytical set, stability for NC 8493 in liver is addressed by measuring NC 20645 in stored liver samples after 6 months storage.

Thus degradation of NC 8493 to a relevant ethofumesate residue would be covered by the proposed residue definition.

**Table 7.1.2-2 Summary of stability data for deep-frozen liver samples fortified with ethofumesate, NC 9607, NC 20645 and NC 8493 – samples fortified at 0.10 mg/kg**

Matrix	Analyte	Storage period months	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
			Individual results (mg/kg)			Individual results (%)			mean (%)	Individual results (%)		mean (%)	
liver	a.s.	0	0.084	0.082	0.087	84	82	87	85	84	82	87	84
		1	0.111 (0.103)	0.112 (0.099)	-	111 (103)	112 (99)	-	111 (101)	114	113	97	113
		3 <sup>a</sup>	0.086	0.084	-	86	84	-	85	81	79		80
		6	0.130	0.136	-	130	136	-	133	119	119	-	119
	NC 9607	0	0.103	0.103	0.097	103	103	97	101	110	109	104	108
		1	0.104 (0.156)	0.114 (0.167)	-	104 (156)	114 (167)	-	109	85	83		84
		3	0.124	0.119	-	124	119	-	121	80	81		80
		6	0.089	0.098		89	98	-	94	100	96	-	98
	NC 20645	0	0.082	0.087	0.085	82	87	85	85	82	87	85	85
		1	0.090 (0.139)	0.082 (0.123)	-	90 (139)	82 (123)	-	86 (131)	110	96		103
		3	0.105	0.108	-	108	108	-	106	113	111		112
		6	0.086	0.087		86	87		87	112	99		106
	NC 8493	0	0.082	0.084	0.085	82	84	85	84	82	84	85	84
		1	0.0001	0.002	-	0.0	1.7	-	<b>0.8</b>	72 (64)	77 (72)	-	74
		3	0.000	0.000	-	0	0	-	<b>0</b>	82	82	-	82
		6	0.000	0.000	-	0	0	-	<b>0</b>	91	91	-	91

Matrix	Analyte	Storage period months	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
			Individual results (mg/kg)			Individual results (%)			mean (%)	Individual results (%)			mean (%)
	NC 8493 <sup>b</sup>	0	0.082	0.084	0.085	82	84	85	84	82	84	85	84
		1	0.100 (0.166)	0.094 (0.152)	-	101 (166)	94 (152)	-	97 (159)	72	77	97	74
		3	0.126	0.116	-	126	116	-	121	82	82		82
		6	0.089	0.107		89	107		98	91	91	-	91

<sup>a</sup> Residues corrected for residues found in control

<sup>b</sup> This analyte is typically detected as NC8493 (256.95/79.10 Da). However, the stored analyte was detected by detecting the NC 20645 ion transitions in this matrix.

Values in brackets were determined for verification

In **kidney**, there was no evidence of any continued degradation of ethofumesate, NC 9607 and NC 20645 when stored deep-frozen over the tested period of 6 months.

A degradation of NC 8493 was also observed in kidney. 6 months of frozen storage, storage stability recoveries decreased to 14 and 18 %. Nevertheless, the sample stored for 6 months were also analysed for NC 20645. Based on these data, it becomes obvious that the samples of the feeding study were analysed still in time, but generally kidney samples should not be stored longer than 6 months in a deep-freezer before analysis. However, degradation of metabolite NC 8493 will result in NC 20645 residues and thus the residues will mainly be monitored since NC 20645 is included in the residue definition.

**Table 7.1.2-3 Summary of stability data for deep-frozen kidney samples fortified with ethofumesate, NC 9607, NC 20645 and NC 8493 – samples fortified at 0.10 mg/kg**

Matrix	Analyte	Storage period	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
		months	Individual results (mg/kg)			Individual results (%)			mean (%)	Individual results (%)			mean (%)
kidney	a.s.	0	0.098	0.095	0.096	98	95	96	96	98	95	96	96
		1	0.103	0.101	-	103	101	-	102	100	102	97	101
		3	0.096	0.107	-	96	107	-	102	101	102	-	102
		6	0.101	0.102	-	101	102		101	105	98	-	101
	NC 9607	0	0.900	0.910	0.940	90	91	95	92	90	91	95	92
		1	0.082	0.083	-	82	83	-	83	91	94	97	93
		3	0.091	0.074	-	92	74	-	83	88	89	-	88
		6	0.089	0.083	-	89	83	-	86	97	97	-	97
	NC 20645	0	0.084	0.077	0.080	84	77	80	80	84	77	80	80
		1	0.079	0.076	-	79	76	-	78	76	77	-	76
		3	0.155	0.079	-	156	79	-	118	77	73	-	75
		6	0.092	0.102	-	92	102		97	76	82	-	79
	NC 8493	0	0.095	0.090	0.089	95	90	89	91	95	90	89	91
		1	0.075	0.075	-	75	75	-	75	91	93	-	92
		3	0.070	0.073	-	71	74	-	72	89	84	-	87
		6	0.018	0.014	-	18	14	-	16	86	99	-	93
		NC 8493 <sup>a</sup>	6	0.069	0.068	-	69	68	-	68	86	99	-

<sup>a</sup> This analyte is typically detected as NC8493 (256.95/79.10 Da). However, the stored analyte was detected by detecting the NC 20645 ion transitions in this matrix.

In **fat**, there was no evidence of any continued degradation of ethofumesate, NC 8493, NC 9607 and NC 20645 when stored deep-frozen over the tested period of 6 months, except for metabolite NC 20645, for which a slight degradation can already be observed after 1 months.

**Table 7.1.2-4 Summary of stability data for deep-frozen fat samples fortified with ethofumesate, NC 9607, NC 20645 and NC 8493 – samples fortified at 0.10 mg/kg**

Matrix	Analyte	Storage period	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
		months	Individual results (mg/kg) <sup>a</sup>			Individual results (%)			mean (%)	Individual results (%)			mean (%)
fat	a.s.	0	0.097	0.095	0.096	97	95	96	96	97	95	96	96
		1	0.092	0.091	-	92	91	-	92	93	91	-	92
		3	0.098	0.097	-	98	97	-	97	100	93	-	97
		6	0.136	0.145	-	136	146	-	141	- <sup>b</sup>	120	-	120
	NC 9607	0	0.088	0.096	0.095	88	96	95	93	88	96	95	93
		1	0.086	0.086	-	86	86	-	86	87	86	-	87
		3	0.086	0.085	-	86	85	-	85	81	82	-	82
		6	0.097	0.098	-	97	98	-	97	- <sup>b</sup>	92	-	92
	NC 20645	0	0.071	0.071	0.078	71	71	78	73	71	71	78	73
		1	0.060	0.068	-	60	68	-	<b>64</b>	72	71	-	72
		3	0.064 (0.051)	0.060 (0.046)	-	64 (52)	60 (46)	-	<b>62</b> <b>(49)</b>	67	62	-	<b>65</b>
		6	0.061	0.060	-	61	60	-	<b>61</b>	72	70	-	71
	NC 8493	0	0.099	0.096	0.104	99	96	104	100	99	96	104	100
		1	0.089	0.084	-	89	84	-	87	86	85	-	85
		3	0.085	0.089	-	85	89	-	87	94	86	-	90
		6	0.099	0.097	-	99	97	-	98	102	102	-	102

Values in brackets were determined for verification

<sup>a</sup> Residues corrected for residues found in control

<sup>b</sup> Sample was not analysed due to being lost during the extraction procedure.

In **milk**, there was no evidence of any continued degradation of ethofumesate, NC 8493, NC 9607 and NC 20645 when stored deep-frozen over the tested period of 6 months.

**Table 7.1.2-5 Summary of stability data for deep-frozen milk samples fortified with ethofumesate, NC 9607, NC 20645 and NC 8493 – samples fortified at 0.10 mg/kg**

Matrix	Analyte	Storage period	residue level in stored spiked samples							Procedural recovery for freshly spiked control sample			
		months	Individual results (mg/kg) <sup>a</sup>			Individual results (%)			mean (%)	Individual results (%)			mean (%)
milk	a.s.	0	0.104	0.101	0.100	104	101	101	102	104	101	101	102
		1	0.095	0.097	-	95	97	-	96	103	99	-	101
		3	0.094	0.088	-	94	87	-	91	97	93	-	95
		6	0.111	0.112	-	111	112	-	111	100	94	-	97
	NC 9607	0	0.084	0.101	0.106	84	102	106	97	84	102	106	97
		1	0.850	0.960	-	85	96	-	91	94	95	-	94
		3	0.095	0.094	-	95	94	-	95	83	88	-	86
		6	0.103	0.102	-	103	102	-	103	89	100	94	-
	NC 20645	0	0.072	0.070	0.079	72	70	79	74	72	70	79	74
		1	0.138	0.124	-	138	124	-	131	133	115	-	124
		3	0.082	0.086	-	82	86	-	84	76	78	-	77
		6	0.092	0.101	-	92	101	-	96	93	93	-	93
	NC 8493	0	0.084	0.097	0.095	86	97	95	93	86	97	95	93
		1	0.091	0.092	-	92	92	-	92	97	97	-	97
		3	0.091	0.094	-	91	94	-	92	93	94	-	93
		6	0.094	0.094	-	94	94	-	94	97	92	-	94

### III. Conclusions

The deep freezer storage stability at -21°C of ethofumesate, NC 8493, NC 9607 (determined as NC 20645) and NC 20645 was investigated in bovine muscle, liver, kidney, fat and milk for a period of about 6 months. According the study design reported in the interim report, the storage stability study is still ongoing up to 24 months.

In **liver and kidney**, metabolite NC 8493 was found to readily convert to NC 9607 or NC 20645, both of which are detected and measured by the analytical method (AD-001-A10-02) 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 in the stored liver samples, if existent. Although residues of NC 8493 were analysed as NC 20645 degradation to recovery results slightly below 70 % (68 %) were observed in kidney.

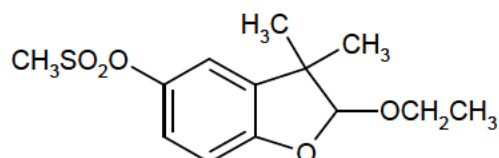
In **muscle, fat and milk**, there was no evidence of any continued degradation of these compounds when stored deep-frozen over the tested period of 6 months, except for metabolite NC 20645 in fat, for which a slight degradation can already be observed after 1 months.

As a result residues in kidney and liver were shown to be stable

#### B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

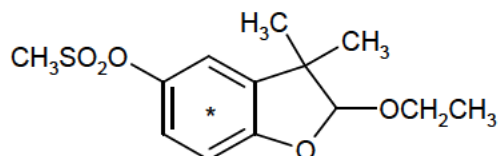
Ethofumesate (company code: NC 8438) is a selective herbicide for pre-emergence and post-emergence use. The chemical structure and nomenclature for the active substance are provided below.

Chemical structure:



Common name:	Ethofumesate
Company code:	NC 8438
IUPAC name:	2-ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methanesulfonate
CAS name:	2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate
CAS #:	26225-79-6
Empirical formula:	C <sub>13</sub> H <sub>18</sub> O <sub>5</sub> S
Molecular weight:	286.34 g/mol

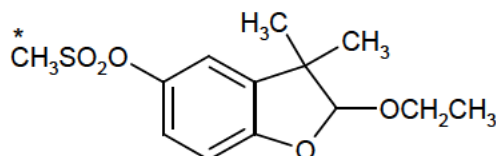
The label positions used in the metabolism studies are shown below:



[<sup>14</sup>C-benzene]-ethofumesate

= phenyl label

\* denotes position of radiolabel



[<sup>14</sup>C-mesyl]-ethofumesate

= mesyl label



Two sugar beet studies were conducted with the  $^{14}\text{C}$ -methylsulfonyl-label (mesyl label) and two preliminary rat studies were conducted with  $^{35}\text{S}$ -ethofumesate. Cleavage of the molecule and formation of metabolites specific to the mesyl label was neither observed in plants nor in animals. Based on this information all other plant and all livestock metabolism studies were conducted with [ $^{14}\text{C}$ -benzene]-ethofumesate, only.

### B.7.2.1. Plants

In the Monograph prepared in the framework of Directive 91/414/EEC the primary crop metabolism of ethofumesate has been investigated in sugar beet and ryegrass. Supportive metabolism studies have been conducted on onion and tobacco.

Most studies were conducted between 1974 and 1994 to support the Annex I inclusion of ethofumesate (Annex I inclusion in 2002). Thus these studies have been conducted and summarized according to former requirements and do not meet the current standards.

To confirm that the data established are still valid and support the proposed residue definition, the relevant plant metabolism studies are presented again in a more comprehensive way. Detailed evaluation reports are provided for the two most recent sugar beet studies and the metabolism study in ryegrass, which were conducted under GLP. New summary tables have been compiled to increase the comprehensibility of the former data/conclusions. The sugar beet studies conducted in the 70ies are only briefly summarized, as well as the studies in onion and tobacco. These studies were conducted before the implementation of GLP certificates, and do not meet the current standards. The results can therefore be only considered as supportive.

An additional study has been submitted in sugar beet along with the present dossier, which has been conducted in 2003. This study was also considered in this assessment to support the proposed residue definition.

All residue values given in mg/kg refer to parent compound equivalents if not indicated otherwise.

An overview on the available plant metabolism studies is given below in Table 7.2.1-1.

**Table 7.2.1-1 Summary of metabolism studies in primary crops presented in the first monograph prepared under Directive 91/414/EEC**

Group	Crop	Label position	Application and sampling details					Remarks
			Method, F or G <sup>(a)</sup>	Rate (kg a.s./ha)	No	Growth stage	Sampling (DAT)	
Root and tuber vegetables	Sugar beet	[ $^{14}\text{C}$ -benzene]	Foliar, G	1.27 or 6.37	1	BBCH 12-13	0+, 10, 30 and 81 and at maturity	KCA 6.2.1 /05, Chapleo, 1992
		[ $^{14}\text{C}$ -benzene]	Foliar, G	1.50 or 7.5	1	BBCH 14	0, 7, 28 and at maturity	KCA 6.2.1 /06; Caley, C. Y.; Chapleo, S.; Haswell, A.; 1994;
		[ $^{14}\text{C}$ -benzene]	Foliar, F	1.5	1	BBCH 14-16	1, 10, 50, 90, 137	KCA 6.2/01, Hennecke, D. 2003
		[ $^{14}\text{C}$ -benzene]	Soil, G	2.00	1	Pre emergence	10, 20, 30, 40 and 50	KCA 6.2.1 /03; Lines, D. S.; Adcock, J. W.; 1978;
			Foliar, G			BBCH 14		
		[ $^{14}\text{C}$ -mesyl]	Soil, F	2.00	1	Pre emergence	50, 75, 125 and 175	KCA 6.2.1 /04; Lines, D. S.;

Group	Crop	Label position	Application and sampling details					
			Method, F or G <sup>(a)</sup>	Rate (kg a.s./ha)	No	Growth stage	Sampling (DAT)	Remarks
			Foliar, F			BBCH 12	50, 75 and 125	Adcock, J. W.; 1979; M-155242-01
	Onion	Not reported	Soil <sup>(b)</sup>	2.00	1	Pre-emergence	22, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 162	Study NC 8438/M30 1976
Cereals	Ryegrass	[ <sup>14</sup> C-benzene]	Foliar, G	2.09 or ~10.45	1	BBCH 12-13	0+, 7, 28, (silage) and 112 (maturity)	KCA 6.2.1 /07 Chapleo, 1992
Leavy vegetables	Tobacco	[ <sup>14</sup> C-benzene]	Soil, G	2.00	1	BBCH 14-16	7, 15, 30, 60, 90 and 120	Study NC 8438/M35 1977
			Foliar, G	2 mg/plant				

(a) Outdoor/field application (F) or glasshouse/protected/indoor application (G)

(b) F or G not reported

### B.7.2.1.1. Sugar beet

#### B.7.2.1.1.1. Sugar beet metabolism 1

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

Report:	KCA 6.2.1 /05 Chapleo, S.;1992 M-155247-01
Title:	THE METABOLISM OF [ <sup>14</sup> C]-ETHOFUMESATE IN SUGAR BEET - A GLASSHOUSE STUDY
Report No:	A82970
Document No(s):	Report includes Trial Nos.: 381174 ENVIR 84B M-155247-01-1
Guidelines:	USEPA (=EPA): subdiv.O, 171-4; Deviation not specified
GLP/GEP:	yes

### I. Summary

The aim of this study was to determine the nature of the residue following application of [<sup>14</sup>C-benzene]-ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methanesulphonate) in sugar beet.

The herbicide was applied as a suspension concentrate (SC) formulation with an application rate of 1.27 kg a.s./ha, and of 6.37 kg a.s./ha (5x the initial rate) for easier metabolite identification. Plants were harvested at the day of application, at 10, 30 and 81 days post application and at maturity.

Total residues in the crop were determined by measurement of the total radioactivity and these were characterised by chromatographic procedures.

The TRR values of the mature crop at harvest are shown in the following table:

**Table 7.2.1-2 TRR values in sugar beet shoot and root after spray application of [<sup>14</sup>C]-ethofumesate**

Matrix	Timing and Application	Sampling event	TRR (mg a.s. equiv./kg)
Shoot (leaves)	2-3 leaf growth stage, 1 x 1.27 kg a.s./ha	Maturity	0.41
Root (body)	1 x 6.37 kg a.s./ha		0.02

At the 1x application rate the mean total residue in mature shoots was 0.41 mg ethofumesate equivalents/kg (6.3% of applied radioactivity). Immature roots the mean total residue was 0.02 mg ethofumesate equivalents/kg (0.65% of applied radioactivity).

At the 5x application rate the mean total residue in mature shoots was 1.06 mg ethofumesate equivalents/kg (0.75% of applied radioactivity). Immature roots the mean total residue was 0.08 mg ethofumesate equivalents/kg (0.13% of applied radioactivity).

The extractable components of the total residue were identified as ethofumesate (0.8%), NC 9607 (58.4%), NC 8493 (4.3%) and NC 20645 (14.4%). A large proportion of the residue was assigned to conjugates present in a polar fraction. Acidic hydrolysis transformed the main amount of the polar radioactivity to the well-known metabolites NC 9607 and NC 8493. Therefore it was assumed that metabolites NC 20645 (the ring opened form of NC 9607) and NC 8493 were present in conjugated form. Before acidic treatment, only 19% of the total residue in shoots was identified, whereas 77.9% of the total residue was identified after the treatment.

Due to the low residue level in roots at final harvest (maturity) and the high levels of interfering matrix compounds (e.g. sugar) metabolite characterisation/identification was not possible.

In general it was shown that ethofumesate was taken up by the plants (via roots and leaves) and metabolized rather quickly. The following metabolic routes were observed:

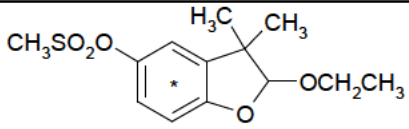
- Cleavage of the ethoxy side chain of the parent compound, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can undergo either conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to form the acid NC 20645 which can also undergo conjugation to give polar metabolites.
- The lactone NC 9607 and the carboxylic acid NC 20645 are interconvertible. Depending on the ambient conditions, the equilibrium is shifted towards one or the other compound.
- Under reflux with 6 M hydrochloric acid, the detected conjugates are hydrolysed to their exocons NC 8493 and NC 20645. As described above, NC 20645 is converted to NC 9607 by an intramolecular ring closure. Therefore NC 9607 is the analytical target detected under acidic conditions.

On the basis of the metabolites identified, a metabolic pathway of [ $^{14}\text{C}$ ]-ethofumesate in sugar beets can be proposed (see Figure 1).

## II. Materials and Methods

### A. Materials

#### 1. Test Material

Chemical structure	 <p>*position of the radiolabel</p>	
Radiolabelled test material	[ $^{14}\text{C}$ ]-ethofumesate (+)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methane-sulphonate	
Specific radioactivity	1x field rate (1.27 kg a.s./ha)	5x field rate (6.37 kg a.s./ha)
	0.3774 MBq/mg (10.2 $\mu\text{Ci/mg}$ )	0.3737 MBq/mg (10.1 $\mu\text{Ci/mg}$ )
Radiochemical purity	> 96% (TLC)	

[<sup>14</sup>C]-ethofumesate was formulated as a suspension concentrate for application at the expected maximum field rate and the 5x maximum field rate. The 1x formulation was prepared by dilution of the 5x formulation with tap water.

## 2. Soil

A sandy loam soil (pH 6-7), 7-8 tonnes, which had been screened through a 10 mm-sieve to remove stones was supplied by the Scottish Agricultural College, Ayr, Scotland. Soil was stored outside upon a fibreglass weave mesh and covered with the mesh. Prior to use, soil was thoroughly weeded. Soil details and characterisation are given in the following table:

**Table 7.2.1-3 Soil characterisation**

<b>Physical characterisation</b>	
Soil type	Brown earth
Soil classification (USDA)	Sandy loam
Sampling location	Baillieston, Scotland (OS Grid Reference = NS 672634)
Sampling depth	Top soil below root mat (10-280 mm)
Sample date	09-04-1991
Previous agrochemical use	None for previous 5 years
Germination test	100% germination within 2 days of sowing (radish)
pH (water)	6.2
pH (KCl)	5.6
% organic matter	6.6
Cation exchange capacity (mequiv./100 g)	11.27
CaCO <sub>3</sub> (mg/kg)	Very low, indeterminate
Moisture content (0.33 bar)	19.94
% Maximum water holding capacity (%)	68.2
Moisture content a pot preparation (%)	24.5
Loss on ignition (%)*	7.7
Clay (< 0.002 mm) (%)**	14.0
Silt (0.002-0.063 mm) (%)**	22.3
Total sand (0.063-2 mm) (%)**	63.7
Very fine sand (0.063-0.125 mm) (%)**	18.4
Fine sand (0.125-0.25 mm) (%)**	24.4
Medium sand (0.25-0.50 mm) (%)**	13.7
Coarse sand (0.50-1.0 mm) (%)**	5.9
Very coarse sand (1-2 mm) (%)**	1.3

\* expressed as % total sample

\*\* expressed as % of total < 2 mm mineral fraction

## 3. Plant

Sugar beet, variety “Beta vulgaris cv Gala”, representative for root vegetables

### B. Study Design

#### Experimental conditions

Plants were grown in pots consisting of approx. 0.1 m<sup>3</sup> capacity lengths of drain pipe (approx. 55 cm high and approx. 50 cm internal diameter) set upon mesh to retain soil. Soil was added to each pot, using a measure calibrated to provide 90.3 ± 1.3 kg fresh weight (72.5 ± 1.0 kg dry weight) of soil, above an approx. 5 cm deep gravel bed. Pots were placed in a glasshouse. To permit free drainage of leachates, without cross contamination between adjacent pots, all pots were placed on a moulded plastic grid system. A fertiliser, Vitax GR112 (N:P:K = 7:7:14), was added and combined with the surface layer of soil. Seed was sown at a rate of 20 seeds/pot at a depth of approx. 15 mm and the soil surface lightly watered. Pots were covered with polythene to maintain

humidity and darkness until emergence (5 days later). Plants were thinned to 8 plants per pot when there were 2-3 fully expanded true leaves per plant (BBCH 12-13).

The plants were grown in the glasshouse under controlled temperature, humidity and light conditions. Pots were watered until the top soil was moist, but were not overwatered. To maintain the good condition of the crop, pesticides were applied if required.

The test item was applied as a foliar spray when sugar beet plants showed 2-3 fully expanded true leaves (BBCH 12-13). A single application of test item was made to 12 pots at the expected 1x field application rate (1.27 kg a.s./ha) and to 4 pots at the 5x field application rate (6.37 kg a.s./ha). The test item was applied as a fine spray to each pot using an all-glass atomiser, with adaptor to fit tubes containing formulation. The spray apparatus used was rinsed 2 times with approx. 1.5 mL of distilled grade water. The rinse was also applied to the plants. Each pot was surrounded by a double sheet of polythene to contain spray. Aqueous washings of the sheets were added to the soil. All control pots were covered with polythene sheeting during spraying to avoid contamination.

### Sampling

Harvesting of plants from pots was conducted on the day of application when the formulation was dry, at 10, 30 and 81 days after application, and at maturity as follows:

On the day of application, all the plants in 4 treated pots (1x rate) and in 4 control pots. In addition 2 plants were harvested from each pot of the 5x rate.

Plants were sampled for analysis by thinning according to the following sampling regime. At each harvest, plants were divided randomly into groups for analysis.

**Table 7.2.1-4 Sampling of plants and preparation of sample groups**

Sample point (DAT)	Treatment	No. of pots sampled	No of plants sampled/pot	No of groups prepared	No of plant/group
0	Control	4	8	4	8
	x1	4	8	4	8
	x5	4	2	4	2
10	Control	4	4	2	8
	Assimilation control	4	4	2	8
	x1	8	4	4	8
	x5	4	2	2	4
30	Control	4	2	1	8
	Assimilation control	4	2	1	8
	x1	8	2	2	8
	x5	4	2	2	4
81	Control	4	1	1	4
	Assimilation control	4	1	1	4
	x1	8	1	2	4
	x5	4	1	2	2
Maturity (approximately 8 months)	Control	4	1	1	4
	Assimilation control	4	1	1	4
	x1	8	1	2	4
	x5	4	1	2	2

Leaf and root components of each plant were separated at the shoot/root junction (0 and 10 days post application) or at the tuber crown (30 and 81 days post application and at maturity). Roots were washed with distilled grade water to remove soil. In addition soil samples were taken on the day of application and at maturity.

## C. Analytical Procedures

### Determination of total residues

#### Shoots

Before homogenisation of the samples, shoots were surface washed with dichloromethane. The total radioactivity in the washes was determined by liquid scintillation counting of a minimum of 3 aliquots. After homogenisation, residues in washed shoots were determined by combustion. Total residues in shoots were calculated by addition of residue components in surface washes and washed tissue.

#### Roots

Before homogenisation of the sample, roots were washed with distilled grade water to remove soil. The washing was discarded. In roots, total residues were calculated from combustion of washed tissue.

#### Extraction of washed tissues:

Washed tissue was macerated in a range of organic solvents of increasing polarity to optimise the extraction of radioactivity. Tissue pools were extracted 3 times with acetonitrile (5 times for day 0), followed by acetonitrile/water (3/1; v/v) (3 times), followed by distilled grade water (3 times). A single final acetonitrile extract was conducted to remove water from the non-extractable residue. The extract was combined with the initial acetonitrile extracts. Extraction involved homogenisation, immersion in an ultrasonic bath and centrifugation to separate extractable and non-extractable components. The acetonitrile and acetonitrile/water extracts were combined and acetonitrile was removed by rotary evaporation. Total radioactivity in each extract was determined by liquid scintillation counting.

#### Identification and characterisation:

##### Initial characterisation:

Dichloromethane surface washes of shoots were reduced to a small volume for chromatographic analysis. The acetonitrile and acetonitrile/water extracts of shoots and roots were combined and the volume was reduced by rotary evaporation. Where necessary, the aqueous remainder was reduced to a small volume for chromatography after addition of ethanol (ethanol/extract: approx. 8/2; v/v).

Surface washes and initial extracts were analysed by TLC with confirmation by HPLC.

##### Hydrolysis of polar metabolites:

Characterisation of initial tissue extracts revealed the presence of polar metabolites, presumably conjugates. The following procedures (enzyme treatment and acidic hydrolysis) were employed in order to attempt to hydrolyse these conjugates and identify the exocons.

##### Enzyme treatment:

Shoot tissue extract (30 days post application, 1x application rate) was examined by TLC after treatment with  $\beta$ -glucosidase or  $\beta$ -galactosidase.

##### Acidic hydrolysis:

During method development, different hydrolysis conditions were tested. As treatment with 1 M HCl or 2 M HCl did not completely hydrolyse the polar conjugates in mature shoots, selected shoot (1x application rate,

mature sample collected at harvest) and root (5x application rate, sample collected 10 days after application) extracts were treated with 6 M HCl for approx. 18 hours under reflux.

For acidic treatment, the combined acetonitrile and acetonitrile/water extracts were extracted 4 times with dichloromethane, after adjustment of the pH to approx. 7 and also to approx. 2. Total radioactivity in each fraction was determined. Dichloromethane extracts were separated from the aqueous fraction and reduced to incipient dryness under a stream of nitrogen gas for chromatography.

The residual aqueous fraction was then hydrolysed with concentrated HCl (final concentration of 6 M HCl, for approx. 18 h under reflux). At this stage, aliquots of the hydrolysate were analysed directly by HPLC. To further characterise the radioactivity in the hydrolysates, aliquots of the reaction mixture were extracted directly 4 times with dichloromethane and also after pH adjustment to approx. 7. Extraction of radioactivity from hydrolysates was shown to be quantitative in roots; no radioactivity remained in the aqueous phase. Dichloromethane extracts were reduced to dryness under a stream of nitrogen gas for analysis by TLC.

#### **Extraction and treatment of fibre-bound residues:**

To solubilise the fibre-bound residue, remaining after conventional solvent extraction, different exhaustive procedures were tested exemplarily with the bound residues of the shoot sample (5x experiment) taken 81 days after application:

Soxhlet extraction using acetonitrile/water (16 h, reflux)

Extraction with 1 M NaCl (16 h, reflux)

Extraction with 1 M HCl (16 h, reflux)

Extraction with 1 M NaOH (16 h, reflux)

Extraction with 5 M NaOH (16 h, reflux)

Extraction with 1 M HCl showed the good results and was therefore applied when high proportions of fibre-bound residue were detected.

After exhaustive extraction, the soluble and non-extractable components were separated by centrifugation. The soluble residues were extracted with dichloromethane. Dichloromethane extracts were reduced to a suitable volume for chromatography.

#### **Quantification:**

Representative extracts were analysed by reversed-phase HPLC with UV- and radio-detection. All samples were co-injected with a standard mixture, fractions collected and quantified by liquid scintillation counting. In addition, representative samples on thin layer chromatography were scraped and quantified by liquid scintillation counting.

### **III. Results and Discussion**

The metabolism of [<sup>14</sup>C]-ethofumesate was investigated in sugar beet (shoot and root) following a single foliar application with an application rate of 1.27 kg a.s./ha, when sugar beet plants had 2-3 fully expanded true leaves. In addition, a 5x overdose experiment was conducted for easier identification of metabolites.

The total radioactive residue (TRR) in shoot and root decreased over time (Table 7.2.1-5 and Table 7.2.1-6) starting with 162.5 mg/kg, 1 h after the application to approx. 0.4 mg/kg at maturity for sugar beet shoots and 0.49 mg/kg 1 h after the application to approx. 0.02 mg/kg at maturity for sugar beet roots. In general the results of the 5x application rate were 5-fold higher than those of the 1x application rate.

**Table 7.2.1-5 Concentration of the the total radioactivity in shoot samples (results expressed as mg a.s. equiv./kg) (if not specified otherwise mean results of two to four replicates are presented)**

Sampling event	day 0	day 10	day 30	day 81		maturity	
1x Application rate							
Replicates	mean (n = 4)	mean (n = 4)	mean (n = 2)	A	B	A	B
Surface wash	116.27	17.19	0.38	0.00	0.00	0.00	0.00
Washed tissue	46.27	17.79	2.87	1.15	0.80	0.46	0.36
TRR	162.54	34.98	3.25	1.15	0.80	0.46	0.36
5x Application rate							
Surface wash	464.97	110.46	5.63	0.04	0.02	0.01	0.02
Washed tissue	114.69	54.79	8.39	3.10	1.40	1.18	0.94
TRR	579.66	165.25	14.02	3.14	1.42	1.19	0.96

**Table 7.2.1-6 Concentration of the total radioactivity in root samples (results expressed as mg a.s. equiv./kg) (if not specified otherwise mean results of two replicates are presented)**

Sampling event	day 0	day 10	day 30	day 81	maturity		
1x Application rate							
Replicates	mean (n = 4)	mean (n = 4)	mean (n = 2)	A	B	A	B
TRR*	0.49	0.25	0.04	0.01	0.02	0.01	0.03
5x Application rate							
TRR*	1.91	0.92	0.21	0.08	0.08	0.08	0.08

\* of washed root tissue

These results given in this table are summarised from Appendix 15 of the original study report (KCA 6.2.1 /05).

**Distribution of activity prior to acidic hydrolysis of polar compounds/fractions****Shoots**

The distribution of the total radioactivity measured in the surface washes and the conventional solvent extracts is presented in Table 7.2.1-7 to Table 7.2.1-11 for samples collected in the 1x application experiment, 1 h, and 10, 30 and 81 days after treatment, as well as at maturity. For a better comparability the results given in the study report were normalised to 100%. Normalization is appropriate since good recoveries were detected for each sample preparation step.

For samples taken early after application (1 h and 10 days after the application), the major portion of radioactivity was present in the surface washes (dichloromethane extract). As expected, the major residue was represented by parent ethofumesate. For samples taken at later time points, high radioactive residues were mainly detected in the acetonitrile or acetonitrile/water extracts; metabolites identified in these extracts were NC 8493 and NC 20645. An unknown polar fraction, showing an increasing proportion of the TRR with time, was detected at the origin of normal phase TLC plates. Due to the high polarity of the fraction, it was assumed that it could be represented by phase II metabolites, i.e. conjugates of either ethofumesate or its metabolites. The polar fraction was characterized in a separate step by acidic hydrolysis (cf. "Distribution of activity after acidic hydrolysis of polar compounds/fractions").

**Table 7.2.1-7 TRR values and distribution of parent compound and metabolites in shoots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 0 (1 h after the application)**

	1x, day 0	
TRR [mg/kg] =	162.54	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	74.2	120.6
Ethofumesate	70.5	114.6
NC 20645	-	-
NC 8493	2.8	4.6



	<b>1x, day 0</b>	
<b>TRR [mg/kg] =</b>	<b>162.54</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
NC 9607	-	-
Unknown (remainder)	0.9	1.4
<b>Conventional extraction</b>		
<b>ACN extract</b>	<b>24.7</b>	<b>40.1</b>
<b>ACN/H<sub>2</sub>O extract</b>	<b>0.4</b>	<b>0.7</b>
<b>Combined extracts</b>	<b>25.1</b>	<b>40.8</b>
Ethofumesate	22.9	37.3
NC 20645	0.8	1.3
NC 8493	0.3	0.4
NC 9607	-	-
Origin (polar fraction)	0.7	1.2
Unknown (remainder)	0.4	0.6
<b>H<sub>2</sub>O extract</b>	<b>0.1</b>	<b>0.2</b>
<b>Solids</b>	<b>0.6</b>	<b>1.0</b>
<b>Total identified</b>	<b>97.3</b>	<b>158.2</b>
<b>Total characterised</b>	<b>2.1</b>	<b>3.4</b>
<b>Total analysed</b>	<b>99.4</b>	<b>161.6</b>
<b>Solids</b>	<b>0.6</b>	<b>1.0</b>
<b>Accountability</b>	<b>100.0</b>	<b>162.5</b>

The results given in this table are summarised from Table 7A and Appendix 20 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

**Table 7.2.1-8 TRR values and distribution of parent compound and metabolites in shoots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 10**

	<b>1x, day 10</b>	
<b>TRR [mg/kg] =</b>	<b>34.98</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	49.5	17.30
Ethofumesate	46.9	16.40
NC 20645	-	-
NC 8493	1.5	0.52
NC 9607	-	-
Unknown (remainder)	1.1	0.38
<b>Conventional extraction</b>		
<b>ACN extract</b>	42.4	14.82
<b>ACN/H<sub>2</sub>O extract</b>	6.8	2.38
<b>Combined extracts</b>	49.2	17.19
Ethofumesate	12.6	4.40
NC 20645	4.1	1.43
NC 8493	-	-
NC 9607	-	-
Origin (polar fraction)	31.2	10.92
Unknown (remainder)	1.3	0.45
<b>H<sub>2</sub>O extract</b>	0.4	0.14
<b>Solids</b>	1.0	0.35
<b>Total identified</b>	<b>65.0</b>	<b>22.75</b>
<b>Total characterised</b>	<b>34.0</b>	<b>11.88</b>
<b>Total analysed</b>	<b>99.0</b>	<b>34.63</b>
<b>Solids</b>	<b>1.0</b>	<b>0.35</b>
<b>Accountability</b>	<b>100.0</b>	<b>34.98</b>

The results given in this table are summarised from Table 10 and Appendix 18/20 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

**Table 7.2.1-9 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 30**

	<b>1x, day 30</b>	
<b>TRR [mg/kg] =</b>	<b>3.25</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	11.7	0.38
Ethofumesate	11.1	0.36
NC 20645	-	-
NC 8493	0.3	0.01
NC 9607	-	-
Unknown (remainder)	0.3	0.01
<b>Conventional extraction</b>		
<b>ACN extract</b>	75.9	2.47
<b>ACN/H<sub>2</sub>O extract</b>	9.8	0.32
<b>Combined extracts</b>	85.7	2.79
Ethofumesate	9.8	0.32
NC 20645	2.7	0.09
NC 8493	-	-
NC 9607	-	-
Origin (polar fraction)	72.7	2.36
Unknown (remainder)	0.5	0.02
<b>H<sub>2</sub>O extract</b>	0.5	0.02
<b>Solids</b>	2.1	0.07
<b>Total identified</b>	<b>24.0</b>	<b>0.78</b>
<b>Total characterised</b>	<b>73.9</b>	<b>2.40</b>
<b>Total analysed</b>	<b>97.9</b>	<b>3.18</b>
<b>Solids</b>	<b>2.1</b>	<b>0.07</b>
<b>Accountability</b>	<b>100.0</b>	<b>3.25</b>

The results given in this table are summarised from Table 10 and Appendix 18/20 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

**Table 7.2.1-10 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 81**

	<b>1x, day 81</b>	
<b>TRR [mg/kg] =</b>	<b>0.98</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	0.4	0.004
Ethofumesate	0.3	0.003
NC 20645	-	-
NC 8493	-	-
NC 9607	-	-
Unknown (remainder)	0.1	0.001
<b>Conventional extraction</b>		
<b>ACN extract</b>	77.2	0.757
<b>ACN/H<sub>2</sub>O extract</b>	9.8	0.096
<b>Combined extracts</b>	87.0	0.853
Ethofumesate	37.2	0.365
NC 20645	-	-
NC 8493	-	-
NC 9607	-	-
Origin (polar fraction includes NC20645)	43.4	0.426
Unknown (remainder)	1.8	0.018
Unknown (non-polar)	4.5	0.044
<b>H<sub>2</sub>O extract</b>	5.5	0.054
<b>Solids</b>	7.1	0.070
<b>Total identified</b>	<b>37.6</b>	<b>0.368</b>
<b>Total characterised</b>	<b>53.4</b>	<b>0.542</b>
<b>Total analysed</b>	<b>91.0</b>	<b>0.910</b>

	1x, day 81	
<b>TRR [mg/kg] =</b>	<b>0.98</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Solids</b>	<b>7.1</b>	<b>0.070</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.980</b>

The results given in this table are summarised from Table 10 and Appendix 18/20 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%. Minor deviations of the values may be caused by rounding.

**Table 7.2.1-11 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – maturity (replicate B)**

	1x, maturity	
<b>TRR [mg/kg] =</b>	<b>0.36</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>0.7</b>	<b>0.003</b>
Ethofumesate	0.1	0.001
NC 20645	0.1	<0.001
NC 8493	0.2	<0.001
NC 9607	0.1	<0.001
Unknown (remainder)	0.2	0.001
<b>Conventional extraction</b>		
<b>ACN extract</b>	<b>51.6</b>	<b>0.186</b>
<b>ACN/H<sub>2</sub>O extract</b>	<b>27.0</b>	<b>0.097</b>
<b>Combined extracts</b>	<b>78.6</b>	<b>0.283</b>
Ethofumesate	-	-
NC 20645	18.5	0.066
NC 8493	-	-
NC 9607	-	-
Origin (polar fraction)	60.1	0.216
<b>H<sub>2</sub>O extract</b>	<b>7.1</b>	<b>0.026</b>
<b>Solids</b>	<b>13.6</b>	<b>0.049</b>
<b>Total identified</b>	<b>19.0</b>	<b>0.068</b>
<b>Total characterised</b>	<b>67.4</b>	<b>0.243</b>
<b>Total analysed</b>	<b>86.4</b>	<b>0.311</b>
<b>Solids</b>	<b>13.6</b>	<b>0.049</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.360</b>

The results given in this table are summarised from Table 10 and Appendix 18/20 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

## Roots

For sugar beet root the distribution of the radioactivity in the different extracts is given in Table 7.2.1-12 to Table 7.2.1-17 for all sampling events of the 1x experiment from 0 to 81 days and for the sample collected in the 5x experiment at 10 days after application and at maturity. The major portion of the radioactivity was always present in the acetonitrile and acetonitrile/water extracts. With time, the amount of radioactivity in the polar fraction (origin of the normal phase TLC systems or solvent front of the reversed-phase system used) increased significantly indicating the formation of polar conjugates. Therefore these fractions were further characterized by hydrolysis (cf. "Distribution of activity after acidic hydrolysis of polar compounds/fractions"). From day 10-samples onwards, identification of metabolites other than the polar ones was only possible in the extracts of the overdose (5x) experiment. In addition the proportion of the bound residues was also rather high in most samples

representing up to 51% of the TRR (1x experiment, day 10). Due to the very low residue levels in roots, especially after day 30, no additional exhaustive extraction steps were applied.

**Table 7.2.1-12 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 0**

	1x, day 0	
TRR [mg/kg] =	0.49	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	not done	not done
<b>Conventional extraction</b>		
ACN extract	57.9	0.28
ACN/H <sub>2</sub> O extract	3.0	0.01
Combined extracts	60.9	0.30
Ethofumesate	32.7	0.16
NC 20645	7.2	0.04
NC 8493	8.5	0.04
NC 9607	-	-
Origin (polar fraction)	2.2	0.01
Unknown (polar)	6.1	0.03
Unknown (remainder)	4.2	0.02
H <sub>2</sub> O extract	1.3	0.01
Solids	37.8	0.19
Total identified	48.4	0.24
Total characterised	13.8	0.07
Total analysed	62.2	0.30
Solids	37.8	0.19
Accountability	100.0	0.49

The results given in this table are summarised from Table 7B of the original study report (KCA 6.2.1 /05).

Recoveries were normalised to 100%.

**Table 7.2.1-13 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 10**

	1x, day 10	
TRR [mg/kg] =	0.25	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	not done	not done
<b>Conventional extraction</b>		
ACN extract	35.9	0.090
ACN/H <sub>2</sub> O extract	7.5	0.019
Combined extracts	43.4	0.109
Origin (polar fraction)	41.9	0.105
Unknown (remainder)	1.5	0.004
H <sub>2</sub> O extract	5.3	0.013
Solids	51.3	0.128
Total identified	-	-
Total characterised	48.7	0.230
Total analysed	48.7	0.230
Solids	51.3	0.128
Accountability	100.0	0.250

The results given in this table are summarised from Table 10 and Appendix 18/21 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%. Minor deviations may occur due to rounding.

**Table 7.2.1-14 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – day 10**

	<b>5x, day 10</b>	
<b>TRR [mg/kg] =</b>	<b>0.92</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	not done	not done
<b>Conventional extraction</b>		
ACN extract	71.4	0.66
ACN/H <sub>2</sub> O extract	8.5	0.08
Combined extracts (ACN + ACN/H <sub>2</sub> O)	80.0	0.74
DCM phase	28.8	0.26
Ethofumesate	10.5	0.10
NC 20645	2.8	0.03
NC 8493	7.2	0.07
NC 9607	4.6	0.04
Unknown	1.8	0.02
Unknown (polar)	1.0	0.01
Unknown (remainder)	0.9	0.01
Aqueous phase	51.2	0.47
H <sub>2</sub> O extract	1.5	0.01
Solids	18.6	0.17
Total identified	25.1	0.23
Total characterised	56.3	0.52
Total analysed	81.4	0.75
Solids	18.6	0.17
Accountability	100.0	0.92

The results given in this table are summarised from Table 14 of the original study report (KCA 6.2.1 /05).

Recoveries were normalised to 100%. Minor deviations may occur due to rounding.

**Table 7.2.1-15 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 30**

	<b>1x, day 30</b>	
<b>TRR [mg/kg] =</b>	<b>0.04</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	not done	not done
<b>Conventional extraction extract</b>		
ACN extract	21.5	0.009
ACN/H <sub>2</sub> O extract	17.0	0.007
H <sub>2</sub> O extract	13.2	0.005
Solids	48.3	0.019
Total identified	-	-
Total characterised	51.7	0.021
Total analysed	51.7	0.021
Solids	48.3	0.019
Accountability	100.0	0.040

The results given in this table are summarised from Table 10 of the original study report (KCA 6.2.1 /05).

**Table 7.2.1-16 TRR values and distribution of parent compound and metabolites in roots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 81**

	1x, day 81	
TRR [mg/kg] =	0.02	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	not done	not done
<b>Conventional extraction extract</b>		
ACN extract	28.3	0.006
ACN/H <sub>2</sub> O extract	13.0	0.003
H <sub>2</sub> O extract	14.5	0.003
Solids	44.3	0.009
Total identified	-	-
Total characterised	55.7	0.011
Total analysed	55.7	0.011
Solids	44.3	0.009
Accountability	100.0	0.020

The results given in this table are summarised from Table 10 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

**Table 7.2.1-17 TRR values and distribution of parent compound and metabolites in roots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – maturity (replicate B)**

	5x, maturity	
TRR [mg/kg] =	0.08	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	not done	not done
<b>Conventional extraction</b>		
ACN extract	34.2	0.027
ACN/H <sub>2</sub> O extract	18.6	0.015
Combined extracts	52.8	0.042
H <sub>2</sub> O extract	21.3	0.017
Solids	25.9	0.021
Total identified	-	-
Total characterised	74.1	0.059
Total analysed	74.1	0.059
Solids	25.9	0.021
Accountability	100.0	0.080

The results given in this table are summarised from Table 10 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

#### **Distribution of activity after enzymatic or acidic hydrolysis of polar compounds/fractions**

Because of the predominance of the polar radioactive components in the acetonitrile and acetonitrile/water extracts, from both shoot and root tissues, experiments (enzyme treatment and acidic hydrolysis) were conducted to attempt to determine if these components were phase II metabolites and resulted from conjugation of the herbicide or its main metabolites.

Treatment of shoot tissue extracts with  $\beta$ -glycosidase ( $\beta$ -glucosidase or  $\beta$ -galactosidase) did not affect the proportions of polar radioactive components and no obvious signs of cleavage were observed. Therefore, it was concluded that the endogenous moiety of the conjugates is unlikely to be  $\beta$ -glucose or  $\beta$ -galactose bound via an oxygen atom to the exocon. As a consequence, subsequent hydrolysis experiments were done with hydrochloric acid.

Selected extracts of shoots and roots were treated with 6 M HCl (18 h, reflux) to hydrolyse the polar components. The harsh conditions were chosen as acidic hydrolysis with 1 M and 2 M HCl showed no complete cleavage of the polar fraction in mature shoots. In contrast, hydrolyses with 2 M HCl of root and shoot extracts from samples taken before maturity, were shown to be exhaustive. After hydrolysis, mainly metabolites NC 8493 (ethofumesate-2-hydroxy) and NC 9607 (ethofumesate-lactone) were detected; traces of metabolite NC 20645 (ethofumesate-carboxylic acid = ring opened NC 9607) were also found. Since NC 9607 and NC 20645 are interconvertible, probably traces of NC 20645 are formed during sample preparation and/or analysis. .

Considering the metabolites formed during hydrolysis, it can be concluded that the majority of the polar fraction is represented by conjugates of metabolites NC 8493 and NC 20645. Under the acidic conditions of the hydrolysis, NC 20645 is transformed into NC 9607 by intra-molecular ring closure. Small amounts of NC 8493 can also result from decomposition of parent compound since ethofumesate itself is acid labile.

#### **Distribution of activity after acidic hydrolysis of bound residues**

As an example, the bound residues of mature shoots were also solubilised by acidic hydrolysis. Exhaustive extraction with 1 M HCl (reflux, approx. 18 h) released a significant portion of the bound residues. The released compounds were assigned to the known metabolites NC 8493, NC 20645 and NC 9607. Based on this result, it can be concluded that at least a part of the bound residues can be assigned to known metabolites, which are not accessible by the conventional solvent extraction.

#### **Characterization of polar compounds/fractions after acidic hydrolysis with 6 M HCl**

##### **Shoots**

The shoot sample taken from the 1x experiment at maturity was used as an example to characterize/identify the compounds forming the polar fractions present in the acetonitrile and acetonitrile/water extracts. The polar fraction was separated by fractionation and treated with 6 M HCl (reflux, 16 h) prior to analysis by TLC and HPLC. Hydrolysis yielded components identified as NC 9607, NC 20645 and NC 8493, with compound NC 9607 representing the predominant proportion. On the basis of the hydrolysis results, in the original Annex II dossier it was concluded that “conjugated NC 9607” is the main metabolite in sugar beet shoots. However, NC 9607 is a lactone and consequently cannot form conjugates. In contrast, the free acid NC 20645, which is formed by hydroxylation of the lactone ring of NC 9607 (ring opening by addition of water), can be conjugated. As a consequence the main metabolite in shoots must be a conjugate of metabolite NC 20645. Under the acidic conditions of the hydrolysis, the conjugate is cleaved and quantitatively transformed to the lactone by an intra-molecular condensation reaction (ring closure to form NC 9607). (Later reports and correspondence has always confirmed that “conjugated NC 9607” is in fact conjugated NC 20645.)

The distribution of the radioactivity in the shoot sample taken at maturity (1x experiment) is summarized in Table 7.2.1-18. Characterization of the polar fraction after acidic hydrolysis and exhaustive extraction of the fibre bound residues are included in the table.

**Table 7.2.1-18 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) after acidic hydrolysis (6 M HCl, 18 h reflux) – maturity (replicate B)**

	1x, maturity	
TRR [mg/kg] =	0.36	
Compound (ethofumesate)	% TRR	mg/kg

	<b>1x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>0.36</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash (DCM)</b>	<b>0.7</b>	<b>0.0025</b>
Ethofumesate	0.1	0.0004
NC 20645	0.1	0.0004
NC 8493	0.2	0.0007
NC 9607	0.1	0.0004
Unknown (remainder)	0.2	0.0007
<b>Conventional extraction</b>		
<b>ACN extract</b>	<b>51.6</b>	<b>0.1858</b>
<b>ACN/H<sub>2</sub>O extract</b>	<b>27.0</b>	<b>0.0972</b>
<b>Combined extracts</b>	<b>78.6</b>	<b>0.2830</b>
<b>DCM phase</b>	<b>2.4</b>	<b>0.0086</b>
Ethofumesate	0.5	0.0018
NC 20645	0.5	0.0018
NC 8493	0.5	0.0018
NC 9607	0.2	0.0007
Unknown (polar)	0.4	0.0014
Unknown (unipolar, bright green pigment fraction)	0.3	0.0011
<b>Aqueous phase (subjected to acidic hydrolysis)</b>	<b>76.2</b>	<b>0.2743</b>
<b>DCM phase (after hydrolysis)</b>	<b>70.5</b>	<b>0.2538</b>
Ethofumesate	-	-
NC 20645	12.2	0.0439
NC 8493	2.7	0.0097
NC 9607	54.0	0.1944
Unknown (polar)	1.6	0.0058
<b>Aqueous phase (remainder after hydrolysis)</b>	<b>5.7</b>	<b>0.0205</b>
<b>H<sub>2</sub>O extract (subjected to acidic hydrolysis)</b>	<b>7.1</b>	<b>0.0256</b>
Ethofumesate	-	-
NC 20645	1.1	0.0040
NC 8493	0.8	0.0029
NC 9607	3.9	0.0140
Unknown (polar)	-	-
Unknown (remainder)	1.3	0.0047
<b>Solids 1 (= bound residues)</b>	<b>13.6</b>	<b>0.0490</b>
<b>Exhaustive extraction of solids (2 M HCl, 16h, reflux)</b>		
<b>Solids 1 (subjected to acidic hydrolysis)</b>	<b>13.6</b>	<b>0.0490</b>
<b>DCM phase (after hydrolysis)</b>	<b>1.0</b>	<b>0.0036</b>
Origin	-	-
Ethofumesate	0.2	0.0007
NC 20645	0.5	0.0018
NC 8493	0.1	0.0004
NC 9607	0.2	0.0007
Unknown (remainder)	-	-
<b>Aqueous phase (after hydrolysis)</b>	<b>2.0</b>	<b>0.0072</b>
<b>Solids 2</b>	<b>10.6</b>	<b>0.0382</b>
<b>Total identified</b>	<b>77.9</b>	<b>0.2806</b>
<b>Total characterised</b>	<b>11.5</b>	<b>0.0413</b>
<b>Solids 2</b>	<b>10.6</b>	<b>0.0382</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.3600</b>

The results given in this table are summarised from Table 13 and Appendix 18 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%. Minor deviations may be caused by rounding.

Table 7.2.1-19 summarizes the identified metabolites and how they occurred in the sample (either free, conjugated or fibre bound). The comparison of the distribution of the metabolites before and after characterization of the polar fraction by hydrolysis and exhaustive extraction of the fibre bound residues reveals



the advantage/necessity of the acidic treatments. After the acidic treatments, the identification rate increased from 20% to 78%, and the metabolic behaviour of ethofumesate in sugar beets was more easily evaluated.

**Table 7.2.1-19 Examination of the nature of the radioactivity in metabolite fractions in mature shoots (1x experiment) (results expressed as % total activity recovered)**

Sampling event	Shoots			
	Maturity (replicate B) (1x rate)			
	before hydrolysis*		after hydrolysis*	
TRR	%	mg/kg	%	mg/kg
	100.0	0.360	100.0	0.360
Ethofumesate (parent compound)	0.1	<0.001	0.6	0.002
Ethofumesate fibre bound	0.2	<0.001	0.2	0.001
NC 8493	0.2	0.001	0.7	0.003
NC 8493 conjugated	-	-	3.5	0.013
NC 8493 fibre bound	0.1	<0.001	0.1	<0.001
NC 9607	0.1	<0.001	0.3	0.001
NC 9607 fibre bound	0.2	0.001	0.2	0.001
NC 20645	18.6	0.067	0.6	0.002
NC 20645 conjugated	-	-	71.2	0.256
NC 20645 fibre bound	0.5	0.002	0.5	0.002
<b>Total identified</b>	<b>20.0</b>	<b>0.072</b>	<b>77.9</b>	<b>0.280</b>
H <sub>2</sub> O extract conventional extraction	7.1	0.026	-	-
Aqueous phase after hydrolysis of H <sub>2</sub> O extract conventional extraction	-	-	5.7	0.021
Unknown 1 (soluble) +	-	-	0.3	0.001
Unknown 2 (soluble) +	60.1	0.216	0.4	0.001
Unknown 3 (soluble) +	-	-	1.6	0.006
Unknown 4 (soluble) +	0.2	0.001		
Aqueous phase after exhaustive extraction of fibre	2.0	0.007	2.0	0.007
<b>Total characterized</b>	<b>69.4</b>	<b>0.250</b>	<b>11.5</b>	<b>0.041</b>
<b>Fibre residue after hydrolysis (solids 2)</b>	<b>10.6</b>	<b>0.038</b>	<b>10.6</b>	<b>0.038</b>

\* before/after hydrolysis of polar fraction present in the acetonitrile and acetonitrile/water extract (= fraction containing the conjugates)

The results given in this table are summarised from Table 16 of the original report.

+ unknowns:

1. associated with pigment fraction;
2. polar component not resolved from the origin (Normal Phase TLC) or solvent front (reversed phase TLC and HPLC) in washes and tissue extracts prior to acidic hydrolysis;
3. polar compound not resolved from origin (Normal Phase TLC) or solvent front (Reversed Phase TLC and HPLC) in acid hydrolysates
4. other unknowns in surface wash of shoots

Remarks:

- treatment of fibre-bound residues was done with 1 M HCl (16 h under reflux) - TRR in mg/kg was recalculated based on the TRR given in %.

## Roots

The root sample taken 10 days post application from the 5x experiment was also used as an example to characterize/identify the compounds forming the polar fraction present in the acetonitrile and acetonitrile/water extracts. As for the shoot sample, the polar fraction was separated by fractionation and treated with 6 M HCl (reflux, 16 h) prior to analysis by TLC and HPLC. Hydrolysis revealed the known metabolites NC 9607, NC 20645 and NC 8493, with compound NC 9607 representing again the predominant proportion. Therefore, it was also shown for roots that metabolite NC 20645 in its conjugated form is a major residue.

The distribution of the radioactivity in the root sample taken 10 days after treatment (5x experiment) is summarized in Table 7.2.1-20. Characterization of the polar fraction after acidic hydrolysis is included in the table.

**Table 7.2.1-20 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) after acidic hydrolysis (6 M HCl, 18 h) – day 10**

Sampling event	Roots	
	5x, day 10	
TRR [mg/kg] =	0.92	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	not done	not done
Conventional extraction		
ACN extract	71.4	0.657
ACN/H <sub>2</sub> O extract	8.5	0.079
Combined extracts (ACN + ACN/H <sub>2</sub> O)	80.0	0.736
DCM phase	28.8	0.265
Ethofumesate	10.5	0.097
NC 20645	2.8	0.026
NC 8493	7.2	0.066
NC 9607	4.6	0.042
Unknown	1.8	0.017
Unknown (polar)	1.0	0.009
Unknown (remainder)	0.9	0.008
Aqueous phase (subjected to acid hydrolysis)	51.2	0.471
NC 20645	6.3	0.058
NC 8493	4.9	0.045
NC 9607	29.3	0.270
Unknown (polar)	8.9	0.082
Unknown 2 (remainder)	1.8	0.017
H <sub>2</sub> O extract	1.5	0.014
Solids	18.6	0.171
Total identified	65.6	0.604
Total characterised	15.9	0.146
Total analysed	81.5	0.750
Solids	18.6	0.171
Accountability	100.0	0.920

The results given in this table are summarised from Table 14 of the original study report (KCA 6.2.1 /05). Recoveries were normalised to 100%.

Table 7.2.1-21 summarizes the identified metabolites and how they occurred in the sample (either free or conjugated). Again, integration of an acidic hydrolysis step allowed the identification of the conjugates by means of their exocons. After the acidic treatments, the identification rate increased from 25% to 66%. The metabolites detected in the roots were the same as detected in the shoots.

**Table 7.2.1-21 Examination of the nature of the radioactivity in metabolite fractions from roots (results expressed as % total activity recovered)**

Sampling event	Roots			
	5x, day 10			
	before hydrolysis*		after hydrolysis*	
TRR	%	mg/kg	%	mg/kg
	100.0	0.92	100.0	0.92
Ethofumesate (parent compound)	10.5	0.10	10.5	0.10
NC 8493	7.2	0.07	7.2	0.07
NC 8493 conjugated	-	-	4.9	0.05
NC 9607	4.6	0.04	4.6	0.04
NC 20645	2.8	0.03	2.8	0.03
NC 20645 conjugated	-	-	35.6	0.33
Total identified	25.1	0.23	65.6	0.60
Aqueous phase after partitioning with DCM	51.2	0.47	-	-
H <sub>2</sub> O extract conventional extraction	1.5	0.01	1.5	0.01

Sampling event	Roots			
	5x, day 10			
	before hydrolysis*		after hydrolysis*	
TRR	%	mg/kg	%	mg/kg
	100.0	0.92	100.0	0.92
Unknown (non-polar, DCM-soluble)#	1.8	0.02	1.8	0.02
Unknown 1 (DCM soluble) +	1.0	0.01	1.0	0.01
Unknown 2 (water soluble) +	-	-	8.9	0.08
Unknown (remainder after partitioning against DCM)	0.9	0.01	0.9	0.01
Unknown (remainder in hydrolysate)	-	-	1.8	0.02
<b>Total characterized</b>	<b>56.3</b>	<b>0.52</b>	<b>15.9</b>	<b>0.15</b>
<b>Solids (bound fibre residue)</b>	<b>18.6</b>	<b>0.17</b>	<b>18.6</b>	<b>0.17</b>

\* before/after hydrolysis of polar fraction present in the acetonitrile and acetonitrile/water extract (= fraction containing the conjugates)

# unknown (non-polar): slightly later retention time than ethofumesate-5-hydroxy (NC 10458) on HPLC and different RF on TLC

+ unknowns (soluble):

1. polar component not resolved from origin (Normal Phase TLC) or solvent front (Reversed Phase TLC and HPLC) in tissue extracts, present in DCM phase

2. polar component not resolved from origin (Normal Phase TLC) or solvent front (Reversed Phase TLC and HPLC) in acidic hydrolysate

Remarks:

TRR in mg/kg was recalculated based on the TRR given in %. Minor deviations may be caused by rounding.

#### IV. Conclusions

Ethofumesate is metabolised in sugar beet to NC 8493, NC 9607 and NC 20645.

NC 8493 and NC 20645 form conjugates which can be hydrolysed with 6 M hydrochloric acid.

The conjugate of metabolite NC 20645 was the main compound found in sugar beet leaves at later sampling times.

The conjugate represents also a predominant portion in sugar beet roots collected 10 days after treatment. Under acidic conditions NC 20645 rearranges to NC 9607.

Based on the metabolites identified the following metabolic routes were deduced:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493 (ethofumesate-2-hydroxy).
- NC 8493 can either undergo conjugation to give polar metabolites, or oxidation to the lactone NC 9607 (ethofumesate-lactone)
- The lactone ring of NC 9607 opens to the acid NC 20645 (ethofumesate-carboxylic acid) which can also undergo conjugation to give polar metabolites.

On the basis of the results of this study it is concluded that the metabolism of [<sup>14</sup>C]-ethofumesate in sugar beet is well understood and a metabolic pathway can be deduced (see pathway in Figure 1 at the end of the chapter).

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##### B.7.2.1.1.2. Sugar beet metabolism 2

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

Report:	KCA 6.2.1 /06; Caley, C. Y.; Chapleo, S.; Haswell, A.; 1994; M-161455-01
Title:	THE METABOLISM OF 14C- ETHOFUMESATE IN SUGAR BEET
Report No:	A87553
Document No(s):	Report includes Trial Nos.: 382445 M-161455-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes

### I. Summary

The aim of this study was to determine the uptake, translocation and metabolism of [ $^{14}\text{C}$ -benzene]- in sugar beet. The herbicide was applied as a solvent-free formulation at the formerly intended maximum field application rate of 1.5 kg a.s./ha and at a 5-fold application rate of 7.5 kg a.s./ha for metabolite identification.

Plants were harvested on the day of application, at 7 and 28 days after the application and at maturity. Total residues in the crop were determined by measurement of total radioactivity. Soluble radioactive residues were characterised and identified by the extraction behaviour and by chromatographic procedures.

The TRR values of the mature crop at harvest are shown in the following table:

**Table 7.2.1-22 TRR values in sugar beet shoot and root after spray application of [ $^{14}\text{C}$ ]-ethofumesate**

Matrix	Timing and Application	Sampling event	TRR (mg a.s. equiv./kg)
Shoot	4 fully expanded true leaves,	Maturity	0.75
Root	1x 1.5 kg a.s./ha 1x 7.5 kg a.s./ha	Maturity	0.01

The tissues were extracted twice with acetonitrile, twice with acetonitrile/distilled-grade water (1/1; v/v) and twice with distilled-grade water.

In shoots at final harvest (maturity) collected in the 1x experiment, the total residue was 0.75 mg/kg. Only 3.1% of the total residue could be identified before acidic treatment. After acidic hydrolysis of extracts containing high amounts of an unidentified polar fraction and exhaustive extraction of the bound residue 83.6% of the total residue was identified, 9.8% was characterized. The results show that the unidentified polar material is largely converted to the derivatives NC 9607 and NC 8493 due to the acidic hydrolysis.

In roots at maturity, the total residue was 0.01 mg/kg and thus the radioactivity level was too low to permit analysis. Characterization of residues was only possible in samples of the 1x experiment collected at early time points (day 0 and 7) or in samples of the overdose experiment.

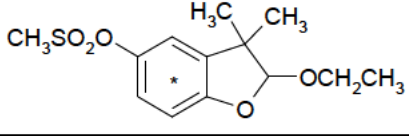
The majority of the radioactivity at day 0 was identified as ethofumesate in the 1x rate root acetonitrile extract. The proportion of ethofumesate declined at day 7 while the concentration of unidentified polar components (polar fraction) increased. Levels of radioactivity in other 1x rate samples taken at day 28 and at maturity were too low to permit analysis. Therefore, samples of 5x rate were analysed.

Based on the results of the 5x rate of root extracts a decrease in ethofumesate concentration is visible that goes along with an increase of polar compounds. At maturity, polar unknown compounds up to 53.8% of the TRR (0.119 mg/kg) were detected. At earlier time points, low levels of the metabolites NC 9607 (4.6%; 0.162 mg/kg) and NC 8493 (6.3%; 0.219 mg/kg) of ethofumesate were identified. These were not consistently detected in samples taken at other sampling events, probably due to the low levels of radioactivity associated with them, but their presence suggests that they may be formed from ethofumesate prior to conjugation.

## II. Materials and Methods

### A. Materials

#### 1. Test Material:

Chemical structure	
Radiolabelled test material	[ <sup>14</sup> C]-ethofumesate 2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methane-sulphonate
Specific radioactivity	8.325 MBq/mg (225 µCi/mg)
Radiochemical purity*	83% (TLC) solvent system 1 79% (TLC) solvent system 2 after purification: > 97%

\*solvent system 1: toluene/ethyl acetate (4/1; v/v); solvent system 2: acetonitrile/water (dest.) (4/1; v/v)

[<sup>14</sup>C]-ethofumesate was formulated as a solvent-free formulation for application at the maximum field application rate of 1.5 kg a.s./ha and 5-fold application rate of 7.5 kg a.s./ha.

The formulations had a specific activity of 9.592 µCi/mg (1x field rate) and 9.176 µCi/mg (5x field rate) which was determined by liquid scintillation counting. The radiochemical purity of the applied formulations was confirmed by TLC. Radiochemical purity was found to be greater than 97%.

#### 2. Soil

A sandy loam soil (UK classification) was used, which had been passed through a 5-10 mm sieve, over a 5 cm deep gravel bed. Soil details and characterisation are given in the following table:

**Table 7.2.1-23 Soil characterisation**

Physical characterisation	
Soil classification (UK)	Sandy loam soil
pH (water)	6.3
pH (KCl)	5.8
% organic carbon	2.84
Cation exchange capacity (mequiv. /100 g)	5.1
Particle size analysis	
Clay (< 0.002 mm) (%)	10
Silt (0.002-0.063 mm) (%)	10
Total sand (0.063-2 mm) (%)	80

#### 3. Plant

Sugar beet, variety "Beta vulgaris cv Gala", representative for root vegetables

### B. Study Design

#### Experimental conditions

Plants were grown in pots filled with sandy loam soil. Fertiliser (Vitax GR 112) was applied at a rate of 27.5 g/pot, sprinkled over the surface and cultivated to a depth of approx. 1 cm, prior to sowing. Twenty seeds were sown in each pot and kept covered until emergence, then exposed to the normal lighting regime. Prior to experimentation, plants were thinned to leave 8 plants per pot.

The study was performed under glasshouse environmental conditions. During cultivation, to maintain the good conditions of plants, pesticides were applied as required.

The test item was applied as a foliar spray when plants had 4 fully expanded true leaves. A single application of test item was made to 6 pots at the 1x field application rate and to 2 pots at the 5x field application rate. The formulation was applied as a fine spray to each pot using an all-glass atomiser. Test pots were sited next to an extraction fan which was kept running continuously, to reduce the concentration of any  $^{14}\text{CO}_2$  possibly released by mineralisation of [ $^{14}\text{C}$ ]-ethofumesate.

### **Sampling**

Plant samples were collected on the day of application when the formulation was dry, at 7 and 28 days after the application and at maturity. On the day of application, all 8 plants in each of 2 treated pots (1x rate) and 2 control pots were sampled. At 7 days after the application, 4 plants were sampled from each of the remaining pots and at 28 days after the application, 3 plants were sampled from each of the remaining pots. This left a single plant close to the centre of each pot which was harvested at maturity.

At each harvest, leaf and root components of each plant were separated at the shoot/root junction and shoots and roots in each group were combined and weighed. Shoots were washed in dichloromethane to remove surface residue and roots were washed in distilled-grade water to remove soil. Total radioactivity was determined in the surface washes and washed tissues. Washed tissue from plants harvested at 0, 7 and 28 days after the application was chopped up using scissors, frozen in dry ice until brittle and homogenised, using a Waring Blender. Maturity shoot samples were homogenised with dry ice using a Retsch-Impeller type grinding mill. Maturity root samples were roughly chopped mixed with dry ice and passed through a mincer. For each sample, the dry ice was allowed to sublime off prior to analysis.

### **C. Analytical Procedures**

#### **Washing:**

The shoot and root tissues were washed in dichloromethane and distilled-grade water, respectively. This was achieved by immersing portions of tissue in solvent for approx. 1-2 min then removing the tissue. Duplicate aliquots of each liquid sample were taken for analysis of radioactivity. The dichloromethane surface washes of the shoots (except for the sample collected at maturity) were directly analysed by TLC (normal phase). If sample concentration was required, subsamples of the surface washes were evaporated to dryness and reconstituted in an appropriate solvent for chromatographic analysis. HPLC analysis was conducted for all surface washes of shoots and roots.

#### **Extraction of tissues**

A portion of each of the tissue samples was extracted twice with acetonitrile, twice with acetonitrile/distilled-grade water (1/1; v/v) and twice with distilled-grade water. The samples were homogenised with each solvent using a Silverson homogeniser at room temperature for several minutes. The supernatant was separated from the residue on each occasion by centrifugation. The two supernatants of each extraction medium were combined. Acetonitrile extracts were reduced to a small volume by rotary evaporation prior to chromatographic analysis. Acetonitrile/water (1/1; v/v) extracts were reduced using a rotary evaporator to leave the aqueous fraction. This was then concentrated by freeze-drying and reconstituted in a small volume of acetonitrile/water (1/1; v/v) prior to chromatographic analysis. Aqueous extracts were not further prepared for chromatography at this stage.

#### **Identification and characterisation:**

##### **Initial characterisation:**

The nature of the radioactivity in the samples of wash and tissue extracts was examined by TLC (generally in a normal phase system and selected samples in a reversed phase system) and HPLC (reversed phase system), whenever a sufficient concentration of radioactivity was present. Some samples were analysed by HPLC only where levels of radioactivity were too low for TLC. For these samples, HPLC fractions were collected at 1 min intervals. Each fraction was analysed by LSC to allow peak assignment and quantification.

Samples were chromatographed alongside standards of non-radiolabelled reference compounds.

#### **Hydrolysis of polar metabolites:**

Characterisation of initial tissue extracts revealed the presence of polar metabolites, presumably conjugates. The following procedures (enzyme treatment and acidic hydrolysis) were employed to selected shoot samples to attempt to characterize these polar metabolites.

#### **Enzyme treatment:**

Subsamples of the maturity 5x rate acetonitrile, acetonitrile/water and water extracts of shoots were reduced to dryness under a stream of nitrogen gas and reconstituted in sodium acetate buffer (pH 5). The samples were each incubated with  $\beta$ -glucosidase at 35°C for 1 h.

#### **Acidic hydrolysis:**

For method development subsamples of the maturity x 5 rate acetonitrile, acetonitrile/water and water extracts of shoots were subjected to acidic hydrolysis (1 M HCl, 1 h, 60°C; 3 M HCl, 1 h, 60°C; and 6 M HCl, 6 h, 95°C). Following incubation, any precipitate that formed was separated out by centrifugation and the supernatant was reduced to dryness by freeze drying and/or under a stream of nitrogen gas. Samples were reconstituted in a small volume of their original solvent. Water was also added to acetonitrile extracts where necessary to assist dissolution.

Subsamples of the maturity 1x rate acetonitrile, acetonitrile/water and water extracts were subjected to 6 M acidic hydrolysis (6 M HCl, 6 h, 95°C). Precipitate was removed by centrifugation and the supernatants taken to dryness by a combination of freeze drying and rotary evaporation techniques. The acetonitrile and acetonitrile/water extract samples were reconstituted in acetonitrile, the acetonitrile soluble fraction removed and any remaining material dissolved in water. The water extract sample was taken up in water, the water-soluble fraction removed and any remaining material dissolved in acetonitrile.

#### **Extraction and treatment of fibre-bound residues:**

Portions of the maturity 5x rate unextracted tissue residue of shoots (following conventional solvent extraction) were subject to acidic hydrolysis (1 M HCl, 1 h, 60°C; 3 M HCl, 1 h, 60°C; and 6 M HCl, 6 h, 95°C). Control samples, containing water in place of the acid, were also incubated. Following incubation, the remaining tissue residue was separated out by centrifugation and the supernatant reduced to dryness by freeze drying and/or under a stream of nitrogen gas. Samples were reconstituted in a small volume of water.

A subsample of the maturity 1x rate unextracted tissue residue of shoots was subjected to 6 M acidic hydrolysis (6 M HCl, 6 h, 95°C). The remaining tissue residue was removed and the sample concentrated as described above.

#### **Quantification:**

The radioactivity in the washes and extracts was quantified using liquid scintillation counting. For a better comparability the results given in the study report were normalised to 100%. The normalization is justified since the single sample preparation steps showed acceptable recoveries.

**Storage stability:**

Acetonitrile extracts of the 1x rate day 0 shoot and root and day 7 shoot, and the x5 rate day 7 root were selected for assessment of storage stability. These samples were stored at approx. -20°C for approximately 6 months following initial analysis. They were then re-examined by TLC (normal phase system).

**III. Results and Discussion**

The metabolism of [<sup>14</sup>C]-ethofumesate was investigated in sugar beet shoots and roots following a foliar spray application with an application rate of 1.5 kg a.s./ha (1x) and an overdose application rate of 7.5 kg a.s./ha (5x) for metabolite identification.

In Table 7.2.1-24 and Table 7.2.1-25 levels of total radioactivity measured in the sugar beet shoots and roots are presented for the different sampling events. Immediately following application of [<sup>14</sup>C]-ethofumesate to sugar beet plants, the vast majority of the radioactive residue was found in the surface wash (>70%) of the shoots. At the 1x rate treatment, a total residue of 126.4 mg/kg was found in the shoots on the day of application. The total radioactive residue declined with time to 0.75 mg/kg (less than 1% of the day 0 value) at the final harvest at maturity. In this final maturity sample only 0.014 mg/kg (2%) was removed by surface washing. A corresponding increase in the proportion present in the washed tissue indicates that translocation into the tissues took place.

The 1x rate root tissue contained a total residue of 1.9 mg/kg on the day of application of which 0.70 mg/kg remained after washing. Levels of radioactivity declined with time to 0.008 mg/kg in the washed tissues at maturity. The radioactivity found in the surface washes of roots is most likely to be derived from the soil present on them rather being a true surface residue. Therefore only the washed roots were considered for analysis.

Overall, the sugar beet shoot contained the predominant proportion of the total radioactive residues, accounting for >99% at the day of application and still >95% at maturity. Therefore identification and characterization of residues was mainly conducted in shoot samples.

**Table 7.2.1-24 Concentration of the total radioactivity in shoot samples (results expressed in mg a.s. equiv./kg fresh weight)**

Sampling event	day 0	day 7	day 28	maturity
<b>1x Application rate</b>				
Surface wash	93.661	42.110	6.292	0.014
Washed tissue	32.716	34.069	20.593	0.734
TRR	126.377	76.179	26.885	0.748
<b>5x Application rate</b>				
Surface wash	n.s.	222.343	44.247	1.238
Washed tissue	n.s.	60.778	61.556	5.362
TRR	n.s.	283.121	105.803	6.600

n.s.: not sampled

**Table 7.2.1-25 Concentration of the total radioactivity in root samples (results expressed in mg a.s. equiv./kg fresh weight)**

Sampling event	day 0	day 7	day 28	maturity
<b>1x Application rate</b>				
surface wash	1.238	0.239	0.016	0.002
washed tissue	0.701	0.478	0.150	0.008
TRR	1.939	0.717	0.166	0.010
<b>5x Application rate</b>				
surface wash	n.s.	1.988	0.304	0.020



Sampling event	day 0	day 7	day 28	maturity
<b>1x Application rate</b>				
washed tissue	n.s.	3.356	3.179	0.202
TRR	n.s.	5.344	3.483	0.222

n.s.: not sampled

### Distribution of activity prior to acidic hydrolysis of polar compounds/fraction

#### Shoots

Chromatographic analysis of shoot washes and extracts showed that almost all the radioactivity present on day 0 up to day 28 was ethofumesate. With time, the proportion of ethofumesate declined and the proportion of unidentified polar material increased.

Results of HPLC measurements for shoots are reported here which are also representative for results measured by TLC (normal phase).

**Table 7.2.1-26 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 0 (HPLC analysis)**

	<b>1x, day 0</b>	
<b>TRR [mg/kg] =</b>	<b>126.377</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>74.1</b>	<b>93.661</b>
Ethofumesate	74.1	93.661
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>25.4</b>	<b>32.078</b>
Ethofumesate	25.4	32.078
<b>ACN/H<sub>2</sub>O extract</b>	<b>0.4</b>	<b>0.493</b>
Ethofumesate	0.4	0.493
<b>H<sub>2</sub>O extract</b>	<b>&lt;0.1</b>	<b>&lt;0.001</b>
<b>Solids</b>	<b>0.1</b>	<b>0.145</b>
<b>Total identified</b>	<b>99.9</b>	<b>126.232</b>
<b>Total characterised</b>	<b>&lt;0.1</b>	<b>&lt;0.001</b>
<b>Total analysed</b>	<b>99.9</b>	<b>126.232</b>
<b>Solids</b>	<b>0.1</b>	<b>0.145</b>
<b>Accountability</b>	<b>100.0</b>	<b>126.377</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1/06). Recoveries were normalised to 100%.

**Table 7.2.1-27 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 7 (HPLC analysis)**

	<b>1x, day 7</b>	
<b>TRR [mg/kg] =</b>	<b>76.179</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>55.3</b>	<b>42.110</b>
Ethofumesate	55.3	42.110
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>41.6</b>	<b>31.718</b>
Unknown 1 (eluting in dead volume)	21.0	16.018
Ethofumesate	20.6	15.701
<b>ACN/H<sub>2</sub>O extract</b>	<b>2.7</b>	<b>2.078</b>
Unknown 1 (eluting in dead volume)	2.4	1.816
Ethofumesate	0.3	0.262
<b>H<sub>2</sub>O extract</b>	<b>&lt;0.1</b>	<b>0.034</b>
<b>Solids</b>	<b>0.3</b>	<b>0.238</b>
<b>Total identified</b>	<b>76.2</b>	<b>58.072</b>

	1x, day 7	
<b>TRR [mg/kg] =</b>	<b>76.179</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Total characterised</b>	<b>23.5</b>	<b>17.868</b>
<b>Total analysed</b>	<b>99.7</b>	<b>75.941</b>
<b>Solids</b>	<b>0.3</b>	<b>0.238</b>
<b>Accountability</b>	<b>100.0</b>	<b>76.179</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1/06). Recoveries were normalised to 100%.

**Table 7.2.1-28 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 28 (HPLC analysis)**

	1x, day 28	
<b>TRR [mg/kg] =</b>	<b>26.885</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>23.4</b>	<b>6.292</b>
Ethofumesate	23.4	6.292
<b>Conventional extraction</b>		
<b>ACN extract</b>	<b>66.2</b>	<b>17.785</b>
Unknown 1 (eluting in dead volume)	55.2	42.053
Ethofumesate	11.0	8.365
<b>ACN/H<sub>2</sub>O extract</b>	<b>9.5</b>	<b>2.559</b>
Unknown 1 (eluting in dead volume)	8.9	6.749
Ethofumesate	1.2	0.913
<b>H<sub>2</sub>O extract</b>	<b>0.4</b>	<b>0.104</b>
<b>Solids</b>	<b>0.5</b>	<b>0.146</b>
<b>Total identified</b>	<b>35.6</b>	<b>27.107</b>
<b>Total characterised</b>	<b>64.5</b>	<b>48.907</b>
<b>Total analysed</b>	<b>99.5</b>	<b>26.739</b>
<b>Solids</b>	<b>0.5</b>	<b>0.146</b>
<b>Accountability</b>	<b>100.0</b>	<b>26.885</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report. Recoveries were normalised to 100%.

**Table 7.2.1-29 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – maturity (HPLC analysis)**

	1x, maturity	
<b>TRR [mg/kg] =</b>	<b>0.748</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>1.9</b>	<b>0.014</b>
Ethofumesate	1.5	0.011
Remainder (unassigned radioactivity)	0.4	0.003
<b>Conventional extraction</b>		
<b>ACN extract</b>	<b>51.5</b>	<b>0.385</b>
Unknown 1 (eluting in dead volume)	44.7	0.334
Ethofumesate	0.8	0.006
Remainder	6.0	0.045
<b>ACN/H<sub>2</sub>O extract</b>	<b>29.7</b>	<b>0.222</b>
Unknown 1 (eluting in dead volume)	23.9	0.179
Remainder	5.8	0.044
<b>H<sub>2</sub>O extract</b>	<b>6.3</b>	<b>0.047</b>
Unknown 1 (eluting in dead volume)	5.6	0.042
Remainder	0.7	0.005
<b>Solids</b>	<b>10.6</b>	<b>0.079</b>
<b>Total identified</b>	<b>2.3</b>	<b>0.017</b>
<b>Total characterised</b>	<b>87.1</b>	<b>0.652</b>
<b>Total analysed</b>	<b>89.4</b>	<b>0.669</b>

	1x, maturity	
TRR [mg/kg] =	0.748	
Compound (ethofumesate)	% TRR	mg/kg
Solids	10.6	0.079
Accountability	100.0	0.748

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-30 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – maturity (TLC analysis)**

	1x, maturity	
TRR [mg/kg] =	0.748	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	1.9	0.014
Origin (polar fraction)	0.1	0.001
Ethofumesate	1.7	0.013
Remainder	<0.1	<0.001
<i>Conventional extraction</i>		
ACN extract	51.5	0.385
Origin (polar fraction)	49.7	0.371
Ethofumesate	1.4	0.010
Remainder	0.5	0.003
ACN/H <sub>2</sub> O extract	29.7	0.222
Origin (polar fraction)	28.8	0.215
Remainder	0.9	0.007
H <sub>2</sub> O extract	6.3	0.047
Solids	10.6	0.079
Total identified	3.1	0.023
Total characterised	86.3	0.645
Total analysed	83.1	0.621
Solids	10.6	0.079
Accountability	100.0	0.748

The results given in this table are summarised from Table 1, 4 and 8 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-31 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – maturity (HPLC analysis)**

	5x, maturity	
TRR [mg/kg] =	6.600	
Compound (ethofumesate)	% TRR	mg/kg
Surface wash	18.8	1.238
Ethofumesate	18.8	1.238
<i>Conventional extraction</i>		
ACN extract	47.0	0.385
Unknown 1 (eluting in dead volume)	40.2	2.652
Unknown 2 (discrete, polar peak)	2.6	0.174
NC8493	0.9	0.062
ethofumesate	3.3	0.217
ACN/H <sub>2</sub> O extract	25.4	1.679
Unknown 1 (eluting in dead volume)	25.4	1.679
H <sub>2</sub> O extract	4.0	0.264
Unknown 1 (eluting in dead volume)	3.5	0.231
Remainder (unassigned radioactivity)	0.5	0.033
Solids	4.8	0.314
Total identified	23.0	1.517
Total characterised	72.2	4.768
Total analysed	95.2	6.283

	5x, maturity	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Solids	4.8	0.317
Accountability	100.0	6.600

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-32 TRR values and distribution of parent compound and metabolites in shoots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – maturity (TLC analysis)**

	5x, maturity	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>18.8</b>	<b>1.238</b>
Origin (polar fraction)	0.4	0.025
Ethofumesate	18.3	1.211
Remainder	<0.1	0.002
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>47.0</b>	<b>3.105</b>
Origin (polar fraction)	44.4	2.928
Ethofumesate	2.7	0.177
<b>ACN/H<sub>2</sub>O extract</b>	<b>25.4</b>	<b>1.679</b>
Origin (polar fraction)	24.0	1.583
Ethofumesate	1.4	0.096
<b>H<sub>2</sub>O extract</b>	<b>4.0</b>	<b>0.264</b>
<b>Solids</b>	<b>4.8</b>	<b>0.314</b>
<b>Total identified</b>	<b>22.5</b>	<b>1.483</b>
<b>Total characterised</b>	<b>72.8</b>	<b>4.802</b>
<b>Total analysed</b>	<b>91.2</b>	<b>6.022</b>
<b>Solids</b>	<b>4.8</b>	<b>0.314</b>
<b>Accountability</b>	<b>100.0</b>	<b>6.600</b>

The results given in this table are summarised from table 1, 4 and 8 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

### Roots

Root tissue extracts contained much lower levels of radioactivity than the shoot tissues and in many cases an insufficient concentration of radioactivity for chromatographic analysis. The majority of the radioactivity at day 0 was identified as ethofumesate in the 1x rate root acetonitrile extract. While the proportion of ethofumesate declined with time an increasing level of unidentified polar components was detected. Radioactivity levels in acetonitrile/water extracts of the 1x experiment were generally low and therefore no further investigations were performed. Levels of radioactivity in other 1x rate samples taken at day 28 and at maturity were too low to permit analysis. Therefore, samples of 5x rate were analysed. These investigations also clearly show a decrease in ethofumesate concentration which goes along with an increase of polar compounds. In 5x samples collected 7 days after the treatment, low levels of the metabolites NC 9607 and NC 8493 were identified. These were not consistently detected in samples taken at other sampling events, probably due to the low residue level - but their presence suggests that they are formed as intermediates prior to conjugation.

Results of HPLC measurements are given below in Table 7.2.1-33 to Table 7.2.1-38, which are generally also representative for results of TLC measurements.

**Table 7.2.1-33 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 0 (HPLC analysis)**

	1x, day 0	
<b>TRR [mg/kg] =</b>	<b>0.701</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>93.7</b>	<b>0.657</b>
Unknown 1 (radioactivity eluting in dead volume)	34.2	0.240
Ethofumesate	59.5	0.417
<b>ACN/H<sub>2</sub>O extract</b>	<b>4.1</b>	<b>0.029</b>
<b>H<sub>2</sub>O extract</b>	<b>0.2</b>	<b>0.001</b>
<b>Solids</b>	<b>2.0</b>	<b>0.014</b>
<b>Total identified</b>	<b>59.5</b>	<b>0.417</b>
<b>Total characterised</b>	<b>38.5</b>	<b>0.270</b>
<b>Total analysed</b>	<b>93.7</b>	<b>0.657</b>
<b>Solids</b>	<b>2.0</b>	<b>0.014</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.701</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-34 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 1x rate) before acidic hydrolysis – day 7 (HPLC analysis)**

	1x, day 7	
<b>TRR [mg/kg] =</b>	<b>0.478</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>78.8</b>	<b>0.376</b>
Unknown 1 (radioactivity eluting in dead volume)	78.8	0.376
<b>ACN/H<sub>2</sub>O extract</b>	<b>7.5</b>	<b>0.036</b>
<b>H<sub>2</sub>O extract</b>	<b>0.5</b>	<b>0.002</b>
<b>Solids</b>	<b>13.3</b>	<b>0.064</b>
<b>Total identified</b>	<b>-</b>	<b>-</b>
<b>Total characterised</b>	<b>86.7</b>	<b>0.414</b>
<b>Total analysed</b>	<b>78.8</b>	<b>0.376</b>
<b>Solids</b>	<b>13.3</b>	<b>0.064</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.047</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-35 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – day 7 (HPLC analysis)**

	5x, day 7	
<b>TRR [mg/kg] =</b>	<b>3.356</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>86.1</b>	<b>2.890</b>
Unknown 1 (radioactivity eluting in dead volume)	22.5	0.754
Unknown 2 (discrete polar peak)	13.9	0.465
NC 8493	6.9	0.231
NC 9707	5.1	0.171
Ethofumesate	35.6	1.194
Remainder	2.2	0.075

	<b>5x, day 7</b>	
<b>TRR [mg/kg] =</b>	<b>3.356</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
ACN/H <sub>2</sub> O extract	5.2	0.176
H <sub>2</sub> O extract	0.4	0.015
Solids	8.2	0.275
<b>Total identified</b>	<b>47.5</b>	<b>1.595</b>
<b>Total characterised</b>	<b>44.3</b>	<b>1.486</b>
<b>Total analysed</b>	<b>86.1</b>	<b>2.890</b>
<b>Solids</b>	<b>8.2</b>	<b>0.275</b>
<b>Accountability</b>	<b>100.0</b>	<b>3.356</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-36 TRR values and distribution of parent compound and metabolites in roots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – day 28 (HPLC analysis)**

	<b>5x, day 28</b>	
<b>TRR [mg/kg] =</b>	<b>3.179</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
ACN extract	76.0	2.417
Unknown 1 (radioactivity eluting in dead volume)	70.9	2.255
Ethofumesate	5.2	0.164
ACN/H <sub>2</sub> O extract	13.4	0.425
Unknown 1	11.0	0.349
Remainder	2.2	0.076
H <sub>2</sub> O extract	2.8	0.088
Solids	7.8	0.249
<b>Total identified</b>	<b>5.2</b>	<b>0.164</b>
<b>Total characterised</b>	<b>87.1</b>	<b>2.768</b>
<b>Total analysed</b>	<b>89.4</b>	<b>2.842</b>
<b>Solids</b>	<b>7.8</b>	<b>0.249</b>
<b>Accountability</b>	<b>100.0</b>	<b>3.179</b>

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-37 TRR values and distribution of parent compound and metabolites in roots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – maturity (HPLC analysis)**

	<b>5x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>0.202</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
ACN extract	46.4	0.094
Unknown 1 (radioactivity eluting in dead volume)	16.1	0.033
Unknown 2 (discrete polar peak)	25.6	0.052
Ethofumesate	4.8	0.010
ACN/H <sub>2</sub> O extract	20.8	0.042
Unknown 1	17.5	0.035
Remainder	3.3	0.007
H <sub>2</sub> O extract	8.6	0.017
Solids	24.2	0.049
<b>Total identified</b>	<b>4.8</b>	<b>0.010</b>
<b>Total characterised</b>	<b>71.1</b>	<b>0.144</b>
<b>Total analysed</b>	<b>67.2</b>	<b>0.136</b>
<b>Solids</b>	<b>24.2</b>	<b>0.049</b>

	5x, maturity	
TRR [mg/kg] =	0.202	
Compound (ethofumesate)	% TRR	mg/kg
Accountability	100.0	0.202

The results given in this table are summarised from Table 1, 4 and 10 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-38 TRR values and distribution of parent compound and metabolites in roots following application of  $^{14}\text{C}$ -ethofumesate to sugar beets (treatment 5x rate) before acidic hydrolysis – maturity (TLC analysis)**

	5x, maturity	
TRR [mg/kg] =	0.202	
Compound (ethofumesate)	% TRR	mg/kg
<i>Conventional extraction</i>		
ACN extract	46.4	0.094
Origin (polar fraction)	37.5	0.076
Ethofumesate	4.9	0.008
ACN/H <sub>2</sub> O extract	20.8	0.042
Origin (polar fraction)	16.4	0.033
Remainder	4.4	0.009
H <sub>2</sub> O extract	8.6	0.017
Solids	24.2	0.049
Total identified	4.9	0.010
Total characterised	70.9	0.143
Total analysed	67.2	0.136
Solids	24.2	0.049
Accountability	100.0	0.202

The results given in this table are summarised from Table 1, 4 and 8 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

#### Characterisation of residues after enzymatic or acidic hydrolysis of polar compounds/fractions

Because of the predominance of the polar radioactive components (polar fractions) in the conventional solvent extracts, from both shoot and root tissues, experiments (enzyme treatment and acidic hydrolysis) were conducted to attempt to determine if these components were phase II metabolites and resulted from conjugation of the herbicide or its main metabolites.

Treatment of shoot tissue extracts with  $\beta$ -glucosidase did not affect the proportions of polar radioactive components and no obvious signs of cleavage were observed. Therefore, it was concluded that the endogenous moiety of the conjugates is unlikely to be  $\beta$ -glucose bound via an oxygen atom to the exocon. As a consequence, subsequent hydrolysis experiments were done with hydrochloric acid.

Treatment of the maturity shoot extracts (to characterize the unknown polar fraction) with various hydrochloric acid concentrations showed acceptable recovery of radioactivity. The 5x rate acetonitrile, acetonitrile/water and water extracts of shoots treated with 1 M and 3 M acid showed most of the radioactivity remained as unidentified polar material. With 6 M acid, a significant effect was seen and this treatment was selected for use with the 1x rate samples. Following treatment with 6 M acid, the major component in all extracts showed co-chromatography with metabolite NC 9607. HPLC co-chromatography also showed the presence of NC8493 in all these samples. Control incubations demonstrated that this change did not take place in the absence of acid. Considering the metabolites formed during vigorous hydrolysis, it can be concluded that the majority of the polar fraction is represented by conjugates of metabolites NC 8493 and NC 20645. Under the acidic conditions of the hydrolysis, NC 20645 is transformed into the analytical target NC 9607 by intra-molecular ring closure.

Small amounts of NC 8493 could also result from the acidic decomposition of the parent compound ethofumesate.

#### Distribution of activity after acidic hydrolysis of bound residues

As an example, the bound residues of mature shoots were also solubilized by acidic hydrolysis. Exhaustive extraction with 6 M HCl (95 °C, approx. 6 h) released a significant portion of the bound residues. The released compounds were assigned to the known metabolites NC 8493 and NC 9607. Based on this result, it can be concluded that at least a part of the bound residues can be assigned to known metabolites, which are not accessible by the conventional solvent extraction.

#### Characterization of polar compounds/fractions after acidic hydrolysis with 6 M HCl

##### Shoots

The shoot sample taken from the 1x experiment at maturity was used as an example to characterize/identify the compounds forming the polar fractions present in the conventional solvent extracts. Subsamples of the extracts were treated with 6 M HCl (95 °C, 6 h) prior to analysis by TLC and HPLC. Hydrolysis yielded components identified as NC 9607 and NC 8493, with compound NC 9607 being the predominant proportion. As a consequence, the main metabolite in shoots must be a conjugate of metabolite NC 20645, which is the ring-opened form of the lactone NC 9607. Under the acidic conditions of the hydrolysis, the conjugate is cleaved and quantitatively transformed to the lactone by an intra-molecular condensation reaction (ring closure to form NC 9607).

Exhaustive extraction (6 M HCl, 95 °C, 6 h) of the bound residue remaining after conventional solvent extraction released further radioactivity. Analysis of the acidic extract by TLC and HPLC revealed additional amounts of metabolites NC 9607 and NC 8493.

As the TRR of the 1x rate root sample accounted for only 0.010 mg/kg, of which 0.008 mg/kg was present in the washed tissue, the nature of the residues of ethofumesate in sugar beet roots was not further investigated by acidic hydrolysis.

The distribution of the radioactivity in the shoot sample taken at maturity (1x experiment) is summarized in Table 7.2.1-39. Characterization of the polar fraction after acidic hydrolysis and exhaustive extraction of the fibre bound residues are included in the table. These results of the HPLC analysis are shown which are also representative for results measured by TLC. For a better comparability all results given in this chapter were normalized to 100% recovery.

**Table 7.2.1-39 TRR values and distribution of parent compound and metabolites in shoots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 1x rate) after acidic hydrolysis – maturity (HPLC analysis)**

	<b>1x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>0.748</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>1.9</b>	<b>0.014</b>
Ethofumesate	1.5	0.011
Remainder (unassigned radioactivity)	0.4	0.003
<i>Conventional extraction</i>		
<b>ACN extract (subjected to hydrolysis)</b>	<b>51.5</b>	<b>0.385</b>
Water soluble phase	3.0	0.023
Unknown	-	-
NC 8493	1.2	0.009



	<b>1x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>0.748</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
NC 9607	1.5	0.011
Remainder (unassigned radioactivity)	0.3	0.002
ACN soluble phase	47.5	0.355
Unknown	-	-
NC 8493	4.3	0.032
NC 9607	39.3	0.294
Remainder (unassigned radioactivity)	3.7	0.028
Precipitate	1.1	0.008
<b>ACN/H<sub>2</sub>O extract</b>	<b>29.7</b>	<b>0.222</b>
Water soluble	3.2	0.024
Unknown	-	-
NC 8493	0.3	0.002
NC 9607	2.4	0.018
Remainder (unassigned radioactivity)	0.5	0.004
ACN soluble	24.8	0.186
Unknown	-	-
NC 8493	13.8	0.103
NC 9607	10.2	0.076
Remainder (unassigned radioactivity)	0.8	0.006
Precipitate	1.7	0.013
<b>H<sub>2</sub>O extract</b>	<b>6.3</b>	<b>0.047</b>
Water soluble	3.0	0.022
Unknown	0.1	0.001
NC8493	0.8	0.006
NC9607	1.9	0.014
Remainder (unassigned radioactivity)	0.2	0.001
ACN soluble	3.1	0.023
Unknown	-	-
NC 8493	1.8	0.013
NC 9607	1.0	0.008
Remainder (unassigned radioactivity)	0.3	0.002
Precipitate	0.3	0.002
<b>Solids 1</b>	<b>10.6</b>	<b>0.079</b>
<i>Exhaustive extraction (6 M HCl)</i>		
<b>Acidic extract</b>	<b>4.0</b>	<b>0.030</b>
Unknown	0.3	0.002
NC 8493	2.1	0.016
NC 9607	1.5	0.011
Remainder (unassigned radioactivity)	0.2	0.001
<b>Solids 2</b>	<b>6.5</b>	<b>0.049</b>
<b>Total identified</b>	<b>83.7</b>	<b>0.626</b>
<b>Total characterised</b>	<b>9.9</b>	<b>0.074</b>
<b>Solids 2</b>	<b>6.5</b>	<b>0.049</b>
<b>Accountability</b>	<b>100.0</b>	<b>0.748</b>

The results given in this table are summarised from Table 1, 4, 5, 7, 10 of the original study report (KCA 6.2.1/06). Recoveries were normalised to 100%. unknown = unresolved from solvent front (eluting in dead volume)

**Table 7.2.1-40 TRR values and distribution of parent compound and metabolites in shoots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 5x rate) after acidic hydrolysis – maturity (HPLC analysis)**

	<b>5x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>18.8</b>	<b>1.238</b>
Ethofumesate	18.8	1.238

	5x, maturity	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<i>Conventional extraction</i>		
<b>ACN extract (subjected to hydrolysis)</b>	<b>47.0</b>	<b>3.105</b>
NC 8493	5.6	0.366
NC 9607	37.7	2.489
Remainder	3.8	0.248
<b>ACN/H<sub>2</sub>O extract</b>	<b>25.4</b>	<b>1.679</b>
NC 8493	4.9	0.326
NC 9607	18.8	1.242
Remainder	1.7	0.112
<b>H<sub>2</sub>O extract</b>	<b>4.0</b>	<b>0.264</b>
NC8493	1.3	0.088
NC9607	2.1	0.135
Remainder	0.6	0.041
<b>Solids 1</b>	<b>4.8</b>	<b>0.314</b>
<i>Exhaustive extraction (6 M HCl)</i>		
<b>Acid extract</b>	<b>2.4</b>	<b>0.16</b>
NC8493	1.3	0.087
NC9607	0.9	0.062
Remainder	0.2	0.013
<b>Solids 2</b>	<b>2.3</b>	<b>0.153</b>
<b>Total identified</b>	<b>91.4</b>	<b>6.033</b>
<b>Total characterised</b>	<b>6.3</b>	<b>0.414</b>
<b>Total analyzed</b>	<b>97.7</b>	<b>6.447</b>
<b>Solids 2</b>	<b>2.3</b>	<b>0.153</b>
<b>Accountability</b>	<b>100.0</b>	<b>6.600</b>

The results given in this table are summarised from Table 1, 4, 5, 7, 10 of the original study report (KCA 6.2.1/06). Recoveries were normalised to 100%.

**Table 7.2.1-41 TRR values and distribution of parent compound and metabolites in shoots following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 5x rate) after acidic hydrolysis – maturity (TLC analysis)**

	5x, maturity	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
<b>Surface wash</b>	<b>18.8</b>	<b>1.238</b>
Origin (polar fraction)	0.4	0.025
Ethofumesate	18.3	1.211
Remainder	<0.1	0.002
<i>Conventional extraction</i>		
<b>ACN extract</b>	<b>47.0</b>	<b>3.105</b>
Origin (polar fraction)	7.4	0.487
NC 8493	4.0	0.261
NC 9607	35.1	2.319
Remainder	0.5	0.034
<b>ACN/H<sub>2</sub>O extract</b>	<b>25.4</b>	<b>1.679</b>
Origin (polar fraction)	2.7	0.178
NC 9607	21.5	1.419
Remainder	1.2	0.082
<b>H<sub>2</sub>O extract</b>	<b>4.0</b>	<b>0.264</b>
Origin (polar fraction)	0.9	0.059
NC 9607	2.3	0.149
Remainder	0.9	0.056
<b>Solids 1</b>	<b>4.8</b>	<b>0.314</b>
<i>Exhaustive extraction (6 M HCl)</i>		
<b>Acidic extract</b>	<b>1.1</b>	<b>0.072</b>
Origin (polar fraction)	0.3	0.020
NC 8493	1.0	0.067
NC 9607	0.0	0.002

	<b>5x, maturity</b>	
<b>TRR [mg/kg] =</b>	<b>6.600</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Remainder	2.3	0.153
<b>Solids 2</b>	<b>2.4</b>	<b>0.161</b>
<b>Total identified</b>	<b>82.5</b>	<b>5.446</b>
<b>Total characterised</b>	<b>15.1</b>	<b>0.999</b>
<b>Total analyzed</b>	<b>97.7</b>	<b>6.447</b>
<b>Solids 2</b>	<b>2.3</b>	<b>0.153</b>
<b>Accountability</b>	<b>100.0</b>	<b>6.600</b>

The results given in this table are summarised from Table 1, 4, 5, 7, 8 of the original study report (KCA 6.2.1 /06). Recoveries were normalised to 100%.

**Table 7.2.1-42 Examination of the nature of the radioactivity in metabolite fractions from mature shoots (treatment 5x rate) expressed as % total activity recovered (HPLC analysis)**

Sampling event	<b>Shoots</b>			
	<b>5x, maturity</b>			
	<b>before hydrolysis*</b>		<b>after hydrolysis*</b>	
<b>TRR</b>	<b>%</b>	<b>mg/kg</b>	<b>%</b>	<b>mg/kg</b>
	100.0	6.600	100.0	6.600
<i>Ethofumesate (parent compound)</i>	22.1	1.455	18.8	1.238
NC 8493	0.9	0.062	0.9	0.062
NC 8493 conjugated	-	-	10.9	0.718
NC 8493 fibre bound	1.3	0.087	1.3	0.087
NC 9607	-	-	-	-
NC 9607 fibre bound#	0.9	0.062	0.9	0.062
NC 20645	-	-	-	-
NC 20645 conjugated	-	-	58.6	3.867
<b>Total identified</b>	<b>25.2</b>	<b>1.666</b>	<b>91.4</b>	<b>6.033</b>
Unknown 1 (eluting in dead volume)	69.1	4.561	-	-
Unknown 2 (discrete polar peak)	2.6	0.174	-	-
Remainder before hydrolysis	0.5	0.033	-	-
Remainder (acidic treatment)	-	-	6.1	0.401
Remainder (exhaustive extraction)	0.2	0.013	0.2	0.013
<b>Total characterized</b>	<b>72.4</b>	<b>4.781</b>	<b>6.3</b>	<b>0.414</b>
Solids (bound fibre residue)	2.3	0.153	2.3	0.153

\* before/after hydrolysis of polar fraction present in the acetonitrile and acetonitrile/water extract (= fraction containing the conjugates)

# identified after exhaustive extraction, precursor could be NC9607, NC20645 or a conjugate of NC20645

#### IV. Conclusions

Ethofumesate is metabolised in sugar beet to NC 8493, NC 9607 and NC 20645. NC 8493 and NC 20645 form conjugates which can be hydrolysed with 6 M hydrochloric acid (6 h at 95 °C).

The conjugate of metabolite NC 20645 was the major compound found in sugar beet leaves at later sampling times.

Under acidic conditions NC 20645 rearranges to NC 9607.

Due to the low residue level in roots no characterization of the polar fraction was conducted, but it is assumed that the same conjugates represent the polar fraction as found in the shoots. This was confirmed in another sugar beet study (cf KCA 6.5.1/ 05), where approx. 36% of the TRR in mature roots was represented by the conjugate of NC 20645 and approx. 5% by the conjugate of NC 8493.

Residues in roots are significant lower compared to residues in shoots (leaves) with residues accounting for less than 0.01 mg/kg in mature roots, which were cleaned from adhering soil.

Based on the metabolites identified in shoots and roots, the following metabolic routes were deduced:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can either undergo conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to the acid NC 20645 which can also undergo conjugation to give polar metabolites.

On the basis of the results of this study it is concluded that the metabolism of [ $^{14}\text{C}$ ]-ethofumesate in sugar beet is well understood and a metabolic pathway can be deduced.

### B.7.2.1.1.3. Sugar beet metabolism 3

#### New data for AIR:

This study has not been evaluated before for EU approval.

Report:	KCA 6.2/01, Hennecke, D. (2003)
Title:	METABOLISM OF ETHOFUMESATE IN SUGAR BEETS
Document No:	GAB-002/7-08
Guidelines:	7028/VI/95 rev. 3
GLP:	Yes

### I. Summary

The main objective of the present study was to investigate the metabolism and the behaviour of ethofumesate after application of  $^{14}\text{C}$ -labelled test item on sugar beet grown in an outdoor area under field conditions.

At the 4-6 leave stage, the herbicide was applied as a suspension concentrate at a rate corresponding to 1500 g ai/ha. Sampling was performed 1, 10, 50, 90 and at maturity at 137 days after application

**Table 7.2.1-43 Application and sampling parameters**

Matrix	Timing and Application	Sampling event (days after application)
Shoot (leaves)	4-6 leaf growth stage 1 x 1.50 kg a.s./ha	1
		10
		50
		90
Root (body)		137 (maturity)

The samples were analysed for total radioactivity, distribution of radioactivity and identity of the recovered radiolabelled compounds.

At harvest the total radioactivity was 0.3 kBq/g in sugar beet leaves and 0.17 kBq/g fw in sugar beet roots corresponding to a reduction of radioactivity of 99.8 % (leaves) and 99.6% (roots), respectively.

At harvest, ethofumesate and its free metabolites were below the limit of detection in both sugar beet leaves and roots (beets). In sugar beet leaves, the major amount of radioactivity was still assigned to sugar conjugated NC20645 (0.13 mg Ethofumesate equivalents/kg fresh weight). In sugar beet roots (beets) only trace amounts of not identifiable mainly fibre bound residues were found. Converted into ethofumesate equivalents a concentration of 0.13 µg g/fresh weight was detected at harvest.

The following metabolic rout was observed.

- Cleavage of the ethoxy side chain of the parent compound, with hydroxylation at the 2 position, to give NC 8493.
- Oxidation of NC 8493 to the lactone NC 9607.
- The lactone ring of NC 9607 opens to form the acid NC 20645 which can also undergo conjugation to give polar metabolites.

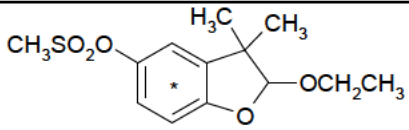
On the basis of the metabolites identified, a metabolic pathway of [ $^{14}\text{C}$ ]-ethofumesate in sugar beets can be proposed.

## II. Materials and Methods

### A. Materials

#### 1. Test Material

The test was performed using a mixture of ethofumesate and  $^{14}\text{C}$ -ethofumesate.

Chemical structure	 <p style="text-align: right;">*position of the radiolabel</p>
Radiolabelled test material	[ $^{14}\text{C}$ ]-ethofumesate (+)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulphonate
Specific radioactivity	2.44 GBq/mmol = 66 mCi/mmol 288.4 g/mol (at this specific activity)
Radiochemical purity	≥ 98.6% (TLC)

#### 2. Soil

The study was performed in the lysimeter outdoor area of the “Fraunhofer Institute for Molecular Biotechnology and Applied Ecology” (Schmallenberg, Germany) in order to enable the application of the test substance at conditions as close as possible to normal agricultural practice. The lysimeter and its surrounding covers an area of approximately 2.5m x 2.0m. The surface of the plot area is approximately 1m<sup>2</sup> (exact value: 1.080 m<sup>2</sup>).

The Lysimeter soil monolith was sampled at Borstel, Neustadt am Rübenberge, Germany, in July 1996. For cultivation of the sugar beets, the first 30 cm of the top layer of the soil monolith were removed and the lysimeter was filled with approx. 300 kg of a Lufa 2.2 standard soil approximately 7 weeks before application of the sugar beet.

**Table 7.2.1-44 Physico-chemical parameters of the Lufa 2.2 soil**

Charge No. Sp 2214302	
Parameter	Value
pH (determined in 0.01 M Ca Cl <sub>2</sub> )	5.6
% organic carbon	2.3
Cation exchange capacity (in mval/100 g DM)	11
Maximum water holding capacity (in g/kg DM)	500
Particle size distribution	
Clay (< 0.002 mm) (%)**	8.1
Silt (0.002-0.063 mm) (%)**	18.3
Total sand (0.063-2 mm) (%)**	73.8

#### 3. Plant

For the study the rizomania resistant sugar beet “Macarena” was used.

### B. Study Design

#### Experimental conditions

The seeds were sown into the lysimeter on 25 April 2002.

Distance between seeds: 10 cm, distance between rows: 10 cm, depth: 5cm

After the first two samplings the plot area was thinned to approximately one plant on each 20 cm.

The application on the lysimeter was performed on 6 June 2002 post emergence on sugar beet (4-6 leaves growths stage) (BBCH 14-16) based on the agricultural practice. The application was performed under a plastic tent in order to prevent contamination of the surrounding area.  $^{14}\text{C}$ -Ethofumesate diluted with unlabelled ethofumesate at a known ratio and suspended in REF JC089 blank formulation was applied at a nominal rate of 1500 g ai/ha.

In the application suspension a radioactivity of 220.66 MBq corresponds to 182.25 mg ethofumesate. Based on these data, the conversion factor to convert radioactivity into mg ethofumesate was 1.211 MBq/mg for the application suspension. The total applied radioactivity was 214.2 MBq. By the means of this value, the total amount (expressed as mg) of ethofumesate applied on the lysimeter (1.080 m<sup>2</sup>) can be calculated by  $214.2/1.211 = 176.8$  mg.

During the first days after application, the lysimeter was covered occasionally by a plastic roof without direct contact to the treated crops, to prevent as much as possible washing off of the applied test item from plant surfaces because unusually heavy rainfalls occurred during this period.

Climatic data, i.e., air and soil temperature, precipitation, additional irrigation and solar irradiation were recorded.

### Sampling

Sugar beets (leaves and roots) were sampled 1, 10, 50, 90, 137 days after application. After sampling the plants were cleaned from adhesive soil with water. After that the plants were separated into leaves and roots.

**Table 7.2.1-45 Sampling of plants and preparation of sample groups**

Samplin g date	Days after application	Number of plants	Fresh weigth of plants (g)	Leaves fresh weight (g)	Roots fresh weight (g)	Leaves/roots ratio
7 June 2002	1	26	84.2	78.1	6.1	12.8
16 June 2002	10	26	184.4	172.6	11.8	14.6
8 August 2002	50	6	925	774.8	150.2	5.2
4 September 2002	90	3	1370	920	450	2.0
21 October 2002 (harvest)	137	10	4990	2381	2609	0.9

The plant organs were washed with methanol in order to wash off remaining ethofumesate from the plant surface. The washing solution was analysed by LSC and by radio-HPLC and, if required by TLC and LC-MS/MS. Radioactivity rinsed from the roots surface is assumed to be due to adherent soil. On the basis of the results of the previous samplings, it was assumed that there were no significant amounts of radioactivity left on the plants surface at the 90 day and the 137 day samplings, so that rinsing of the surfaces was no longer performed.

### C. Analytical Procedures

#### Determination of total residues

After separation of the plants into leaves and roots the plants were cut into pieces, which were mixed and divided in several subsamples. These subsamples were introduced into a solvent mixture of chloroform/methanol/water (1:2:0.8 (v/v/v)). It was assumed, that the water content of the plant material was generally 90% of the fresh weight, so that the volume of water added to the mixture was reduced according to the fresh weigth of the respective sample.

The extraction mixture was stored in a freezer for at least 24 hours to unlock cell membranes. Then the mixture was shaken for 10 min and the solvent was decanted after centrifugation. Fresh solvent was added to the residue and the extraction was repeated twice. In a fourth step solvent was added and the mixture was homogenised by an Ultraturrax-dispenser. The extracts were filtered. The extracts and the residual material were stored deep frozen at -20°C until further analysis.

Clean-up of the extracts was carried out by separation into an organic and an aqueous fraction adding chloroform and water to the extracts. Following this procedure two phases were formed, which were separated by means of a separation funnel. Generally after all clean up steps aliquots of the extracts were measured by LSC in order to detect if there were some losses of radioactivity due to the sample preparation steps.

The organic leaf extracts were strongly coloured by the chlorophyll. In order to the green colour the extracts were evaporated up to 1 mL. The samples were then evaporated to dryness and re-dissolved in dichloromethane/cyclohexane (1:1, v:v) and treated with gel permeation chromatography (GPC). After GPC the samples were evaporated to dryness and redissolved in water/acetonitrile 3:7 (v/v) before analysis. Organic extracts of roots were treated by GPC in the same way as described above.

Aliquots of the aqueous extract were evaporated in order to remove the residuary methanol. This extract was analysed without further clean up.

To a second aliquote of the aqueous extract aqueous NaOH-solution was added. The solution was shaken thrice with n-hexane. After separation of the aqueous phase and the n-hexane phase, the n-hexane phases were combined, evaporated and the residues were redissolved with HPLC mobile phase solvent and analysed. By this procedure only the free ethofumesate was removed while the conjugated metabolites remained in the aqueous phase.

#### **Identification of conjugated metabolites**

After removal of the free ethofumesate, the alkaline aqueous phase was acidified and put into an oven at 100°C for 16 hours. By means of this acidic hydrolysis step, the sugar conjugated residues of ethofumesate and its metabolites were released. After cooling down, the reaction mixture was extracted thrice by liquid/liquid extraction with chloroform. Then hydrolysis of the aqueous phase and the chloroform extraction step were repeated once. The combined chloroformic extracts were measured by LSC, evaporated to dryness, redissolved with HPLC-mobile phase solvent, and analysed.

The chemical pulping method had a considerable disadvantage: In the acidic media NC 20645 will be transformed partially into NC 9607 and NC 8493. Therefore no direct quantitation of sugar conjugated metabolites could be performed because the analysed distribution of metabolites did not reflect the distribution of the released metabolites. Enzymatic cleavage of the sugar conjugated residues was not applicable because of the naturally higher sugar excess of the aqueous extract fraction. The only way to identify the bound residues was an analysis by means of the MS/MS detector without prior cleavage. The following conjugates were conceivable:

1. Glucose conjugated NC 8493, Molecular mass: 420.3 g/mol
2. Glucose conjugated NC 20645, Molecular mass: 436.3 g/mol

#### **Quantification**

The samples were analysed for total radioactivity, distribution of radioactivity and identity of the recovered radiolabelled compounds.

The analysis of Ethofumesate and potential metabolites was carried out by LSC, by reversed-phase-radio-HPLC, by radio TLC and by LC-MS/MS using the reference substances NC 9607, NC 8493 and NC 20645.

#### Mass Balance

For each sampling a mass balance was performed by adding the radioactivity detected in all analytical steps. Then this sum was compared with the radioactivity detected at the individual steps considering the results of radio-HPLC, TLC and LC-MS/MS. The detected radioactivity was expressed as % of the totally recovered radioactivity (TRR). In addition the results of the several samplings were compared among each other. The data were not corrected for the percentage of recovery, which had been achieved for the test item as a result of the validation experiments.

#### Conversion of Bq into µg for ethofumesate and metabolites

The specific radioactivity of the applied ethofumesate was calculated to be 1,2 MBq per mg ethofumesate or 1211 Bq per µg ethofumesate, respectively. Based on this information and taking into account the molecular masses of the detected metabolites, for each metabolite a corresponding conversion factor could be calculated.

**Table 7.2.1-46 Calculation of conversion factors for the detected metabolites**

Substance	Molecular mass [g]	Mass ratio	Conversion factor [Bq/µg]
Ethofumesate	286.3	1	1211
NC 8493	258.3	0.902	1342
NC 9607	256.3	0.895	1352
NC 20645	274.3	0.958	1264

### III. Results and Discussion

#### Plants dry weight

In order to determine the dry weight of the plants after dividing them into leaves and roots, two subsamples were treated in a drying oven at 90°C until a constant weight. With increasing plant age the water content decreased slightly.

**Table 7.2.1-47 Dry weight of plant organs (in % of fresh weight)**

Sampling	leaves (%)	roots (%)
1 day	6,5	16,7
10 days	7,1	11,7
50 days	8,4	14,3
90 days	10,1	19,1
137 days (harvest)	12,4	23

Loss of radioactivity due to drying was calculated by subtraction of the total radioactivity detected by LSC after combustion from the totally recovered radioactivity (TRR). Unexpectedly strong fluctuations related to the total recovered radioactivity were observed, which could not be explained. Since no further analysis of the dried plants has been carried out, these findings did not influence the overall results of the study.

#### Total recovered radioactivity

**Table 7.2.1-48 Recovery of radioactivity after determination of dry weight**

Sampling	leaves		roots		leaves	roots
	[mg/kg] dried	[mg/kg] TRR <sup>1</sup>	[mg/kg] dried	[mg/kg] TRR <sup>1</sup>	%TRR	%TRR
1 day	939,83	2.416,11	70,76	198,37	38,90	35,70
10 days	457,94	627,01	37,07	56,11	73,00	66,10
50 days	28,27	22,13	2,59	1,49	127,80	174,60 <sup>2</sup>
90 days	3,11	3,91	0,56	1,15	79,60	48,70
137 days (harvest)	1,54	1,97	0,37	0,59	77,80	61,70



1 mean values of parallel subsamples

2 measuring error assumed

The total radioactivity detected in the plant specimens decreased strongly from 0 days after application to 137 days after application. One day after application, the total amount of radioactivity was 190 kBq/g fw (fresh weight) in sugar beet leaves and 39 kBq/g fw in sugar beet roots, respectively. At harvest, 137 days after application, the total radioactivity decreased to 0.3 kBq/g for sugar beet leaves and to 0.17 kBq/g fw for sugar beet roots corresponding to a reduction of radioactivity of 99.8 % (leaves) and 99.6% (roots), respectively.

#### Distribution of radioactivity (mass balances)

The distribution of radioactivity detected in sugar beet was determined in the rinsing solution (of leaves and roots), in the plant extracts and the non-extractable radioactivity (NER) after combustion of the extraction residues.

Due to the natural growth of sugar beet plants, the weight of the sampled roots was very low up to day 50. In addition only minor amounts of the test item were assumed to be in the roots at the first sampling dates simply because of the application technique (spraying on the surface of the leaves). Therefore all roots from the respective sampling date were pooled. By this procedure a well detectable signal was achieved which enabled further analysis.

In all other cases quadruplicate analysis of the samples was performed. The data showed partially large differences in terms of total detected radioactivity (up to a factor of 6 for the 50 d sampling of leaves). This was explained by the inhomogeneous distribution of the radioactivity onto the leaves. The leaves, which were already present at application, received more radioactivity than those leaves, which were grown later. At the 50 d sample the amount of leaves, which were already present at application was only small relative to the other leaves and inhomogeneity of the sample subdivision became much more important compared with the 1d and 10d sampling. But the distribution of radioactivity among the parallel samples related to the corresponding total radioactivity (%TRR) was in good accordance. The results are expressed in mg/kg ethofumesate equivalents. In the original study report the results are presented in Becquerel/g. As such study results should be expressed in mg/kg in this report the analytical results were re-calculated as follows:

$$1 \text{ Becquerel/g} = 8.25763831544178 \cdot 10^{-7} \text{ mg ethofumesate/g}$$

or

$$1 \text{ Becquerel/kg} = 8.25763831544178 \cdot 10^{-4} \text{ mg ethofumesate/kg}$$

**Table 7.2.1-49 Distribution of radioactivity in the samples**

Sample identification	Sample weight		Methanol rinsing		Extraction (Bligh-Dyer)		NER		Total activity	
	fresh (g)	dry (g)	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR
Leaves 1 d	11,30	0,74	1158,09	66,4	577,19	33,1	8,80	0,5	1744,08	100,0
	12,70	0,83	1133,67	55,2	904,78	44,1	15,18	0,7	2053,64	100,0
	12,20	0,79	1318,60	65,7	677,35	33,8	10,47	0,5	2006,43	100,0
	12,20	0,79	1133,98	62,6	668,64	36,9	9,82	0,5	1812,45	100,0
Roots (pooled) 1 d	3,70	0,61	100,69	83,5	17,61	14,6	2,32	1,9	120,63	100,0
Leaves 10d	24,10	1,70	17,08	1,3	1254,36	95,2	46,18	3,5	1317,61	100,0
	20,90	1,47	17,48	1,6	1009,04	95,1	34,00	3,2	1060,52	100,0
	16,00	1,13	10,79	2,9	355,81	94,6	9,40	2,5	376,01	100,0
	22,90	1,61	5,44	0,6	915,99	96,3	29,61	3,1	951,05	100,0

Sample identification	Sample weight		Methanol rinsing		Extraction (Bligh-Dyer)		NER		Total activity	
	fresh (g)	dry (g)	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR	(mg a.s. equiv./kg)	% TRR
Roots (pooled) 10 d	5,90	0,68	7,16	18,7	22,92	59,8	8,26	21,6	38,34	100,0
Leaves 50 d	160,40	13,43	0,00		64,15	85,4	10,97	14,6	75,12	100,0
	138,10	11,56	0,00		226,78	89,1	27,71	10,9	254,49	100,0
	160,20	13,41	2,94	0,6	404,13	82,0	85,50	17,4	492,58	100,0
	120,50	10,08	0,00		218,34	87,2	32,12	12,8	250,45	100,0
Roots (pooled) 50 d	94,40	13,50	4,73	10,4	23,25	51,1	17,52	38,5	45,50	100,0
Leaves 90 d	86,60	8,75	0,00		14,03	69,9	6,03	30,1	20,05	100,0
	88,50	8,94	0,00		27,53	76,5	8,45	23,5	35,99	100,0
	89,00	8,99	0,00		19,30	80,0	4,81	20,0	24,11	100,0
	89,20	9,01	0,00		52,39	88,5	6,80	11,5	59,19	100,0
Roots 90 d	55,00	10,51	0,00		5,64	45,0	6,89	55,0	12,53	100,0
	55,60	10,63	0,00		4,68	39,4	7,19	60,6	11,87	100,0
	55,30	10,55	0,00		6,51	45,7	7,74	54,3	14,25	100,0
	55,70	10,63	0,00		4,18	42,1	5,75	57,9	9,93	100,0
Leaves (harvest) 137 d	80,40	9,97	0,00		12,01	71,8	4,30	28,2	16,30	100,0
	82,10	10,18	0,00		8,13	64,6	4,46	35,4	12,59	100,0
	82,20	10,19	0,00		21,09	73,9	7,45	26,1	28,54	100,0
	89,20	11,06	0,00		17,32	72,5	6,56	27,5	23,88	100,0
Roots (harvest) 137 d	80,50	18,52	0,00		4,87	40,1	7,26	59,9	12,13	100,0
	80,00	18,40	0,00		3,86	39,4	5,93	60,6	9,79	100,0
	80,00	18,40	0,00		4,36	40,5	6,40	59,5	10,75	100,0
	80,20	18,45	0,00		4,98	45,1	6,06	54,9	11,05	100,0

The following table shows details from the distribution of radioactivity measured in the Bligh-Dyer extracts.

**Table 7.2.1-50 Distribution of extractable radioactivity in the samples**

Sample identification	Extraction (Bligh-Dyer)		Aqueous phase		Organic phase	
	(mg a.s. equiv./kg)	%TRR	(mg a.s. equiv./kg)	%TRR	(mg a.s. equiv./kg)	%TRR
Leaves 1d	577,19	33,1	248,76	14,3	356,37	20,4
	904,78	44,1	372,78	18,2	557,68	27,2
	677,35	33,8	326,37	16,3	384,51	19,2
	668,64	36,9	296,03	16,3	398,94	22,0
Roots (pooled) 1d	17,61	14,6	4,17	3,5	0,23	0,2
Leaves 10d	1254,36	95,2	1319,44	100,1	18,77	1,4
	1009,04	95,1	1006,12	94,9	16,31	1,5
	355,81	94,6	369,02	98,1	6,40	1,7
	915,99	96,3	969,79	102,0	11,09	1,2
Roots (pooled) 10d	22,92	59,8	17,80	46,4	2,98	7,8
Leaves 50d	64,15	85,4	64,45	85,8	2,54	3,4
	226,78	89,1	239,87	94,3	4,38	1,7
	404,13	82,0	381,16	77,4	27,78	5,6
	218,34	87,2	228,92	91,4	4,04	1,6
Roots (pooled) 50d	23,25	51,1	20,38	44,8	2,18	4,8
Leaves 90d	14,03	69,9	11,83	59,0	1,35	6,7
	27,53	76,5	25,03	69,6	2,06	5,7
	19,30	80,0	17,57	72,9	1,39	5,8
	52,39	88,5	49,76	84,1	1,52	2,6
Roots 90d	5,64	45,0	5,29	42,3	0,25	2,0
	4,68	39,4	5,11	43,0	0,26	2,2
	6,51	45,7	6,17	43,3	0,26	1,8
	4,18	42,1	4,09	41,2	0,18	1,8
Leaves (harvest) 137 d	12,01	71,8	10,13	60,6	0,96	5,7
	8,13	64,6	7,20	57,2	0,49	3,9
	21,09	73,9	17,25	60,4	1,19	4,2
	17,32	72,5	14,72	61,6	1,40	5,9
Roots (harvest) 137 d	4,87	40,1	4,25	35,0	0,53	4,4
	3,86	39,4	3,81	38,9	0,18	1,8

Sample identification	Extraction (Bligh-Dyer)		Aqueous phase		Organic phase	
	(mg a.s. equiv./kg)	%TRR	(mg a.s. equiv./kg)	%TRR	(mg a.s. equiv./kg)	%TRR
	4,36	40,5	3,88	36,1	0,22	2,1
	4,98	45,1	4,57	41,4	0,23	2,0

Generally it was observed that extractable radioactivity from the roots related to total recovered radioactivity in the roots (%TRR) was lower than extractable radioactivity (%TRR) from the leaves.

### Identification of detected radioactivity

#### Methanol rinsing solution

The TRR in the methanol rinsing solution was measured by radio-HPLC. There was a fraction at the 10 d and at the 50 d samples that could not be identified. Therefore these samples were measured by LC-MS/MS.

**Table 7.2.1-51 Identification of washable radioactivity on the leaves/roots surfaces**

Sample identification	Methanol rinsing <sup>1</sup>		Ethofumesate (%) <sup>2</sup>	NC 9607 (%) <sup>2</sup>	NC 8493 (%) <sup>2</sup>	NC 20645 (%) <sup>2</sup>	Not identified (%) <sup>2</sup>
	(mg a.s. equiv./kg)	%TRR					
Leaves 1 d	1158,09	66,4	100,0	--	--	--	--
	1133,67	55,2	100,0	--	--	--	--
	1318,60	65,7	100,0	--	--	--	--
	1133,98	62,6	100,0	--	--	--	--
Roots (pooled) 1 d	100,69	83,5	100,0	--	--	--	--
Leaves 10 d	17,08	1,3	79,8	--	--	7,0	13,2
	17,48	1,6	62,9	--	--	7,9	29,2
	10,79	2,9	91,7	--	--	3,9	4,4
	5,44	0,6	83,3	--	--	9,9	6,8
Roots (pooled) 10 d	7,16	18,7	65,7	--	--	21,0	13,3
Leaves (sample 3) 50 d	2,94	0,6	34,8	--	--	5,4	59,8
Roots (pooled) 50 d	4,73	10,4	31,2	4,3	6,1	24,4	34,1

<sup>1</sup> total activity in rinsing solution

<sup>2</sup> related to total radioactivity in the methanol rinsing

The total radioactivity of the surface wash of leaves and roots one day after application was due to unchanged ethofumesate (55% and 66% of the TRR of the leaves and 84% of the TRR of the roots).

10 days after application, the situation had significantly changed. The TRR rinsed of the leaves decreased to about 1% to 3% TRR. About 70% to 92% of this radioactivity could be identified as unchanged ethofumesate. Up to 10% could be identified by LC-MS/MS as NC 20645 and 5% to 30% are unknown.

The TRR rinsed of the roots decreased to 19% TRR. The roots rinsing solution contained 21% of NC 20645.

After 50 days the non-identifiable amount of this radioactivity increased to about 34% (roots) and 60% (leaves). Furthermore trace amounts of NC 9607 and NC 8493 were detected in the roots rinsing solution.

#### Organic phase of Bligh-Dyer extraction

The TRR in the organic phase was measured by TLC. Where possible the samples were reanalysed by means of LC-MS/MS in order to ensure the identification obtained by TLC. However, for the 137 day sample no valid chromatogram could be determined at all. In this case the whole detected radioactivity in this fraction was categorized as “not identified”.

**Table 7.2.1-52 Identification of the radioactivity detected in the organic fraction**

Sample identification	Organic phase <sup>1</sup>		Ethofumesate (%) <sup>2</sup>	NC 9607 (%) <sup>2</sup>	NC 8493 (%) <sup>2</sup>	NC 20645 (%) <sup>2</sup>	not identified (%) <sup>2</sup>
	(mg a.s. equiv./kg)	%TRR					
Leaves 1d	356,37	20,4	94,60	--	2,80	2,60	--
	557,68	27,2	94,60	--	3,00	2,40	--
	384,51	19,2	93,80	1,50	2,40		2,30
	398,94	22,0	93,20	2,00	2,30	2,50	
Roots (pooled) 1d	0,23	0,2	95,10		0,80	0,30	3,60
Leaves 10d <sup>3</sup>	18,77	1,4	44,30	4,00	4,40	34,10	13,30
	16,31	1,5	39,50	2,70	3,40	39,90	14,60
	6,40	1,7	42,50	2,10	4,80	35,60	15,00
	11,09	1,2	26,80	5,20	5,20	16,00	46,90
Roots (pooled) 10d	2,98	7,8	83,20	4,90	5,00	1,40	--
Leaves (pooled) 50d	38,74	12,3	40,60	10,70	4,30	35,20	9,20
Roots (pooled) 50d	2,18	4,8	98,50	--	--	--	--
Leaves (pooled) 90d	6,32	20,8	31,00	15,30	9,70	10,50	33,50
Roots (pooled) 90d	0,95	7,8	32,30	9,00	10,40	7,10	32,90
Leaves (pooled) 137 d	4,04	19,7	--	--	--	--	100 <sup>4</sup>
Roots (pooled) 137 d	1,15	10,3	--	--	--	--	100 <sup>4</sup>

<sup>1</sup> total activity in organic phase

<sup>2</sup> % related to total radioactivity in column 2

<sup>3</sup> analytical mistake assumed for sample No. 4

<sup>4</sup> no valid chromatogram obtainable

One day after application unchanged ethofumesate was found as expected. But then the amount of ethofumesate decreased strongly and as a main metabolite NC 20645 was identified in the leaves. At later sampling the amount of NC 20645 related to the total activity decreased again and the other known metabolites NC 9607 and NC 8493 as well as a non-identifiable fraction became more important.

#### Aqueous phase of Bligh-Dyer extraction

The TRR in the aqueous phase was measured by TLC. Since the known free metabolites were already detected in the organic phase, it was not expected to find more than trace amounts of these substances in the aqueous fraction.

**Table 7.2.1-53 Identification of the radioactivity detected in the aqueous fraction**

Sample identification	Aqueous phase <sup>1</sup>		Ethofumesate (%) <sup>2</sup>	NC 9607 (%) <sup>2</sup>	NC 8493 (%) <sup>2</sup>	NC 20645 (%) <sup>2</sup>	Not identified (%) <sup>2</sup>
	(mg a.s. equiv./kg)	%TRR					
Leaves 1d	248,76	14,3	3,50	--	--	6,80	89,70
	372,78	18,2	3,10	--	--	7,50	89,40
	326,37	16,3	3,00	--	--	7,50	89,50
	296,03	16,3	3,20	--	--	8,30	90,30
Roots (pooled) 1d	4,17	3,5	--	--	--	64,60	35,40
Leaves 10d <sup>3</sup>	1319,44	100,1	--	--	--	--	100,00
	1006,12	94,9	--	--	--	--	100,00
	369,02	98,1	--	--	--	--	100,00
	969,79	102,0	--	--	--	--	100,00
Roots (pooled) 10d	17,80	46,4	--	--	--	--	100,00
Leaves (pooled) 50d	914,40	87,2	--	--	--	--	100,00
Roots (pooled) 50d	20,38	44,8	--	--	--	--	100,00
Leaves (pooled) 90d	104,20	71,4	--	--	--	--	100,00
Roots (pooled) 90d	20,67	42,5	--	--	--	21,60	78,40
Leaves (pooled) 137 d	49,29	60,0	--	--	--	--	100,00
Roots (pooled) 137 d	16,51	37,9	--	--	--	--	100,00

1 total activity in aqueous fraction

2 % related to total radioactivity in column 2

### Identification and distribution of the recovered radioactivity

One day after application, the radioactivity, which was washable from the plant surface, amounted for leaves 60% and for roots 84%, respectively. This radioactivity corresponded to unchanged ethofumesate. In addition, considerable amounts of sugar conjugated NC 20645 as major metabolites were already found one day after treatment. Free (not sugar-conjugated) metabolites were only found in trace amounts. The main free metabolite was NC 20645, which amounted for approximately 2% of the initial ethofumesate concentration.

Ten days after application, the radioactivity, which was washable from the plant surface decreased to approximately 2% of total recovered radioactivity (TRR) whereas the amount of extractable radioactivity increased to more than 90% TRR. The maximum amounts of sugar conjugated and fibre bound residues in the leaves as well as in the roots were detected ten days after application, indicating that Ethofumesate was metabolised only after uptake. Ten days after sampling, approximately 92% of the total recovered radioactivity was identified as sugar conjugated NC 20645 in sugar beet leaves. In sugar beet roots, sugar conjugated NC20645 amounted to 26% of the total recovered radioactivity at this sampling date.

From ten days after application until harvest (137 days after treatment) a rapid reduction of all identified metabolites and unknown fractions was observed. The high amounts of sugar conjugated metabolites indicate that this was mainly due to natural elimination from the plant after prior metabolism. The effect of thinning of the applied radioactivity due to increasing plant size was assumed to be only a minor effect.

At harvest, Ethofumesate and its free metabolites were below the limit of detection in both sugar beet leaves and roots (beets). In sugar beet leaves, the major amount of radioactivity was still assigned to sugar conjugated NC20645 (0.13 mg a.s. equal./kg). In sugar beet roots only trace amounts of not identifiable mainly fibre bound residues were found.

The results are summarised in the table below.

**Table 7.2.1-54 Distribution of Ethofumesate and its metabolites in sugar beets (in µg/kg fresh weight)**

Sampling time after application	Commodity of sugar beet	Ethofumesate	NC 9607	NC 8493	NC 20645	NC 20645 glucose-conjugated	Unknown (extractable)	Fibre bound residues
1 d	Leaves	131.968	0.255	0.842	2.508	20.026	2.188	0.909
	Roots	31.112	< 0.001	0.027	0.721	0.085	0.318	0.638
10 d	Leaves	0.710	0.020	0.024	0.235	40.640	1.429	1.358
	Roots	1.223	0.022	0.023	0.253	1.577	1.561	1.406
50 d	Leaves	0.034	0.006	0.003	0.024	1.406	0.113	0.267
	Roots	0.038	0.002	0.003	0.012	0.167	0.042	0.186
90 d	Leaves	0.006	0.002	0.002	0.002	0.276	0.007	0.074
	Roots (beet)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.073	0.124
137 d	Leaves	< 0.001	< 0.001	< 0.001	< 0.001	0.138	0.004	0.069
	Roots (beet)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.051	0.080

## IV. Conclusions

Based on the new study, it can be proposed that conjugated (and non-conjugated) open-ring-2-keto-ethofumesate (NC 20645) should be included in the residue definition of Ethofumesate as it was a major metabolite in roots up to 50 days after application and in leaves up to harvest.

The proposed residue definition is: sum of Ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate.

Additional plant metabolism studies are not considered to be necessary to support the use in sugar and fodder beets.

All metabolites which have been identified in the plant metabolism studies (ethofumesate, 2-OH-ethofumesate (NC 8493), 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) were found in rats as well.

#### **B.7.2.1.1.4. Sugar beet metabolism 4 (supportive information)**

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

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Report:	KCA 6.2.1 /03;Lines, D. S.; Adcock, J. W.;1978;M-155241-01
Title:	THE METABOLISM OF ETHOFUMESATE BY SUGAR BEET UNDER GREENHOUSE CONDITIONS
Report No:	A82964
Document No:	M-155241-01-1
Guidelines:	Deviation not specified
GLP/GEP:	<u>no</u>

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### **I. Summary**

The metabolism of [ $^{14}\text{C}$ -methylsulfonyl]-ethofumesate in sugar beet was investigated following pre- and post-emergence treatment in the greenhouse. The pre-emergence application was conducted at the time of sowing; post-emergence application was done at the 4-leaf stage (BBCH 14) of the crop. The application rate was 2 kg a.s./ha in each trial.

Whole plants with roots were sampled 10, 20, 30, 40 and 50 days after the applications. Plants treated with ethofumesate post-emergent were washed with methanol before extraction, whereas plants treated pre-emergent were extracted without prior surface wash.

For extraction, the plants were chopped and homogenised in methanol, the fibre was filtered off and the process repeated. The radioactivity in the extracts was determined by LSC and in the non-extracted residues by combustion and LSC. The methanol extracts were reduced to dryness, made up in water and extracted twice with dichloromethane (DCM). The organic phase was separated and backwashed with water. The combined aqueous phases were subjected to acidic hydrolysis with 6 M HCl (the aqueous phase was diluted with approx. the same volume of conc. HCl). Hydrolysis was performed under reflux for 1 hour. The reaction mixture was extracted with ethyl acetate (EtoAc) and backwashed with water. The extracts before (DCM) and after the hydrolysis step (EtoAc and aqueous remainder) were concentrated and analysed by TLC (thin layer chromatography). TLE (thin layer electrophoresis) and HPLC (high performance liquid chromatography) were used to confirm the identity of some of the components. Clean-up and concentration steps with C18 columns were introduced where necessary.

Identification of the radiolabelled components was done in different chromatographic systems by comparison with unlabelled standards. The presence of methanesulphonic acid (MSA) was excluded in the extracts by TLC and TLE. The extraction and analysis processes were validated beforehand with a radiolabelled reference compound ( $^{14}\text{C}$ -MSA). Recoveries of MSA were above 97% and confirmed that the compound would be detected, if present.

The TRR values declined significantly with time, independent if ethofumesate was applied pre- or post-emergent. After 50 days, the total residues accounted for 1.4 mg/kg or 2.59 mg/kg, respectively.

In general, similar metabolic profiles were observed in both the pre- and post-emergent beet extracts and were in agreement with the findings of previous studies. Ethofumesate was extensively metabolised following uptake from the soil and absorption from leaf surfaces. The major metabolites were conjugated NC 20645 (0.05-12.33 mg/kg, corresp. to 60-80% of total recovered radioactivity) and conjugated NC 8493 (0.05-0.83 mg/a.s. equiv./kg, corresp. up to 5% of the total recovered radioactivity). Ethofumesate concentrations declined significantly with time, highest residues were detected in samples taken 10 days after application. Non-conjugated AE C 520645 (ring opened AE C 509607) was detected at minor extent. The exocons of the conjugates were identified after acidic hydrolysis with 6 M HCl. Under the acidic conditions, the exocon NC 20645 was immediately transformed to NC 9607 by an intramolecular condensation step. Exocon NC 8493 was stable under the acidic conditions.

Between 6-25% of the TRR could not be identified. Generally, the higher proportions of unidentified materials occurred at later sampling times, where the residue level was low and the native sugar content in the plants was high. Most probably, the majority of the unidentified products were unhydrolysed conjugates of NC 20645 and NC 8493. In some of the later samples it was necessary to repeat the hydrolysis. Re-hydrolysis always resulted in the release of additional amounts of NC 9607 and NC 8493, indicating that the high sugar content hindered proper cleavage of the conjugates.

The TRR values and the distribution of the radioactivity are shown in the following table:

**Table 7.2.1-55 Distribution of radioactivity in the extracts of sugar following pre-emergent application**

Sampling event (day)	10		20		30		40**		50	
TRR	%	mg/ kg	%	mg/ kg	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	15.99	100.0	3.33	100.0	2.27	100.0	0.05	100.0	1.40
Ethofumesate	16.6	2.66	3.9	0.13	0.9	0.02			2.9	0.04
NC 20645, conj. *	58.7	9.39	80.8	2.69	80.6	1.83			72.9	1.02
NC 8493, conj.	1.4	0.23	3.0	0.1	2.2	0.05			5.0	0.07
NC 20645	0.9	0.14	3.9	0.13	4.8	0.11			5.7	0.08
unknown	14.6	2.33	8.1	0.27	9.3	0.21			10.0	0.14
Total identified	77.7	12.42	91.6	3.05	88.5	2.01			86.4	1.21
Total uncharacterised	14.6	2.33	8.1	0.27	9.3	0.21			10.0	0.14
Solids	7.8	1.24	0.3	0.01	2.2	0.05			3.6	0.05
Accountability	100.0	15.99	100.0	3.33	100.0	2.27	100.0	0.05	100.0	1.40

\* identified after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC

\*\* no analysis was performed due to the low residue level

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

**Table 7.2.1-56 Distribution of radioactivity in the extracts of sugar beet following post-emergent leaf application**

Sampling event (day)	10		20		30		40		50	
TRR	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	17.50	100.0	9.65	100.0	9.87	100.0	5.37	100.0	2.59
Ethofumesate*	13.7	2.40	5.5	0.53	3.5	0.35	4.1	0.22	2.3	0.06
NC 20645, conj.**	70.5	12.33	62.7	6.05	66.7	6.58	61.6	3.31	67.2	1.74
NC 8493, conj.**	4.7	0.83	6.2	0.60	4.8	0.47	3.7	0.20	2.3	0.06
NC 20645***	3.0	0.52	8.1	0.78	-	-	-	-	-	-
unknown	6.0	1.05	13.0	1.25	21.1	2.08	25.1	1.35	19.3	0.50
Total identified	91.9	16.08	82.5	7.96	75.0	7.40	69.5	3.73	71.8	1.86
Uncharacterised	6.0	1.05	13.0	1.25	21.1	2.08	25.1	1.35	19.3	0.50
Solids	2.1	0.37	4.6	0.44	4.0	0.39	5.4	0.29	8.9	0.23
Accountability	100.0	17.50	100.0	9.65	100.0	9.87	100.0	5.37	100.0	2.59

- not detected

\* identified by TLC (toluene/ethyl acetate 4/1; v/v)

\*\* identified as exocon after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)

\*\*\* identified after treatment with cold HCl (2 M) for 1 hour and extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)  
Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

#### B.7.2.1.1.5. Sugar beet metabolism 5 (supportive information)

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

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Report:	KCA 6.2.1 /04;Lines, D. S.; Adcock, J. W.;1979;M-155242-01
Title:	THE METABOLISM OF ETHOFUMESATE (98% PURE <sup>14</sup> C-ETHOFUMESATE) BY SUGAR BEET UNDER FIELD CONDITIONS
Report No:	A82965
Document No:	M-155242-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

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### I. Summary

The metabolism of [<sup>14</sup>C-methylsulfonyl]-ethofumesate in sugar beet was investigated following pre- and post-emergence treatment of 2 kg a.s./ha in the field. The pre-emergence application was conducted at the time of sowing; post-emergence application was done at the 2-leaf stage (BBCH 12) of the crop.

This study was conducted to confirm the metabolic degradation of ethofumesate in sugar beets as evaluated in a greenhouse study (cf. KCA 6.2.1 /03; Lines, D. S.; Adcock, J. W.; 1978; M-155241-01) and to confirm that no methanesulphonic acid (MSA) is formed in sugar beets, even under field conditions. The increased ease of the metabolite identification in the greenhouse study, coupled with the greater relevance of the field study, was intended to lead to a fuller understanding of the metabolic behaviour of ethofumesate in sugar beet.

Whole plants with roots were sampled 50, 75, 125 and 175 (pre-emergent, only) days after application. Plants of the pre-emergent treatment were washed free of soil with water and acetone before homogenization. The foliage of plants of the post-emergent treatment was additionally washed with methanol (leaf wash fraction). For extraction, the whole plants were chopped and homogenised in methanol, the fibre was filtered and re-homogenized with methanol. The radioactivity in the extracts was determined by LSC and in the non-extracted residues by combustion and LSC. The methanol extracts were reduced to dryness, made up in water and extracted twice with dichloromethane (DCM). The organic phase was separated and backwashed with water. The combined aqueous phases were subjected to acidic hydrolysis with conc. HCl (the aqueous phase was diluted with approx. the same volume of conc. HCl). Hydrolysis was performed under reflux conditions for 1 hour. The reaction mixture was extracted with ethyl acetate (EtoAc) and backwashed with water. The extracts before (DCM) and after the hydrolysis step (EtoAc and aqueous remainder) were concentrated and analysed by TLC (thin layer chromatography). In addition, a number of aqueous extracts were examined for the presence of MSA using TLE (thin layer electrophoresis). To separate the extracted sugar from the metabolites, several extracts were subjected to a clean-up step with Amberlite XAD 2 resin.

Identification of the radiolabelled components was done in different chromatographic systems by comparison with unlabelled standards. The absence of methanesulphonic acid (MSA) in the extracts was confirmed by TLC and TLE.

The TRR values declined after pre-emergent application of ethofumesate from 0.24 mg /kg at day 50 to 0.01 mg/kg at day 175. Total residues following post-emergent application were 1.28 mg/kg at day 50 and 0.03 mg/kg at day 125.



In general, similar metabolic profiles were observed in both the pre- and post-emergent beet extracts and were in agreement with the findings of previous studies. The concentration of ethofumesate declined sharply after application and the major portion of the characterized residues was identified as conjugates of NC 20645 (25-50% of TRR), together with conjugates of NC 8493 (1-20% of the TRR). Trace levels of ethofumesate and free NC 20645 were occasionally found in plants treated post-emergent. The leaf washing of post-emergent treated plants contained mainly conjugated NC 20645 and unidentified material. The low concentration of ethofumesate in the leaf washes suggest that the active substance is readily absorbed through the leaf surface.

The TRR values and the distribution of the radioactivity are shown in the following table:

**Table 7.2.1-57 Distribution of radioactivity in the extracts of sugar beets following pre-emergent application**

Sampling event (day)	50		75		125		175**	
TRR	%	mg/ kg	%	mg/ kg	%	mg/kg	%	mg/kg
	100.0	0.237	100.0	0.086	100.0	0.058	100.0	0.006
Ethofumesate	-	-	-	-	-	-	-	-
NC 20645, conj.*	48.1	0.114	37.2	0.032	25.9	0.015	-	-
NC 8493, conj.*	12.2	0.029	10.5	0.009	5.2	0.003	-	-
NC 20645**	-	-	-	-	-	-	-	-
unknown	18.6	0.044	23.3	0.020	20.7	0.012		
Total identified	60.3	0.143	47.7	0.041	31.0	0.018		
Total uncharacterised	18.6	0.044	23.1	0.020	20.7	0.012	33.3	0.002
Solids	21.1	0.05	29.1	0.025	48	0.028	66.7	0.004
Accountability	100.0	0.237	100.0	0.086	100.0	0.058	100.0	0.006

- not detected

\* identified as exocon after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC

\*\* no analysis was performed due to the low residue level

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

**Table 7.2.1-58 Distribution of radioactivity in the extracts of sugar beet following post-emergent leaf application**

Sampling event (day)	50		75		125	
TRR	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	1.285	100.0	0.228	100.0	0.026
Ethofumesate	0.2	0.002	-	-	-	-
NC 20645, conj.*	47.6	0.612	36.8	0.084	23.1	0.006
NC 8493, conj.*	20.6	0.265	19.7	0.045	3.8	0.001
NC 20645*	0.1	0.001	2.2	0.005	-	-
unknown	16	0.205	7.5	0.017	19.2	0.005
Total identified	68.5	0.880	58.8	0.134	29.6	0.007
Uncharacterised	16.0	0.205	7.5	0.017	19.2	0.005
Solids	15.6	0.200	33.8	0.077	53.8	0.014
Accountability	100.0	1.285	100.0	0.228	100.0	0.026

- not detected

\* identified as exocon after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

## B.7.2.1.2. Ryegrass

### B.7.2.1.2.1. Ryegrass metabolism 4

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

Report: KCA 6.2.1 /07  
Chapleo, S.;1992  
M-155248-01

Title: THE METABOLISM OF [<sup>14</sup>C]-ETHOFUMESATE IN ANNUAL RYEGRASS - A

Report No:	GLASSHOUSE STUDY A82971
Document No(s):	Report includes Trial Nos.: 381169 ENVIR 85B M-155248-01-1
Guidelines:	USEPA (=EPA): subdiv.O, 171-4; Deviation not specified
GLP/GEP:	yes

### I. Summary

The metabolism of [phenyl-UL-<sup>14</sup>C]-ethofumesate in ryegrass was investigated according to the formerly envisaged use pattern. A single foliar spray application of [<sup>14</sup>C]-ethofumesate was made on ryegrass at the 2-3 leaf growth stage at an application rate of 2.09 kg a.s./ha. An additional overdose experiment (5x) was conducted to ease identification of metabolites. The formulation used was an SC formulation. The experiments were conducted in the greenhouse.

Samples were taken directly after the application (as soon as the applied formulation was dry), 7 days after the application, at silage stage (28 days after application) and at maturity. The TRR values are shown in the following table:

**Table 7.2.1-59 Total residues in ryegrass samples Concentration of the total radioactivity in ryegrass samples (results expressed as mg a.s. equiv./kg)**

Sampling event	day 0	day 7	day 28 (silage stage)	16 weeks (maturity) <sup>a</sup>
1x Application rate				
Surface wash	501.68	33.78	0.73	0.22
Washed tissue	64.25	13.84	2.28	0.87
TRR	565.93	47.62	3.01	1.09
5x Application rate				
Surface wash	1907.3	295.5	14.32	2.24
Washed tissue	116.0	97.5	15.00	7.20
TRR	2023.3	393.0	29.32	9.44

<sup>a</sup> results of replicate B are presented.

Residues were well solubilized by foliar washes and conventional solvent extraction steps; an exhaustive extraction with 1 M HCl (16 h at reflux) was only applied in samples of mature ryegrass. Overall, more than 90% of the total radioactive residue of all samples of the 1x experiment was subjected to chromatographic analysis.

Ryegrass samples, taken early after application, showed mainly residues of parent ethofumesate. With time, ethofumesate concentrations decreased and the concentration of the metabolites NC 20645 (free and conjugated form), NC8493 (free and conjugated form) and NC 9607 increased. At final harvest, metabolite NC 20645 (mainly in conjugated form) was the predominant component, followed by parent compound and metabolite NC 8493 in free and conjugated form. Metabolite NC 9607 represented only a minor proportion.

On the basis of the metabolites identified, the following metabolic routes were deduced:

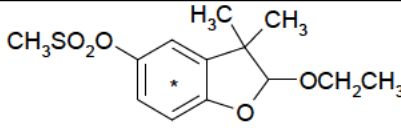
- Cleavage of the ethoxy side chain of the parent compound, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can undergo either conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to form the carboxylic acid NC 20645 which can also undergo conjugation to give polar metabolites.

Under reflux with 6 M hydrochloric acid, the detected conjugates are hydrolysed to their exocons NC 8493 and NC 20645. Metabolites NC 20645 and NC 9607 are inter-convertible. Under acidic conditions metabolite NC 2064 is transformed to metabolite NC 9607 due to an intra-molecular ring closure. Small amounts of NC 8493 probably result from the acidic decomposition of parent compound ethofumesate.

## II. Materials and Methods

### A. Materials

#### 1. Test Material:

Chemical structure	 <p style="text-align: right;">* position of the radiolabel</p>
Radiolabelled test material	[ <sup>14</sup> C]-ethofumesate 2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methane-sulphonate
Specific radioactivity	7.178 MBq/mg (194 µCi/mg)
Radiochemical purity*	98% (TLC)

[<sup>14</sup>C]-ethofumesate was formulated as a 50% suspension concentrate. The formulations had a specific activity of 10.3 µCi/mg (1x field rate) and 10.2 µCi/mg (5x field rate).

#### 2. Soil

A sandy loam soil (USDA) was used, which had been passed through a 10 mm sieve to remove stones. Soil details and characterisation are given in the following table:

**Table 7.2.1-60 Soil characterisation**

Physical characterisation	
Soil classification (USDA)	Sandy loam soil
pH (water)	6.2
pH (KCl)	5.6
% organic carbon	6.6
Cation exchange capacity (mequiv. /100 g)	11.27
Moisture content (0.33 bar)	19.94
Maximum water holding capacity* (%)	68.2
Moisture content at pot preparation* (%)	22.1
Particle size analysis	
Clay (< 0.002 mm) (%)	14.0
Silt (0.002-0.063 mm) (%)	22.3
Total sand (0.063-2 mm) (%)	63.7

\* conducted by IRI (Inveresk Research International, Tranent, Scotland))

#### 3. Plant

Annual ryegrass, variety *Lolium westerwoldicum* auct cv “Billion”

### B. Study Design

#### Experimental conditions

Plants were grown in pots filled with sandy loam soil. Seed was sown at a rate of approx. 3 g/m<sup>2</sup> (0.18 ± 0.1 g seeds per pot). The plants were grown under controlled greenhouse conditions: Supplementary lighting (16 h) was provided, temperature (monthly mean: 17-23 °C) and relative humidity (monthly mean: 50-

74%) was controlled. To maintain the good conditions of plants, water, fertilizers and pesticides were applied as required.

Test material was applied as a foliar spray when plants were at the 2-3 leaf growth stage. A single application of test material was made to 16 pots at the 1x field application rate and to 10 pots at the 5x field application rate. Formulation was applied as a fine spray to each pot using an all-glass atomiser. All control plants were moved to a separate glasshouse during spraying to avoid contamination.

### **Sampling**

Plant samples were collected on the day of application when the formulation was dry, at 7 and 28 days after the application and at maturity. For each pot, foliage was sampled at the soil level and the fresh weight determined.

## **C. Analytical Procedures**

### **Determination of total residues**

At each harvest, foliage was washed in distilled grade water followed by dichloromethane to remove surface residue. Total radioactivity in washes was determined by liquid scintillation counting (LSC) of a minimum of 3 aliquots. Washed foliage was chopped up using scissors and/or kitchen knives, frozen in cardice until brittle, and milled in cardice. Residues remaining in washed tissues were determined by combustion of a minimum of 6 aliquots. Total residues were calculated by addition of residue components in foliar washes and washed tissue.

### **Extraction of washed tissues**

Washed tissue was macerated in a range of organic solvents of increasing polarity to optimise the extraction of radioactivity. Tissue pools were extracted 3 times with acetonitrile (5 times for day 0), followed by acetonitrile/water (3/1; v/v) (3 times), followed by distilled grade water (3 times). A single final acetonitrile extract was conducted to remove water from the non-extractable residue. The extract was combined with the initial acetonitrile extracts. Extraction involved homogenisation, immersion in an ultrasonic bath and centrifugation to separate extractable and non-extractable components. The acetonitrile and acetonitrile/water extracts were combined and acetonitrile was removed by rotary evaporation.

### **Extraction of fibre-bound residues**

The following attempts to further solubilize the bound residue which remained after conventional solvent extraction were conducted.

- Soxhlet extraction with acetonitrile/water (16 h)
- Extraction under reflux using 1 M NaCl (16 h)
- Extraction under reflux using 1 M HCl (16 h)
- Extraction under reflux using 1 M NaOH (16 h)
- Extraction under reflux using 5 M NaOH (16 h)

Extraction with hydrochloric acid released an additional amount of radioactivity which was partitioned with dichloromethane for subsequent chromatographic analysis.

### **Identification and characterisation**

Extracts and washes were examined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) in at least 2 contrasting systems with confirmation in a third. Reference compounds (non-labelled) were analysed individually and compared to the retention times of the detected compounds. In

addition a standard mixture of reference compounds was co-chromatographed with each wash/extract. When polar metabolites formed a significant proportion of the extractable residue, the combined acetonitrile and acetonitrile/water extracts were either analysed directly by HPLC or partitioned with dichloromethane and the aqueous phase was subjected to an acidic hydrolysis step (6 M HCl, 18 h under reflux). Again, the hydrolysates were either analysed directly by HPLC or were partitioned with dichloromethane and the dichloromethane extract was analysed by TLC. Enzymatic treatment (with  $\beta$ -glucosidase and  $\beta$ -galactosidase) of the tissue extract was also conducted to characterize the polar fraction.

### Quantification

Representative extracts were analysed by reversed-phase HPLC with UV- and radio-detection. All samples were co-injected with a standard mixture, fractions collected and quantified by liquid scintillation counting. In addition, representative samples on thin layer chromatography were scraped and quantified by liquid scintillation counting.

## III. Results and Discussion

The metabolism of [ $^{14}\text{C}$ ]-ethofumesate was investigated in ryegrass following a single foliar application at the 2-3 leaf growth stage with an application rate of 2.09 kg a.s./ha. The total radioactive residue (TRR) in ryegrass foliage declined over time starting with 565.9 mg a.s. equiv./kg, at the day of application to approx. 1.09 mg/kg at maturity (approx. 16 weeks after application), which represents less than 1% of the day 0 value. In general the results of the 5x application rate were 5-fold higher (or even more) than those of the 1x application rate and were used for the identification of metabolites.

At the day of application the main fraction of radioactivity (89%) was detected in the surface wash. The proportion of radioactivity in the surface wash declined with time and only 20% of the radioactivity was removed by washing from the final sample collected at harvest. A corresponding increase of the radioactivity in the washed tissue indicates that translocation into the tissues took place.

Distribution of activity prior to acidic hydrolysis of polar compounds/fraction

At 1 h after the application, 96.1% of the total radioactivity (565.9 mg/kg) detected in/on ryegrass was identified as ethofumesate, most of it was found in the foliar washes. Minor amounts of metabolite NC 8493 were detected in the aqueous surface wash and minor amounts of metabolite NC 20645 were detected in the tissue extracts.

At day 7 and 28 after the application the radioactivity in foliage decreased to 47.6 mg/kg and 3.01 mg/kg, respectively. 70.9% and 24.8% of the total activity was detected in the surface washes and 19.2% and 71.6% in the tissue extracts. Thus overall, 90.1% and 95.7% of the radioactivity was soluble and was subjected to chromatographic investigations. At day 7 and 28, 80.9% and 26.9%, respectively of the total foliage residue was identified as ethofumesate, most of it was found in foliar washes. Other compounds were identified as NC 9607 (0.4%), NC 8493 (2.4%) and NC 20645 (1.0%) at day 7, while at day 28 only NC 8493 (1.6%) and NC 20645 (1.2%) were identified. The percentage of unidentified polar compounds, which were not resolved from the origin of the normal phase TLC system used, nor from the solvent front of the reversed-phase TLC system used, increased from 4.0% at day 7 to 62.0% at day 28.

At maturity the radioactivity in foliage amounted to 1.09 mg/kg (replicate B). 91.7% of the total activity was released in the foliar washes or the tissue extracts leaving 8.3% of the TRR as bound residue. 13.2% of the total residue was identified as ethofumesate, all detected in foliar washes. Compounds identified as NC 9607,

NC 8493 and NC 20645 accounted for 2.6%, 6.7% and 20.1% of the total residue, respectively. Unidentified polar components accounted for 36.2% (0.39 mg/kg) of the total residue.

The distribution of radioactivity detected in ryegrass samples collected after the 1x application rate is summarized in the following tables.

**Table 7.2.1-61 Characterisation of surface washes and extracts following application of  $^{14}\text{C}$ -ethofumesate to ryegrass (treatment 1x rate) before acidic hydrolysis of the tissue extracts – day 0 (results expressed as % total activity recovered)**

	1x, day 0	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>565.9</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	65.0	367.9
Ethofumesate	64.1	362.8
Origin	-	-
NC 20645	-	-
NC 8493	0.8	4.5
NC 9607	-	-
Remainder	0.1	0.6
DCM wash	23.7	134.1
Ethofumesate	22.9	129.6
Origin	-	-
NC 20645	-	-
NC 8493	-	-
NC 9607	-	-
Remainder	0.8	4.5
Conventional tissue extracts		
ACN extract	8.8	49.8
ACN/H <sub>2</sub> O (3/1; v/v) extract	1.3	7.4
Combined extracts	10.1	57.2
Ethofumesate	9.1	51.5
Origin	0.2	1.1
NC 20645	0.6	3.4
NC 8493	-	-
NC 9607	-	-
Remainder	0.2	1.1
H <sub>2</sub> O extract	0.1	0.6
Solids	1.1	6.2
Total identified	97.5	551.8
Total characterised	1.4	7.9
Total analysed	98.9	559.7
Solids	1.1	6.2
Accountability	100.0	565.9

The results given in this Table are summarised from Table 7 of the original report.

**Table 7.2.1-62 Characterisation of solvent washes and extracts following application of  $^{14}\text{C}$ -ethofumesate to ryegrass (treatment 1x rate) before acidic hydrolysis of the tissue extracts – day 7 (results expressed as % total activity recovered)**

	1x, day 7	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>47.62</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	53.6	25.52
Ethofumesate	52.4	24.95
Origin	-	-
NC 20645	-	-
NC 8493	0.6	0.29

	1x, day 7	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>47.62</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
NC 9607	-	-
Unknowns (polar)	-	-
Remainder	0.6	0.29
DCM wash	17.3	8.24
Ethofumesate	16.0	7.62
Origin	-	-
NC 20645	-	-
NC 8493	0.6	0.29
NC 9607	-	-
Remainder	0.7	0.33
Conventional tissue extracts		
ACN extract	18.8	8.95
ACN/H <sub>2</sub> O (3/1; v/v) extract	0.4	0.19
Combined extracts	19.2	9.14
Ethofumesate	12.5	5.95
Origin	1.9	0.90
NC 20645	1	0.48
NC 8493	1.2	0.57
NC 9607	0.4	0.19
Unknown (definite polar peak)	2.1	1.00
Remainder	0.1	0.05
H <sub>2</sub> O extract	0.1	0.05
Solids	9.8	4.67
Total identified	84.7	40.33
Total characterised	5.5	2.62
Total analysed	90.2	42.95
Solids	9.8	4.67
Accountability	100.0	47.62

The results given in this table are summarised from Appendix 17 of the original report.

**Table 7.2.1-63 Characterisation of solvent washes and extracts following application of <sup>14</sup>C-ethofumesate to ryegrass (treatment 1x rate) before acidic hydrolysis of the tissue extracts – day 28 (silage stage) (results expressed as % total activity recovered)**

	1x, day 28	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>3.01</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	9.3	0.28
Ethofumesate	8.0	0.24
Origin	-	-
NC 20645	1.2	0.04
NC 8493	-	-
NC 9607	-	-
Unknowns (polar)	-	-
Remainder	0.1	<0.01
DCM wash	14.8	0.45
Ethofumesate	14.5	0.44
Origin	-	-
NC 20645	-	-
NC 8493	0.3	0.01
NC 9607	-	-
Remainder	<0.1	<0.01
Conventional tissue extracts		
ACN extract	37.7	1.13
ACN/H <sub>2</sub> O (3/1; v/v) extract	32.2	0.97
Combined extracts	69.9	2.10
Ethofumesate	4.4	0.13

	<b>1x, day 28</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>3.01</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Origin	60.3	1.82
NC 20645	-	-
NC 8493	1.3	0.04
NC 9607	-	-
Unknown (definite polar peak)	1.7	0.05
Remainder	2.2	0.07
H <sub>2</sub> O extract	1.7	0.05
Solids	4.3	0.13
Total identified	29.7	0.89
Total characterised	66.0	1.99
Total analysed	95.7	2.88
Solids	4.3	0.13
Accountability	100.0	3.01

The results given in this table are summarised from Appendix 17 of the original report.

**Table 7.2.1-64 Characterisation of solvent washes and extracts following application of <sup>14</sup>C-ethofumesate to ryegrass (treatment 1x rate) before acidic hydrolysis of the tissue extracts – 16 weeks (maturity) (replicate B) (results expressed as % total activity recovered)**

	<b>1x, 16 weeks, maturity</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>1.09</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	24.2	0.26
Ethofumesate	12.1	0.13
Origin	-	-
NC 20645	4.1	0.05
NC 8493	6.4	0.07
NC 9607	-	-
Unknowns (polar)	-	-
Remainder	1.6	0.02
DCM wash	2.2	0.02
Ethofumesate	1.1	0.01
Origin	0.1	<0.01
NC 20645	0.3	<0.01
NC 8493	0.3	<0.01
NC 9607	0.3	<0.01
Remainder	0.1	<0.01
Conventional tissue extracts		
ACN extract	42.6	0.46
ACN/H <sub>2</sub> O (3/1; v/v) extract	12.4	0.14
Combined extracts	55.0	0.60
Ethofumesate	-	-
Origin	36.1	0.39
NC 20645	15.7	0.17
NC 8493	-	-
NC 9607	2.3	0.03
Remainder	0.9	0.01
H <sub>2</sub> O extract	10.3	0.11
Solids	8.3	0.09
Total identified	42.6	0.46
Total characterised	49.1	0.54
Total analysed	91.7	1.00
Solids	8.3	0.09
Accountability	100.0	1.09

The results given in this table are summarised from Appendix 17 of the original report.



**Distribution of activity after enzymatic or acidic hydrolysis of polar compounds/fraction**

Because of the predominance of the polar radioactive components (which were not resolved from the origin of the normal phase TLC system used, nor from the solvent front of the reversed-phase TLC or HPLC systems used), hydrolysis experiments were conducted to characterize the radioactivity. Selected tissue extracts were subjected to enzymatic and acidic treatment in order to determine if the polar components were the result of conjugation of the herbicide or its main metabolites.

Treatment with  $\beta$ -glycosidase activities ( $\beta$ -glucosidase or  $\beta$ -galactosidase) did not affect the proportions of polar radioactive components and no obvious signs of deconjugation were observed. Therefore the conjugates are most likely no O-glucosides. However acid hydrolysis of the polar fraction using 2 M HCl or 6 M HCl yielded components identified as NC 9607, NC 8493 and NC 20645. Thus the unidentified polar components are acid-labile and can be transformed to discrete moieties originating from the parent pesticide or its close metabolite derivatives.

At silage stage and at maturity, polar metabolites in tissue extracts were also hydrolysed using 6 M HCl. The distribution of ethofumesate and its metabolites in the different extracts is given in Table 7.2.1-65 and Table 7.2.1-66. Table 7.2.1-67 and **Fehler! Verweisquelle konnte nicht gefunden werden.** summarise the identified metabolites and how they occurred in the sample at silage stage and the mature sample (either free, conjugated or fibre bound).

**Table 7.2.1-65 Characterisation of solvent washes and extracts following application of  $^{14}\text{C}$ -ethofumesate to ryegrass (treatment 1x rate) after acidic hydrolysis of the tissue extracts (6 M HCl, 18 h under reflux) – day 28 (silage stage)**

	<b>1x, day 28</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>3.01</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	9.3	0.28
Ethofumesate	8.0	0.24
Origin	-	-
NC 20645	1.2	0.04
NC 8493	-	-
NC 9607	-	-
Unknowns (polar)	-	-
Remainder	0.1	<0.01
DCM wash		
Ethofumesate	14.8	0.45
Ethofumesate	14.5	0.44
Origin	-	-
NC 20645	-	-
NC 8493	0.3	0.01
NC 9607	-	-
Unknowns (polar)	-	-
Remainder	0.0	0.00
Conventional tissue extracts		
Combined extracts (ACN + ACN/H <sub>2</sub> O)	69.9	2.10
DCM phase	14.6	0.44
Ethofumesate	11.2	0.34
NC 20645	0.7	0.02
NC 8493	0.9	0.03
NC 9607	0.7	0.02
Other unknown (bright green pigment fraction)	0.3	0.01
Unknown (remainder)	0.8	0.02
Aqueous phase (subjected to acidic hydrolysis)	55.3	1.66
DCM phase (after hydrolysis)	52.1	1.57

	<b>1x, day 28</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>3.01</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Ethofumesate	-	-
NC 20645	9.8	0.30
NC 8493	2.7	0.08
NC 9607	39.6	1.19
Unknown (polar)	-	-
Aqueous phase (remainder)	3.2	0.10
H <sub>2</sub> O extract	1.7	0.05
Solids	4.3	0.13
Total identified	89.6	2.70
Total characterised	6.1	0.18
Total analysed	95.7	2.88
Solids	4.3	0.13
Accountability	100.0	3.01

The results given in this table are summarised from Table 13 of the original report.

**Table 7.2.1-66 TRR values and distribution of parent compound and metabolites in ryegrass foliage following application of <sup>14</sup>C-ethofumesate to sugar beets (treatment 1x rate) after acidic hydrolysis (6 M HCl, 18 h reflux) – maturity (replicate B); HPLC analysis**

	<b>1x, maturity</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>1.09</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Surface washes		
H <sub>2</sub> O wash	24.2	0.264
Unknown (polar, eluting with HPLC solvent front)	7.3	0.080
Ethofumesate	8.6	0.094
NC 20645	4.6	0.051
NC 8493	3.3	0.036
NC 9607	0.3	0.004
DCM wash	2.2	0.024
Origin (unresolved radioactivity on TLC plate)	0.1	0.001
Ethofumesate	1.1	0.012
NC 20645	0.3	0.003
NC 8493	0.3	0.003
NC 9607	0.3	0.004
Unknown (remainder)	0.1	0.001
Conventional tissue extracts		
Combined extracts (ACN + ACN/H <sub>2</sub> O)	55.1	0.601
DCM phase	17.5	0.191
Ethofumesate	7.9	0.086
NC 20645	1.9	0.021
NC 8493	1.0	0.011
NC 9607	4.6	0.050
Other unknown (bright green pigment fraction)	-	-
Unknown (remainder)	2.1	0.023
Aqueous phase (subjected to acidic hydrolysis)	37.6	0.410
Ethofumesate	-	-
NC 20645	6.2	0.068
NC 8493	4.2	0.045
NC 9607	24.0	0.262
Unknown (remainder)	3.2	0.035
H <sub>2</sub> O extract (after hydrolysis)	10.3	0.112
Unknown (polar, eluting with solvent front)	0.3	0.003
NC 20645	1.7	0.019
NC 8493	1.2	0.013
NC 9607	6.4	0.070
Unknown (remainder)	0.8	0.008
Solids 1	8.2	0.090
Exhaustive extraction (1 M HCl)		
Solids 1	8.2	0.090

	<b>1x, maturity</b>	
<b>TRR [mg a.s. equiv./kg] =</b>	<b>1.09</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Acid extract after partitioning	4.8	0.052
DCM extract	1.7	0.019
Origin (unresolved radioactivity on TLC plate)	<0.1	<0.001
NC 20645	0.1	0.001
NC 8493	1.2	0.013
NC 9607	0.4	0.005
Unknown (remainder)	<0.1	<0.001
Solids 2	1.7	0.019
Total identified	79.6	0.868
Total characterised	18.7	0.203
Total analysed	93.5	1.019
Solids	1.7	0.019
Accountability	100.0	1.090

The results given in this table are summarised from Table 14 of the original report.

**Table 7.2.1-67 Examination of the nature of the radioactivity in metabolite fractions from ryegrass foliage at silage stage (treatment 1x rate) expressed as % total activity recovered**

Sampling event	Ryegrass			
	1x, day 28			
	before hydrolysis*		after hydrolysis*	
TRR	%	mg/kg	%	mg/kg
	<b>100.0</b>	<b>3.01</b>	<b>100.0</b>	<b>3.01</b>
Ethofumesate (parent compound)	26.9	0.81	33.7	1.01
NC 8493	1.6	0.05	1.2	0.04
NC 8493 conjugated	-	-	2.7	0.08
NC 9607	-	-	0.7	0.02
NC 20645	1.2	0.04	1.9	0.06
NC 20645 conjugated#	-	-	49.4	1.49
Total identified	29.7	0.89	89.6	2.70
Unknown (discrete peak)	1.7	0.05	0.3	0.01
Unknown (non-resolved from origin/solvent front)	60.3	1.82	-	-
Remainder	2.3	0.07	0.9	0.03
Not analyzed	1.7	0.05	4.9	0.15
Total characterized	66.0	1.99	6.1	0.18
Solids (bound fibre residue)	4.3	0.13	4.3	0.13
Accountability	100.0	3.01	100.0	3.01

\* before/after hydrolysis of polar fraction present in the acetonitrile and acetonitrile/water extract (= fraction containing the conjugates)

# identified as NC9607 after acidic hydrolysis, no differentiation possible if formed from conjugated or non-conjugated  
NC 20645

**Table 7.2.1-68 Examination of the nature of the radioactivity in metabolite fractions from mature ryegrass foliage (treatment 1x rate) expressed as % total activity recovered**

Sampling event	Ryegrass			
	1x, maturity (replicate B)			
	before hydrolysis*		after hydrolysis*	
TRR	%	mg/kg	%	mg/kg
	<b>100.0</b>	<b>1.090</b>	<b>100.0</b>	<b>1.090</b>
Ethofumesate (parent compound)	13.2	0.144	17.6	0.192
NC 8493	6.7	0.073	4.6	0.050
NC 8493 conjugated	-	-	5.4	0.058
NC 8493 fibre bound	1.2	0.013	1.2	0.013
NC 9607	2.6	0.033	5.2	0.058
NC 9607 fibre bound#	0.4	0.005	0.4	0.005
NC 20645	20.1	0.218	6.8	0.074
NC 20645 conjugated##	-	-	38.3	0.418
NC 20645 fibre bound	0.1	0.001	0.1	0.001

Total identified	44.3	0.487	79.6	0.868
Unknown (non-resolved from origin/solvent front)	36.2	0.392	7.7	0.084
Remainder	2.6	0.028	6.2	0.067
Not analyzed	15.1	0.163	4.8	0.052
Total characterized	53.9	0.583	18.7	0.203
Solids (bound fibre residue)	1.7	0.019	1.7	0.019
Accountability	100.0	1.090	100.0	1.090

\* before/after hydrolysis of polar fraction present in the acetonitrile and acetonitrile/water extract (= fraction containing the conjugates)

# identified after exhaustive extraction, precursor could be NC9607, NC20645 or a conjugate of NC20645

## identified as NC9607 after acidic hydrolysis, no differentiation possible if formed from conjugated or non-conjugated NC 20645

Acidic hydrolysis transformed the main amount of the polar radioactivity (which was either non-resolved from the origin using normal phase systems or eluted with the solvent front using reverse phase systems) to discrete known moieties. The decline of the polar fraction correlated with an increase of the concentration of metabolites NC 9607 and NC 8493 indicating that these metabolites - or in the case of NC 9607 its carboxy analogue NC 20645 - are present in conjugated forms. Small amounts of NC 8493 may also result from the acidic decomposition of parent compound ethofumesate. Thus, with the help of an acidic hydrolysis step, the predominant amount of the ethofumesate related residues in ryegrass can be assigned to four compounds (parent ethofumesate and metabolites NC 8493, NC9607 and NC 20645). Metabolites NC 9607 and NC 20645 are inter-convertible; the presence of acid favours the formation of NC 9607.

#### IV. Conclusions

The total residue in ryegrass is represented by four compounds: Parent ethofumesate and the metabolites NC 9607, NC 8493 and NC 20645, of which the two latter were detected in free and conjugated forms. The conjugates were mainly detected in the samples collected at later time points (silage stage and maturity) and can be hydrolysed with 6 M hydrochloric acid. The main residue in the mature sample consisted of metabolite NC 20645, mainly in conjugated form, followed by parent ethofumesate and metabolite NC 8493 in free and conjugated form. Metabolite NC 9607 represented only a minor proportion. (The high concentration of metabolite NC 9607 after acidic hydrolysis can be attributed to the fact that cleavage of the conjugate of NC 20645 leads to metabolite NC 9607 due to an intramolecular condensation when applying acidic condition).

#### Based on the metabolites identified the following metabolic routes were deduced

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can either undergo conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to the acid NC 20645 which can also undergo conjugation to give polar metabolites.

On the basis of the results of this study it is concluded that the metabolism of [<sup>14</sup>C]-ethofumesate in ryegrass is well understood and is identical to the metabolism of sugar beet. A common metabolic pathway is shown in Figure 1.

*Supportive studies (studies which are used to support the metabolic pathway elucidated for plant matrices):*

#### B.7.2.1.3. Onion

##### B.7.2.1.3.1. Onion metabolism 7 (supportive information)

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

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Title:	Adcock, J. W.; Warner, P. A., Challis, I. R.;1976 M-155236-01 THE METABOLISM OF <sup>14</sup> C-ETHOFUMESATE IN THE ONION
Report No:	A82959
Document No:	M-155236-01-1
Guidelines:	Deviation not specified
GLP/GEP:	No; study performed prior to GLP

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### I. Summary

The metabolism of ethofumesate was investigated in onion after a pre-emergence treatment with [<sup>14</sup>C]-ethofumesate at an application rate of 2 kg a.s./ha. The position of the radiolabel was not reported, however it is assumed that the active substance used was [phenyl-UL-<sup>14</sup>C]-ethofumesate.

Plant samples were taken at 22, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 162 days (harvest) after treatment. Samples were washed free of soil and stored at -12°C until analysis.

Whole plants were homogenised in methanol (3 x 50 mL), the fibre being filtered off and re-homogenised. The extract was concentrated to low volume for chromatographic analysis. The radioactivity in the extracts was determined by liquid scintillation counting (LSC) and the non-extracted radioactive residues were determined by combustion and LSC of the sub-samples of the extracted plant residues. Plant extracts from samples collected between 22 and 80 days were analysed by normal phase TLC using a number of different solvent systems. Identification of the metabolites was done by comparison with the position of unlabelled standards applied to the TLC plates and detected by viewing the TLC plate under UV light at 254 nm. The position of the radioactive components was determined by use of a radio-scanner, the amount of each spot was determined by desorbing the radioactive spot from the TLC plate and liquid scintillation counting of the extract.

Acidic hydrolysis of polar material was carried out by removing the area from the TLC plate and boiling in 6 M hydrochloric acid for 1 hour. The resulting solution was then liquid-liquid partitioned with ethyl acetate and the extracts analysed by LSC and TLC.

The TRR in the onion plants reached a maximum after 50 days (3.2 mg/kg) and decreased thereafter with time. At harvest (day 162) the TRR in the whole plant accounted for only 0.025 mg/kg (80% of the residue (0.02 mg/kg) was extractable - but due to the low residue level no further identification/characterization of the residue was performed - and 20% (0.005 mg/kg) was assigned to fibre bound residue).

Identification of metabolites was performed in the samples collected 22 to 80 days after the treatment; samples collected at later time points showed insufficient activity (too low residue levels) for identification.

In the respective sample extracts three compounds were identified: parent ethofumesate, metabolite NC 20645 (ethofumesate-carboxylic acid = ring-opened NC 9607) and a conjugate of metabolite NC 20645. The elucidation of the exocon of the conjugate was achieved after acidic hydrolysis with 6 M HCl. The identified hydrolysis product was metabolite NC 9607. Since NC 9607 is a lactone which cannot form conjugates, it was concluded that the exocon has to be metabolite NC 20645 which was immediately transformed to NC 9607 by an intramolecular condensation step in the presence of the acid.

The TRR values and the distribution of the radioactivity are shown in the following table:

**Table 7.2.1-69 Distribution of radioactivity in the samples of onion (part 1)**

Sampling day (day after treatm.)	22		30		40		50		60		70	
TRR	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	1.34	100.0	1.55	100.0	2.96	100.0	3.18	100.0	1.06	100.0	0.96
Ethofumesate	41.2	0.55	39.9	0.62	42.3	1.25	59.4	1.89	55.7	0.59	39.8	0.38
NC 20645	25.5	0.34	19.3	0.30	46.0	1.36	18.6	0.59	20.8	0.22	33.5	0.32
NC 20645, conj.*	5.2	0.07	12.9	0.20	8.5	0.25	17.0	0.54	18.9	0.20	23.0	0.22
unknown	25.5	0.34	24.5	0.38	-	-	-	-	-	-	-	-
Total identified	71.9	0.96	72.1	1.12	96.8	2.86	95.0	3.02	95.3	1.01	96.3	0.92
Total characterised	25.5	0.34	24.5	0.38	-	-	-	-	-	-	-	-
Solids	2.6	0.04	3.4	0.05	3.2	0.10	5.0	0.16	4.7	0.05	3.7	0.04
Accountability	100.0	1.34	100.0	1.55	100.0	2.96	100.0	3.18	100.0	1.06	100.0	0.96

- not detected

\* identified as NC 9607 after acidic hydrolysis

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

**Table 7.2.1-70(cont'd): Distribution of radioactivity in the samples of onion (part 2)**

Sampling day (day after treatm.)	80		90**		100**		110**		120**		162**	
TRR	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	0.89	100.0	0.09	100.0	0.08	100.0	0.07	100.0	0.07	100.0	0.025
Ethofumesate	11.2	0.10										
NC 20645	12.4	0.11										
NC 20645, conj.*	69.7	0.62										
unknown	-	-										
Total identified	93.3	0.83										
Total characterised	-	-	88.9	0.08	87.5	0.07	85.7	0.06	89.6	0.06	80.0	0.020
Solids	6.7	0.06	11.1	0.01	12.5	0.01	14.3	0.01	10.4	0.01	20.0	0.005
Accountability	100.0	0.89	100.0	0.09	100.0	0.08	100.0	0.07	100.0	0.07	100.0	0.025

- not detected

\* identified as NC 9607 after acidic hydrolysis

\*\* samples were extracted, but insufficient activity was present for identification

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

**B.7.2.1.4. Tobacco****B.7.2.1.4.1. Tobacco metabolism 8 (supportive information)***This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I.*

Report:	KCA 6.2.1 /02 Warner, P. A.; Adcock, J. W.; 1977 M-155240-01
Title:	METABOLISM OF ETHOFUMESATE IN TOBACCO
Report No:	A82963
Document No:	M-155240-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

**I. Summary**

The metabolism of [phenyl-UL-<sup>14</sup>C]-ethofumesate in tobacco was investigated following soil application and foliar application. The soil application, as well as the foliar application (application to the leaves of the plants using a syringe), was done at BBCH 14-16 (4-6 leaf stage) at an application rate of 2 kg a.s./ha. The studies were conducted in the greenhouse.

Whole plants without roots were sampled 7, 15, 30, 60, 90 and 120 days after the application.

The plants were chopped and homogenised in methanol, the fibre was filtered off and the process repeated. The radioactivity in the extracts was determined by LSC and in the non-extracted residues by combustion and LSC. Concentrated extracts were analysed by TLC. The position and quantity of the radiolabelled components was determined using a radio-scanner. Identification of the radiolabelled components was done by comparison with unlabelled standards applied to the TLC plates. Acidic hydrolysis of polar material was carried out by removing the area from the TLC plate and boiling in 6 M hydrochloric acid for 1 hour. The resulting solution was partitioned by liquid-liquid extraction with ethyl acetate and the extracts were analysed by LSC and TLC. Prior to TLC analysis some plant extracts were purified by column chromatography.

The TRR for the soil treated plants was found highest after 7 days and decreased over time to 1.2 mg/kg at day 120. In the foliar treated plants the residue levels were consistently lower than in the soil treated plants and were highest after 15 days (5.6 mg/kg) and then decreased with time to 0.82 mg/kg at day 120.

Ethofumesate was extensively metabolised in tobacco following uptake from the soil and absorption from leaf surfaces. The metabolic pathway was consistent between the two application techniques with conjugated NC 20645 (0.28-1.87 mg/kg) and conjugated NC 8493 (0.05-1.05 mg/a.s. equiv./kg) being the major components 7 days after application and afterwards. Non-conjugated NC 20645 (ring opened NC 9607) was detected only at one interim sampling point (15 days) in each study with an amount of 0.2-0.4 mg/a.s. equiv./kg. Parent ethofumesate was detected in the extracts of the samples collected up to 90 days, however in decreasing percentages.

The exocons of the conjugates were identified after acidic hydrolysis with 6 M HCl. Under the acidic conditions, the exocon NC 20645 was immediately transformed to NC 9607 by an intramolecular condensation step. Exocon NC 8493 was stable under the acidic conditions.

The TRR values and the distribution of the radioactivity are shown in the following table:

**Table 7.2.1-71 Distribution of radioactivity in the extracts of tobacco after soil application**

Sampling event (day)	7		15		30		60		90		120	
TRR	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	10.3	100.0	4.6	100.0	3.8	100.0	1.94	100.0	2.79	100.0	1.20
Ethofumesate*	58.3	6.0	45.3	2.1	39.7	1.5	22.2	0.43	4.7	0.13	-	-
NC 20645, conj. **	11.7	1.2	30.2	1.4	27.8	1.05	14.4	0.28	67.0	1.87	83.3	1.00
NC 8493, conj. **	-	-	10.8	0.5	21.2	0.8	54.1	1.05	14.3	0.40	4.2	0.05
NC 20645***	-	-	4.3	0.2	-	-	-	-	-	-	-	-
unknown	30.1	3.1	2.2	0.1	-	-	-	-	-	-	-	-
Total identified	69.9	7.2	90.5	4.2	88.7	3.4	90.7	1.76	86.0	2.40	87.5	1.05
Total uncharacterised	30.1	3.1	2.2	0.1	6.6	0.25	-	-	6.5	0.18	-	-
Solids	-	-	7.3	0.3	4.8	0.18	9.3	0.18	7.5	0.21	12.5	0.15
Accountability	100.0	10.3	100.0	4.6	100.0	3.8	100.0	1.94	100.0	2.79	100.0	1.20

- not detected

\* identified by TLC (toluene-ethyl acetate 4/1; v/v)

\*\* identified as exocon after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)

\*\*\* identified after treatment with cold 2 M HCl for 1 hour and following extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

**Table 7.2.1-72 Distribution of radioactivity in the extracts of tobacco after foliar application**

Sampling event (day)	7		15		30		60		90		120	
TRR	%	mg/k g	%	mg/k g	%	mg/k g	%	mg/k g	%	mg/k g	%	mg/k g
	<b>100.0</b>	<b>5.1</b>	<b>100.0</b>	<b>5.6</b>	<b>100.0</b>	<b>2.92</b>	<b>100.0</b>	<b>0.94</b>	<b>100.0</b>	<b>0.90</b>	<b>100.0</b>	<b>0.82</b>
Ethofumesate*	72.5	3.7	53.9	3.0	36.0	1.05	31.9	0.30	-	-	-	-
NC 20645, conj.**	9.8	0.5	12.6	0.7	34.2	1.00	40.4	0.38	52.2	0.47	43.9	0.36
NC 8493, conj.**	13.7	0.7	18.0	1.0	17.8	0.52	20.2	0.19	22.2	0.20	8.5	0.07
NC 20645***	-	-	7.2	0.4	-	-	-	-	-	-	-	-
unknown	3.9	0.2	7.2	0.4	7.9	0.23	-	-	14.4	0.13	35.4	0.29
Total identified	96.1	4.9	91.6	5.1	88.0	2.57	92.6	0.87	74.4	0.67	52.4	0.43
Uncharacterised	3.9	0.2	7.2	0.4	7.9	0.23	-	-	14.4	0.13	35.4	0.29
Solids	-	-	1.3	0.1	4.1	0.12	7.4	0.07	11.1	0.10	12.2	0.10
Accountability	100.0	5.1	100.0	5.6	100.0	2.9	100.0	0.94	100.0	0.90	100.0	0.82

- not detected

\* identified by TLC (toluene/ethyl acetate 4/1; v/v)

\*\* identified as exocon after acidic hydrolysis (6 M HCl, under reflux 1 hour) and following extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)

\*\*\* identified after treatment with cold HCl (2 M) for 1 hour and extraction with ethyl acetate by TLC (toluene/ethyl acetate 4/1; v/v)

Remark: recalculation of radioactive residue in % of the TRR was done based on residues results in mg/kg.

#### B.7.2.1.5. Summary of the metabolism of ethofumesate in plants

Metabolism of ethofumesate was investigated in root and tuber vegetables (sugar beet and onions), cereals (ryegrass) and leafy crops (tobacco) following application of [<sup>14</sup>C-benzene]- or [<sup>14</sup>C-methylsulfonyl]-ethofumesate. Application was conducted either as a pre-emergent or a post-emergent spray. The application rate in the sugar beet studies ranged between approx. 1.3-2.0 kg a.s./ha and between 2.0 and 2.1 kg a.s./ha in the other crops. Comparison of pre- and post-emergent treatment revealed that ethofumesate is taken-up via roots and leaves. The metabolism in the plants is independent from the route of uptake.

The most recent studies on sugar beet (Chapleo, S.; 1992; M-155247-01, Caley, C. Y.; Chapleo, S.; Haswell, A.; 1994; M-161455-01 and Hennecke, D., 2003, GAB-002/7-08) and on ryegrass (Chapleo, S.; 1992; M-155248-01) show a conclusive picture on the metabolic behaviour of ethofumesate. Two additional studies on sugar beet and the studies on onion and tobacco were conducted before the implementation of GLP certificates and are therefore considered as supportive data only. However, these studies are in very good agreement with the GLP studies and confirm the results of these studies. Nevertheless, the metabolism study which should cover the cereal group was conducted on ryegrass and therefore no information on cereal grains is available from this study.

The major metabolic pathway for ethofumesate in plants was identified as follows:



- cleavage of the ethoxy side chain, with hydroxylation at the 2 position to give metabolite NC 8493 (ethofumesate-2-hydroxy = 2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methanesulfonate).
- This metabolite can either undergo conjugation to give polar metabolites or oxidation to the lactone NC 9607 (ethofumesate-lactone = 2,3-dihydro-3,3-dimethyl-2-oxobenzofuran-5-yl methanesulfonate).
- The lactone ring opens to the carboxylic acid NC 20645 (ethofumesate-carboxylic acid = 2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methyl propionic acid) which can also undergo conjugation to give polar metabolites.

The lactone NC 9607 and the carboxy analogue NC 20645 are inter-convertible and depending on the ambient conditions, either one or the other metabolite will predominate. Under acidic conditions, metabolite NC 20645 is converted to metabolite NC 9607 by an intramolecular ring closure.

Cleavage of the molecule under release of methanesulphonic acid was excluded in two sugar beet studies using two different chromatographic systems (TLC and TLE). The extraction and analysis processes were validated beforehand with a radiolabelled reference compound ( $^{14}\text{C}$ -MSA). Recoveries of MSA were above 97% and confirmed that the compound would be detected, if present.

According to the GLP studies, the extractability of radioactive residues was high in sugar beet shoots and ryegrass; surface wash and conventional solvent extraction released between 86.4-99.9% of the total radioactive residue. If an additional exhaustive extraction step with hydrochloric acid was applied, the extraction efficiency was always >90%. Exhaustive extraction released generally the known moieties NC 8493 and NC 9607. The extractability in roots was generally lower due to the high sugar and starch content of the tissue. Nevertheless, in the most recent sugar beet study (Caley, C. Y.; Chapleo, S.; Haswell, A.; 1994; M-161455-01), 75.8-93.7% of the radioactivity was released by conventional solvent extraction.

Identification rates were always high in samples collected early after the treatment with parent ethofumesate being the predominant residue. However, ethofumesate metabolised rather quickly and an increasing amount of polar components (a polar fraction that was eluted with the solvent front in reversed phase chromatographic systems or was not resolved from the origin in normal phase TLC) was detected with time, besides metabolites NC 8493 and NC 20645. Minor amounts of metabolite NC 9607 were also detected in several samples. The amount of metabolite NC 8493 decreased with time, indicating a further degradation/conjugation. Vigorous acidic treatment (3 M or 6 M HCl) of the extracts or the polar fraction itself showed that the polar compounds were acid-labile and were transformed to discrete known moieties (NC 8493 and NC 9607). Thus, the polar fraction was assigned to conjugates of the metabolites NC 20645 (the carboxy analogue to metabolite NC 9607) and NC 8493. Under the acidic conditions of the hydrolysis, metabolite NC 20645 is immediately converted to metabolite NC 9607.

With the help of the acidic treatment, the main proportion of the total radioactivity was assigned to known compounds. The following table shows the distribution of the radioactivity in the mature crops sugar beet and ryegrass:

**Table 7.2.1-73 Distribution of the radioactivity in mature *sugar beet shoots* and *ryegrass foliage* expressed as % total activity recovered**

Sampling event	1x, maturity (replicate B)		5x, maturity		1x, maturity (replicate B)	
	KCA 6.2.1 /05		KCA 6.2.1 /06		KCA 6.2.1 /07	
	sugar beet (leaves)		sugar beet (leaves)		ryegrass (foliage)	
TRR	%	mg/kg	%	mg/kg	%	mg/kg
	100.0	0.360	100.0	6.600	100.0	1.090
Ethofumesate (a.s.)	0.6	0.002	18.8	1.238	17.6	0.192
Ethofumesate fibre bound	0.2	0.001	-	-	-	-
NC 8493	0.7	0.003	0.9	0.062	4.6	0.050
NC 8493 conjugated	3.5	0.013	10.9	0.718	5.4	0.058
NC 8493 fibre bound	0.1	0.000	1.3	0.087	1.2	0.013
NC 9607	0.3	0.001	-	-	5.2	0.058
NC 9607 fibre bound <sup>#</sup>	0.2	0.001	0.9	0.062	0.4	0.005
NC 20645	0.6	0.002	-	-	6.8	0.074
NC 20645 conjugated <sup>##</sup>	71.2	0.256	58.6	3.867	38.3	0.418
NC 20645 fibre bound	0.5	0.002	-	-	0.1	0.001
Total identified	77.9	0.280	91.4	6.033	79.6	0.868
Unknown compounds ( $\geq 5$ comp.)	3.8	0.014	-	-	7.7	0.084
Remainder	-	-	6.3	0.414	6.2	0.067
Not analysed	7.7	0.028	-	-	4.8	0.052
Total characterized	11.5	0.041	6.3	0.414	18.7	0.203
Solids (bound fibre residue)	10.6	0.038	2.3	0.153	1.7	0.019
Accountability	100.0	0.360	100.0	6.600	100.0	1.090

<sup>#</sup> identified after exhaustive extraction, precursor could be NC9607, NC20645 or a conjugate of NC20645

<sup>##</sup> identified as NC9607 after acidic hydrolysis, no differentiation possible if formed from conjugated or non-conjugated NC 20645

Very low residues were always detected in mature roots of sugar beets after application of 1.3-2.0 kg a.s./ha. The total radioactive residue ranged between <0.01-0.03 mg/kg at harvest when considering all studies conducted (non-GLP and GLP). Due to the low residue level at maturity, identification of single compounds in the final extracts was very difficult or not possible. However, identification of the residues was possible in a root sample of the overdose experiment collected 10 days after treatment. The nature of the residues in this sample was intensively examined - before and after hydrolysis of the extracts - and it was clearly shown that the residues in roots are identical with those of shoots. No exhaustive extraction of the bound residues was conducted, however on the basis of all other experiments; it can be assumed that additional amounts of the known metabolites NC 8493 and NC 9607 could be released.

Analysis of mature roots of the one overdose experiment revealed the presence of parent ethofumesate and high amounts of the polar fraction, indicating the presence of the polar conjugates of metabolites NC 20645 and NC 8493 besides the parent compound. Thus it was shown that the nature of the residue was identical for all crop matrices investigated.

**Table 7.2.1-74 Distribution of the radioactivity in mature *sugar beet roots*, sampled at maturity expressed as % total activity recovered**

Sampling event	5x, maturity, before hydrolysis			
Application rate	6.35 kg a.s./ha		7.5 kg a.s./ha	
	KCA 6.2.1 /05		KCA 6.2.1 /06	
TRR	%	mg/kg	%	mg/kg
	100.0	0.08	100.0	0.202
Conventional extraction				
ACN extract	34.2	0.027	46.4	0.094
Origin (polar fraction)	n.a.	n.a.	37.5	0.076
Ethofumesate	n.a.	n.a.	4.9	0.008
ACN/H <sub>2</sub> O extract	18.6	0.015	20.8	0.042
Origin (polar fraction)	n.a.	n.a.	16.4	0.033
Remainder	n.a.	n.a.	4.4	0.009
H <sub>2</sub> O extract	21.3	0.017	8.6	0.017
Total identified	-	-	4.9	0.010
Total characterized	74.1	0.059	70.9	0.143
Solids (bound fibre residue)	25.9	0.021	24.2	0.049
Accountability	100.0	0.080	100.0	0.202

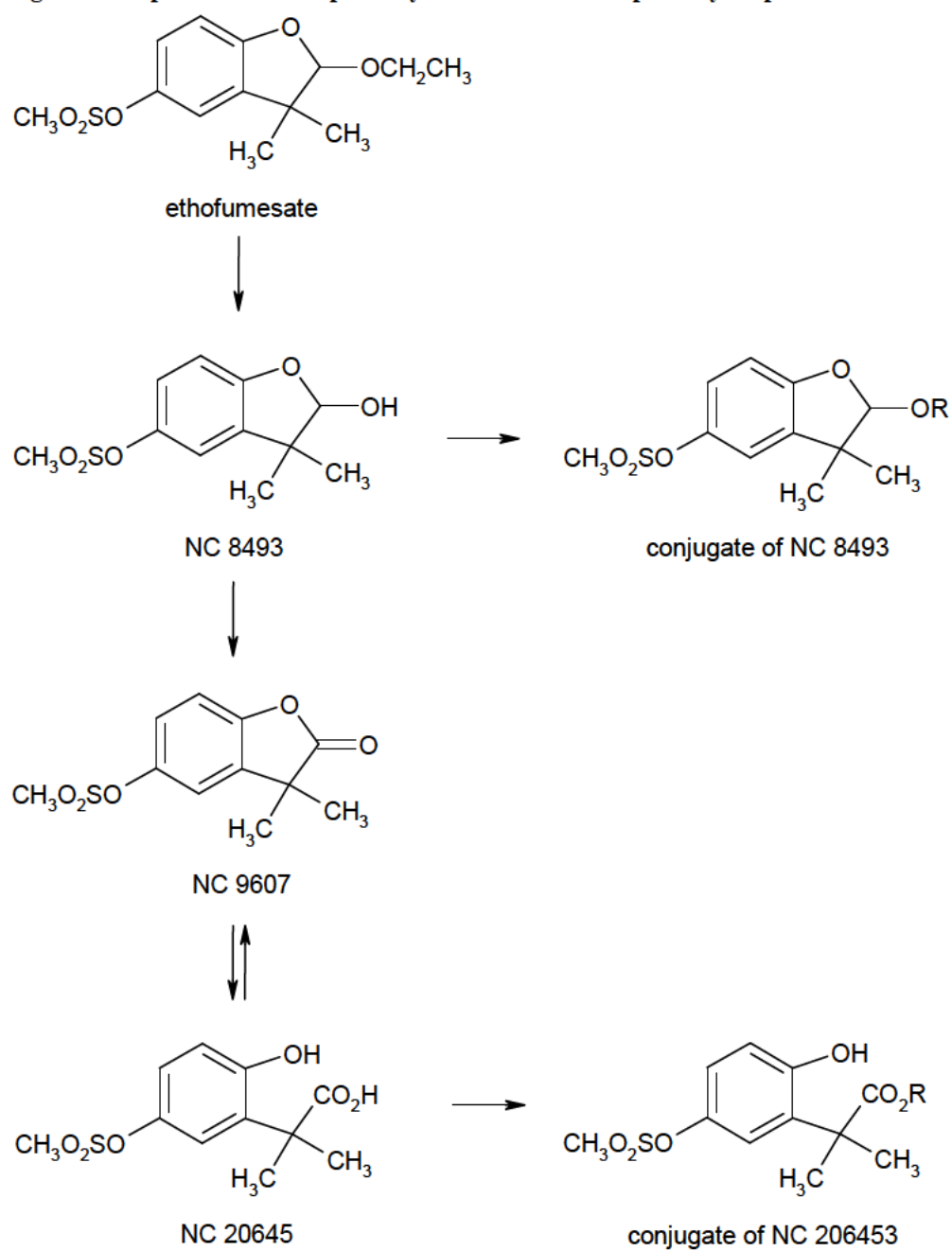
n.a. not analysed since residue level was too low for chromatographic analysis

Considering the residue levels detected in mature sugar beet roots at harvest (<0.01-0.03 mg/kg) it can be assumed that the residues in supervised field studies will be even lower when applying ethofumesate according to the intended GAP. Thus most probably, no ethofumesate related residues will be detectable in the roots, or if only known compounds will be present.

Overall, the plant metabolism studies showed that all crops under investigation followed the same metabolic path. Final residues were always dominated by a polar fraction. The polar fraction was acid labile and could be converted to the common moiety NC 9607 (major amount) and metabolite NC 8493 by vigorous treatment with hydrochloric acid. Since ethofumesate itself is also acid-labile under these vigorous conditions (decomposition to NC 8493 is possible), parent compound should be separated (e.g. by partitioning with dichloromethane) before the acidic treatment. Since major amounts of metabolite NC 8493 were only detected in intermediate growth stages it is not necessary to include this metabolite in the residue definition for mature crops, as well as its conjugate which was always a minor metabolite. Thus with parent compound ethofumesate and the common moiety NC 9607 form the relevant residue for ethofumesate in mature crops. Since the common moiety NC 9607 and NC 20645 are interconvertible, the pH value in the final extract determines which of the compounds the analytical target is.

In conclusion, plant metabolism was investigated in two crop groups have shown that there is extensive metabolism of ethofumesate in plants from both pre- and post-emergence uses. No significant quantities of uncharacterised or unidentified metabolites have been found. Although the route of metabolism of ethofumesate in all crops investigated is very similar it has to be pointed out that the definition of residue can be established for root crops only as the metabolism conducted on ryegrass gives no information on the metabolism in cereal grains. The metabolism of ethofumesate in plants is also very similar to that observed in livestock and rats. The proposed metabolic pathway in plants is given in Figure 1.

Figure 1 Proposed metabolic pathway of ethofumesate in primary crops



(R = unknown conjugate)

### B.7.2.2. Livestock

The metabolic fate of ethofumesate has also been investigated in livestock (lactating cows and laying hens), in addition to the rat. Available metabolism studies in livestock are presented in Table 7.2.2-1.

The metabolism studies in livestock showed that the metabolic pathway of ruminants and poultry are very similar. Ethofumesate should be considered as not fat soluble due to a log POW < 3.

**Table 7.2.2-1 Summary of metabolism studies in livestock presented in the first monograph prepared under Directive 91/414/EEC**

Group	Species	Label position	No of animals	Application and sampling details				Remarks
				Rate (mg/kg bw/day)	Duration (days)	Commodity	Time	
Laying poultry	Hens	[ <sup>14</sup> C-benzene]	6	0.6	14	Egg	Daily	
						Excreta	Daily	
						Tissue	After sacrifice	
			13	0.78	10	Egg	Twice daily	
						Excreta	Daily	
						Tissue	After sacrifice	
Lactating ruminants	Cow	[ <sup>14</sup> C-benzene]	1	0.3-0.36	7	Milk	Twice daily	
						Urine and faeces	Day-1, 1 and 7	
						Tissue	After sacrifice	
			1	5	4	Milk	Twice daily	
						Urine and faeces	Daily	
						Tissue	After sacrifice	
	Sheep	[ <sup>14</sup> C-benzene]	1	0.2	1	Milk	Not analysed	
						Urine and faeces	Daily	
						Tissue	After sacrifice (4 days after dosing)	

#### B.7.2.2.1. Poultry

Ethofumesate is authorized for use on beet crops. Since sugar beet tops may be fed to poultry two metabolism studies were conducted to investigate the fate of ethofumesate related residues in poultry matrices. These studies were submitted and evaluated during the Annex I inclusion process and were considered acceptable. Therefore, no additional data was considered necessary.

However to increase the readability and comprehensibility of the present dossier section, main data and results (on the basis of normalized recoveries) of the most recent (and most conclusive) study are summarized in the following paragraph. The previous hen study is only briefly summarized since it confirms the results of the more recent study.

**B.7.2.2.1.1. Metabolism in Poultry 1***Studies submitted and evaluated for the first inclusion of ethofumesate on Annex I:*

Report:	KCA 6.2.2 / [REDACTED] 1992; M-155246-01
Title:	THE METABOLISM OF <sup>14</sup> C ETHOFUMESATE IN LAYING HENS
Report No:	A82969
Document No(s):	Report includes Trial Nos.: SMS 297/920431 TOX 90542 M-155246-01-1
Guidelines:	USEPA (=EPA): Subdiv. O, 171-4; Deviation not specified
GLP/GEP:	yes

**I. Summary**

The metabolism and excretion of ethofumesate were investigated with [phenyl-UL-<sup>14</sup>C]-ethofumesate in laying hens as a model for poultry. Six hens were orally dosed once for 14 consecutive days at a rate of 1 mg a.s./bird/day which corresponded to a feeding level of approx. 9-10 mg a.s./kg dry feed/day. The animals were sacrificed within 24 hours after the last administration. Total radioactive residues (TRR) were determined daily in the eggs and excreta, and at sacrifice in the dissected organs and tissues (thigh and breast muscle, fat, liver, skin and the gastro-intestinal tract and contents). At sacrifice, mean concentrations of radioactivity in the edible tissues were always below the limit of detection (0.01 mg/kg) except for the liver, where a mean concentration of 0.03 mg/kg was detected. Therefore only liver and excreta were extracted and analysed for parent compound and metabolites.

**Recovery and Elimination of Radioactivity**

A mean total of 85.8% of the total dose was recovered in the excreta and the cage wash. The rate of excretion was rapid with a mean of 82.5% of the dose being eliminated during 0-24 hours after the first dose. At sacrifice, the mean radioactive residues in the organs and tissues were calculated to be less than 0.1% of the total dose. Residues in eggs were negligible and were also far below 0.1% of the dose.

**Total Radioactive Residues in Eggs, Organs and Tissues**

Concentrations of radioactivity in the pooled egg samples collected during the 14 day dosing period were always below 0.01 mg/kg (0.003 mg/kg or less). At day 8 a “plateau level” of 0.003 mg/kg was reached, however due to the low residue level it is rather difficult to define a clear residue peak (Table 7.2.2-2). Also the mean concentration of radioactivity in all dissected organs and tissues was always below 0.01 mg/kg, except for liver. Considering the fact that the real dietary exposure of hens is significantly lower than 10 mg ethofumesate/kg dry feed, a negligible transfer of ethofumesate related residues in edible matrices can be seen expected. The distribution of radioactivity in matrices of laying hen administered with an average daily dose of 1.0 mg <sup>14</sup>C-ethofumesate per day on fourteen consecutive days is presented in Table 7.2.2-2.

**Table 7.2.2-2 Time course of total radioactivity in eggs following oral administration of 14 daily doses of <sup>14</sup>C-ethofumesate at a dose rate of 1 mg/bird/day (mean of 6 birds)**

Time after the first admin. [d]	TRR (mg a.s. equivalents/kg)
1	<0.004
2	<0.003
3	<0.003
4	<0.003

Time after the first admin. [d]	TRR (mg a.s. equivalents/kg)
5	<0.003
6	<0.003
7	<0.003
8	0.003
9	0.003
10	0.003
11	<0.003
12	0.003
13	0.003
14	0.003

**Table 7.2.2-3 Residues in eggs, whole blood, muscle (thigh and breast), skin, fat, liver and gastro intestinal tract and gastro intestinal tract contents of laying hens following oral administration of 14 daily doses of <sup>14</sup>C-ethofumesate at a dose rate of 1 mg/bird/day**

	Radioactivity recovered (% of dose)							
	Replicate						Mean	SD
	1	2	3	4	5	6		
Egg (day 8)	-	-	-	-	-	-	0.003	-
Whole blood	0.0082	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	0.0039	-
Thigh muscle	<0.0101	<0.0099	<0.0094	<0.0096	<0.0099	<0.0098	<0.0098	-
Breast muscle	<0.0093	<0.0089	<0.0094	<0.0091	<0.0094	<0.0093	<0.0092	-
Skin	<0.0087	<0.0094	<0.0081	<0.0090	<0.0091	<0.0084	<0.0088	-
Fat	<0.0088	<0.0085	<0.0090	<0.0087	<0.0083	<0.0085	<0.0086	-
Liver	0.0300	0.0393	0.0225	0.0337	0.0261	0.0167	0.0281	0.0081
Gastro intestinal tract (GIT)	0.0616	0.0201	0.0411	0.0480	0.0285	0.0152	0.0358	0.0177
GIT contents	0.3638	0.1203	0.2003	0.1121	0.1111	0.0540	0.1603	0.1101

In the organs and tissues, the highest radioactivity concentration was determined in liver (0.0281 mg/kg). Only the gastro intestinal tract showed higher radioactivity concentrations indicating that the last dose was not completely absorbed and distributed.

### Metabolism

Since liver was the only organ that showed radioactive residues, extraction and characterization of residues was performed. Liver was subjected to an enzymatic treatment before extraction with ethanol. A total of 86.9% of the liver radioactivity was extracted from protease and  $\beta$ -glucuronidase-treated liver samples, which was associated with polar compounds as shown by TLC analysis. For further characterization of the residue, the sample was boiled in 5 M HCl for 2 h under reflux. The subsequent ethyl acetate extraction released approximately 64.6% (0.018 mg a.s. equivalents/kg) of the liver radioactivity. Approximately half of this radioactivity was associated with the metabolites NC 8493 and NC 9607, as shown by TLC and confirmed by HPLC.

Extraction and analysis of the pooled excreta samples showed the presence of ethofumesate, NC 20645, NC 9607 and NC 8493. Metabolite NC 20645 was by far the main constituent and represented approx. 60% of the extracted radioactivity.

**B.7.2.2.1.2. Metabolism in Poultry 2**

*This study was submitted and evaluated for the first inclusion of ethofumesate on Annex I:*

Report:	KCA 6.2.2 /02 [REDACTED] 1999;M-185380-01
Title:	Poultry - Metabolism, Distribution and nature of the residues in eggs and edible tissues Code AE B049913
Report No:	C002998
Document No(s):	Report includes Trial Nos.: Tox97227 M-185380-01-1
Guidelines:	EU (=EEC): 01/414/EEC; USEPA (=EPA): OPPTS 860.1300; Deviation not specified
GLP/GEP:	yes

**I. Summary**

The metabolism and excretion of  $^{14}\text{C}$ -ethofumesate was investigated in laying hens as a model for poultry. Three laying hens were orally dosed with [phenyl-UL- $^{14}\text{C}$ ]-ethofumesate of radiochemical purity >98%. The active substance was administered orally in a gelatine capsule to the hens for 10 consecutive days. The dose, 1.5 mg a.s./bird/day (approx. 0.8 mg/kg bw), was equivalent to approximately 11 to 12 mg/kg in the diet (11.4 mg/kg dry feed), approximately 19x the maximum predicted daily exposure (0.6 mg/kg dry feed, cf. CA 6.4), in accordance with current international regulatory guidelines.

Excreta, cage washings and eggs were collected at approximately 24 hours after the initial dose and eggs were collected twice daily. Eggs were divided into yolks and whites.

Approximately 6 hours after administration of the final dose, the hens were sacrificed. At necropsy, liver, abdominal and subcutaneous fat, skin, skeletal muscle, undeveloped eggs and gastro-intestinal tract were removed for determination of the distribution and magnitude of  $^{14}\text{C}$ -ethofumesate residues.

Identification of the metabolite residues was carried out in eggs and edible tissues containing residues  $\geq 0.01$  mg/kg, namely in egg yolk, abdominal fat, skin and liver. In addition, a subsample of muscle was investigated, although its residue level was lower than the trigger value of 0.01 mg/kg.

The radioactive content of the samples was analysed by liquid scintillation counting. Metabolites were characterised by HPLC and/or TLC.

In egg yolk residues reached a plateau by day 8 of dosing at a concentration of approx. 0.019 mg/kg. The residue level in egg whites was an order of magnitude lower, with a maximum concentration of 0.002 mg/kg seen on day 5 of dosing. The highest residue levels in edible tissues were found in the liver (0.095 mg/kg), while lowest residues were found in muscle (0.007 mg/kg).

Ethofumesate was present in all tissues and was the major residue identified in egg yolk, fat and skin. NC 20645 (the carboxy analogue of NC 9607) was the major residue identified in muscle and liver and was also present in the skin and egg yolk. NC 9607 (the lactone) was present in all tissues. The hydroxy derivative NC 8493 was present at low levels in the muscle only. Some unidentified metabolites were also detected at low levels.

The metabolism of ethofumesate in the hen occurs by hydrolysis of the ether bond to form the hydroxy metabolite NC 8493, which is then oxidized to the lactone derivative NC 9607 which can form the carboxy analogue NC 20645 due to hydrolytic cleavage of the lactone ring.



## II. Materials and Methods

### A. Materials

#### 1. Test Material

IUPAC Name	2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate
Code name	AE B049913
Common name	Ethofumesate
Empirical formula	C <sub>13</sub> H <sub>18</sub> O <sub>5</sub> S
Molar mass	286.3 g/mol
Labelling position	<sup>14</sup> C phenyl ring
Specific radioactivity	7.84 MBq/mg = 212 µCi/mg
Radiochemical purity	98.93% by radio-HPLC
Radioactive test substance	Batch 901B-1
Dose level	10 oral doses of 1.50 mg/bird/day = 12 mg/kg feed intake/day*
Vehicle	Radiolabelled ethofumesate dissolved in acetone, applied directly onto ground feed contained in a gelatine capsule

\*mean feed intake: 127.8 + 17.1 g feed/bird/day; mean bird weight: 1796 g

#### 2. Test Animals

Species	Laying hen ( <i>Gallus gallus domesticus</i> )
Strain	“Isa Brown”
Breeding facility	Hilside Poultry Farm, Newmarket Road, Royston
Sex and numbers involved	3 out of 6 hens (based on the egg laying record)
Age	18 weeks
Body weight	1.5-2 kg
Identification	Study number from DEBRA database
Housing	Individually in stainless steel wire mesh metabolism (room temperature 20 + 3°C, automatically illuminated with a 17 h photoperiod of fluorescent lighting; relative humidity 50-56%.)
Feed and water	The hens were allowed water ad libitum and the daily intake of feed was measured. The diet was standard layers ration obtained from Special Diet Services, Witham, Essex.

### B. Study Design

#### Dosing

Radiolabelled ethofumesate was dissolved in a small volume of acetone to give a concentration of 11.2 mg/mL (2.37 mCi/mL). Appropriate amounts of the dosing solution were applied directly onto ground feed contained in a gelatine capsule that absorbed the dosing solution. The capsules were prepared, sealed and used daily without storage.

Hens were dosed orally with one capsule per day for 10 consecutive days.

#### Sampling of eggs and excreta

Following administration of the first dose, excreta and eggs were collected approximately 24 hours post-initial dose and thereafter the eggs only were collected twice daily at the same 24-hour intervals for the duration of the study. The eggs were divided into yolks and whites.

Cages were rinsed with distilled water at the time of excreta collection and these washings were combined with excreta.

#### Sacrifice and sampling of organs and tissues

Approximately six hours after administration of the final dose the hens were killed by neck extension and dislocation of the spinal cord. The following tissues were collected and sampled: skin, skeletal muscle from the breast and thigh, liver, abdominal fat, any unlaidd developing eggs (from ovary and oviduct) and the gastro-

intestinal tract. Unless analysed immediately, all samples were stored under deep-frozen conditions until analysis.

### Sample preparation

Preparation for measurement of the total radioactive residue

Aliquots of sample materials of egg yolk, unlaidd developing eggs, skin, fat, muscle, liver and excreta (including cage washings) were diluted with water and solubilised with SHT (mixture of 10 N sodium hydroxide (200 mL), Triton X-450 (100 mL), methanol (300 mL) and water (400 mL)). Sample material of egg white was directly solubilised in SHT. After solubilisation the samples were acidified with acetic acid prior to addition of scintillate.

The gastro-intestinal tract was solubilised in 10 N NaOH, following homogenisation. Aliquots were acidified with acetic acid a prior to addition of scintillant.

Radioactivity was measured by liquid scintillation counting with automatic external standard quench correction.

The limit of quantification (LOQ) was for all matrices within <0.0001 mg/kg (egg yolk, white and undeveloped) and 0.0013 mg/kg (liver).

All samples handled for determination of radioactive content were initially completed within 4 weeks of dose termination. All samples were stored under deep-frozen conditions until analysis.

### Metabolite analysis

Where a total radioactive residue was 0.01 mg/kg or less, no analysis was required. For residues above the trigger value organic extraction was performed and each extract quantified. Where the extract was below 0.01 mg/kg no identification was required, between 0.01 and 0.05 mg/kg, the extract was characterised. The nature of each of the residues present in the extract at levels higher than 0.05 mg/kg were identified.

The different extracts were (if necessary) concentrated and cleaned-up. Qualitative analysis was done by TLC in two different solvent systems. Identification and quantification was done by HPLC using a UV (224 nm) and a radioisotope detector. The chromatographic system consisted of a reversed phase column (C18) and the eluting solvents water (containing 0.1% formic acid) and acetonitrile in the gradient mode. Metabolite assignment was done by comparison with non-radiolabelled reference compounds.

## III. Results and Discussion

### A. Recovery and Elimination of Radioactivity

The distribution of radioactivity in laying hens after administration of an average daily dose of 1.5 mg <sup>14</sup>C-ethofumesate per bird/day on 10 consecutive days is presented in Table 7.2.2-4.

The excreta collected following the first dose only was combined with cage washings and quantified as one.

**Table 7.2.2-4 Residues in eggs, muscle, skin, fat, liver and in the gastro intestinal tract of laying hens following oral administration of 10 daily doses of <sup>14</sup>C-ethofumesate at a dose rate of 1.5 mg/bird/day (mean of 3 replicates), equivalent to 11.4 mg/kg dry feed**

Sample	TRR [mg/kg]	Transfer of total residues
Egg yolk (steady state: day 8)	0.019 + 0.001	0.002
Egg white (steady state: day 6)	0.002 + 0.002	<0.001
Undeveloped eggs (eggs from ovary/oviduct)	0.024 + 0.003	0.002

Sample	TRR [mg/kg]	Transfer of total residues
Muscle	0.007 + 0.005	<0.001
Skin	0.020 + 0.006	0.002
Fat, abdominal	0.019 + 0.003	0.002
Fat, subcutaneous	0.016 + 0.003	0.001
Liver	0.095 + 0.034	0.008
Gastro intestinal tract	0.362 + 0.153	0.032

### B. Levels and Time Course of Total Radioactive Residues in Eggs

In egg yolk and white, residue levels of ethofumesate were detectable within 24 hours after the initial dose administration, with residue levels in egg yolk continuing to rise to reach a plateau by day 8 of dosing at a concentration of 0.019 + 0.01 mg/kg. The residue level in egg white was an order of magnitude lower, with a maximum concentration of 0.002 mg/kg seen on day 5 of dosing.

In undeveloped eggs, the mean concentration of ethofumesate-derived residue was 0.024 + 0.03 mg/kg, which reflected the residue levels of eggs yolk.

**Table 7.2.2-5 Time course of total radioactivity in egg yolk following oral administration of 10 daily doses of  $^{14}\text{C}$ -ethofumesate at a dose rate of 1.50 mg/bird/day**

Time after the first admin. [d]	Admin. no.	TRR (mg a.s. equiv./kg)				
		001 F	002F	003F	Mean	SD
1	1	<0.001	0.001	<0.001	0.001	<0.001
2	2	0.002	N.S.	0.001	0.002	0.001
3	3	0.003	N.S.	0.003	0.003	<0.001
4	4	0.010	0.005	0.007	0.008	0.002
5	5	0.014	0.011	0.013	0.013	0.002
6	6	0.016	0.011	0.017	0.015	0.003
7	7	0.013	0.016	0.018	0.016	0.002
8	8	0.020	0.018	0.019	0.019	0.001
9	9	0.020	0.017	0.020	0.019	0.002
10	10	0.020	0.019	0.022	0.020	0.002

N.S. = no sample available; SD = standard deviation For the calculation of mean value and standard deviation non-rounded values were used.

**Table 7.2.2-6 Time course of total radioactivity in egg white following oral administration of 10 daily doses of  $^{14}\text{C}$ -ethofumesate at a dose rate of 1.50 mg/bird/day.**

Time after the first admin. [d]	Admin. no.	TRR (mg a.s. equiv./kg)				
		001 F	002F	003F	Mean	SD
1	1	0.001	0.001	0.001	0.001	<0.001
2	2	0.001	N.S.	0.001	0.001	<0.001
3	3	0.001	N.S.	0.002	0.001	0.001
4	4	0.001	0.002	0.001	0.001	0.001
5	5	0.001	0.001	0.003	0.002	0.001
6	6	0.001	0.001	0.004	0.002	0.002
7	7	0.002	0.002	0.001	0.002	<0.001
8	8	0.001	0.001	0.002	0.001	<0.001
9	9	0.002	0.002	0.001	0.002	<0.001
10	10	0.002	0.003	0.002	0.002	<0.001

N.S. = no sample available; SD = standard deviation For the calculation of mean value and standard deviation non-rounded values were used.

### C. Total Radioactive Residues in Dissected Organs and Tissues

The concentration of the total radioactivity in the dissected organs and tissues at sacrifice is summarized in Table 7.2.2-4. The highest residues of ethofumesate and its metabolites were found in the gastro-intestinal tract

(0.362 mg/kg), followed by residues in liver (0.095 mg/kg), reflecting the significance of these organ for excretion and metabolism.

The residue level of liver was followed in decreasing order by those determined in the skin (0.020 mg/kg), abdominal fat (0.019 mg/kg), subcutaneous fat (0.016 mg/kg) and muscle (0.007 mg/kg).

#### **D. Extraction Efficiency of Residues**

##### **Egg yolk, abdominal fat and skin:**

Homogenised pooled samples of egg yolk, abdominal fat and skin were extracted with hexane overnight at 37°C by stirring. The remaining solids following hexane extraction were re-suspended and extracted in acetonitrile. The remaining residues were extracted with methanol. For abdominal fat the residues in the solids were found below the trigger value of 0.01 mg/kg after conventional solvent extraction. In an attempt to further characterise the remaining residues in egg yolk and skin the corresponding solids were soxhlet extracted with methanol. For both matrices it was not necessary to further extract the resulting remainder since the total residues were found below the trigger value of 0.01 mg/kg.

The hexane extract was back extracted with acetonitrile. All acetonitrile extracts and all methanol extracts resulting from the extraction of the solids were combined and reduced in volume using a Turbopap.

##### **Muscle and liver:**

Homogenised pooled samples of muscle and liver were extracted three times with acetonitrile by stirring. After centrifugation the acetonitrile extracts were decanted and combined. The solids which remained after the acetonitrile extraction were re-suspended and extracted with methanol. In order to extract more activity residues were soxhlet extracted with methanol. For muscle the residues in the solids were found below the trigger value of 0.01 mg/kg and no further extractions were performed.

The soxhlet extract of liver was further incubated with protease to attempt to recover any protein-bound radioactive residue and followed by addition of ethanol. The remaining residues after protease incubation were incubated with Helix Pomatia Juice to hydrolyse any conjugates of radioactive residues and followed by addition of ethanol. The remaining solids were extracted with 0.1 M HCl overnight at room temperature, followed by an additional extraction under mild alkali conditions (0.1 M NaOH, overnight at room temperature). The solids after mild alkali extraction were not further extracted since the total residues were found at 0.012 mg/kg.

The combined acetonitrile extract was reduced in volume using a Turbopap and back-extracted with hexane. The acetonitrile extract was further reduced in volume.

#### **E. Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues**

In general, where a total radioactive residue was 0.01 mg/kg or less no analysis was performed. For residues above this trigger value, organic extraction was performed and each extract was quantified. Where the residue of the extract was between 0.01 and 0.05 mg/kg the extract was characterised. Attempts were made to identify the nature of each residue present in the extract at levels greater than 0.05 mg/kg.

The distribution of the parent compound and metabolites in eggs, organs and tissues is summarised in Table 7.2.2-7 to Table 7.2.2-11. Presented results were corrected for procedural losses during the solvent extractions to present worst-case residue conditions. Therefore, deviations to the data given in the report may occur.

**Metabolites in eggs**

Residues in egg white were <0.01 mg/kg and were therefore not further analysed.

In egg yolk the mean residue levels of ethofumesate and its metabolites were 0.019 +0.001 mg/kg at the plateau. Overall, 70.3% of the TRR was organo-extractable (0.013 mg/kg), with 40.3% of the TRR characterised and 30.0% identified. Parent compound ethofumesate was the major component of the radioactive residue, accounting for 13.9% of the TRR (0.003 mg/kg). NC 20645 (6.9% of TRR, 0.001 mg/kg), and NC 9607 (9.2% of TRR, 0.002 mg/kg) were also present, as well as polar material accounting for 10.2% of the TRR (0.002 mg/kg). The residue levels in eggs of the ovary and the oviduct accounted for 0.024+0.003 mg/kg reflecting the levels seen in egg yolk. No further analyses were performed.

**Metabolites in abdominal fat**

Mean residue levels of ethofumesate and its metabolites in abdominal fat were 0.019 mg/kg. Overall, 88.1% of all the residues in abdominal fat was organo-extractable (0.017 mg/kg), with 21.2% of the TRR characterised (0.004 mg/kg) and 66.9% (0.013 mg/kg) identified. Unchanged parent compound (53.3% of TRR, 0.010 mg/kg) accounted for the majority of the <sup>14</sup>C-residue. NC 9607 accounted for 13.6% of the TRR (0.003 mg/kg), with polar material (0.5%; <0.001 mg/kg) and an unknown metabolite also seen (3.2%; 0.001 mg/kg).

**Metabolites in liver**

The mean residue level of ethofumesate and its metabolites in liver was 0.095 mg/kg of tissue. Overall, 88.7% (0.084 mg/kg) of the residue in liver was extractable after conventional as well as soxhlet extraction with organic solvents followed by enzymatic digestion with protease and helix pomatia juice and additional exhaustive extraction with mild acid and alkali. The carboxy-derivative NC 20645 was the main compound identified in the conventional acetonitrile extracts and accounted for 24.9% (0.024 mg/kg). Parent compound, NC 9607, a polar fraction and an additional unknown accounted each for less than 3% of the TRR. Soxhlet extraction released additional amounts of ethofumesate, NC9607 and NC 20645. Minor amounts of NC 8493 were also assigned. Due to the low residue level, only a qualitative assignment of metabolites was done.

**Metabolites in skin**

The mean residue level of ethofumesate-derived residues in skin was 0.020 mg/kg. Overall, 87.45% of the residue in skin was organo-extractable (0.017 mg/kg), with 29.4% of TRR (0.006 mg/kg) characterized and 58.0% (0.012 mg/kg) identified. Ethofumesate accounted for the majority of the residue (42.8% of TRR, 0.009 mg/kg). Minor metabolites NC 20645 and NC 9607 and a polar compound each represented between 6.6-8.7% of the TRR (0.001-0.002 mg/kg). Two additional unknown metabolites accounted for 2.3 and 2.8% of the TRR (<0.001-0.001 mg/kg).

**Metabolites in muscle**

The mean residue level of ethofumesate related residues in muscle was 0.007 mg/kg. Although this was below the trigger value for residue characterisation, the residue was extracted and a final extract was analysed in order to obtain a metabolite profile of the residues in muscle. Overall, 89.2% of the residue was organo-extractable (0.006 mg/kg), with 21.5% of the TRR characterised (0.004 mg/kg) and 67.8% (0.005 mg/kg) identified. The carboxy derivative NC 20645 was the main compound identified and accounted for 55.0% of the TRR (0.004 mg/kg). Ethofumesate accounted for 6.4% of the TRR (<0.001ppm). Minor metabolites NC 8493 and

NC 9607 were also seen at 1.2% and 5.2% of the TRR (both <0.001 mg/kg). Unknown metabolites accounted in total for 10.2% of the TRR (0.001 mg/kg).

**Table 7.2.2-7 Radioactive residues of parent compound and metabolites in egg yolk at steady state (day 8; pooled sample)**

Extract	Egg yolk	
<b>TRR [mg/kg]</b>	<b>0.019</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Conventional extraction		
Hexane extract (A)*	2.55	<0.001
Hexane (E) (#)	0.03	<0.001
ACN (F)	2.52	<0.001
Solids (R1)	97.45	0.019
ACN(B)	42.71	0.008
Solids (R2)	54.74	0.010
ACN extraction		
ACN(B+F) (G)	45.23	0.009
ACN (H/J)	40.23	0.008
Ethofumesate	13.89	0.003
NC 20645	6.89	0.001
NC 9607	9.22	0.002
Unknown (polar)	10.24	0.002
Hexane (I) (#)	4.99	0.001
MeOH extraction of R2		
Solids (R2)	54.74	0.010
MeOH (C)*	14.09	0.003
Solids (R3)*	40.65	0.008
Soxhlet extraction of R3		
MeOH (D)	10.95	0.002
MeOH (C+D) (K)	25.04	0.005
MeOH (L)	22.45	0.004
Hexane (M)	2.59	<0.001
Solids (R4)	29.70	0.006
Total identified	30.00	0.006
Total characterised	40.30	0.008
Total analysed	62.69	0.012
Solids	29.70	0.006
Accountability	100.00	0.019

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to figure 13 (extraction procedure) of the original report.

**Table 7.2.2-8 Radioactive residues of parent compound and metabolites in abdominal fat (pooled sample) sampled six hours after final dosage**

Extract	Abdominal fat	
<b>TRR [mg/kg]</b>	<b>0.019</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Conventional extraction		
Extraction with hexane		
Hexane (A)*	70.80	0.013
Hexane 1(#)	2.03	0.000
ACN (D)	68.76	0.013
Solids (R1)*	29.20	0.006
Extraction of R1 with ACN		
ACN (B)	6.47	0.001
Solids (R2)	22.73	0.004
MeOH (C) (#)	10.87	0.002

Extract	Abdominal fat	
<b>TRR [mg/kg]</b>	<b>0.019</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Solids (R3)	11.86	0.002
ACN extraction		
ACN (D + B)	75.24	0.014
Hexane 2 (#)	0.62	<0.001
ACN (F/G)	74.62	0.014
Oily residue (I) (#)	4.03	0.001
ACN (H)	70.58	0.013
Ethofumesate	53.28	0.010
NC 9607	13.63	0.003
Unknown (polar)	0.47	<0.001
Unknown	3.20	0.001
Total identified	66.91	0.013
Total characterised	21.23	0.004
Total analysed	70.58	0.017
Solids	11.86	0.002
Accountability	100.00	0.019

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to figure 10 (extraction procedure) of the original report.

**Table 7.2.2-9 Radioactive residues of parent compound metabolites in liver (pooled sample) sampled six hours after final dosage**

Extract	Liver	
<b>TRR [mg/kg]</b>	<b>0.095</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Conventional extraction		
ACN combined (A)*	34.00	0.032
Turbovap trap 1(#)	0.36	<0.001
Hexane 1 (#)	0.55	0.001
ACN (H)	33.09	0.031
Hexane 2 (#)	0.55	0.001
ACN (I)	32.55	0.031
Turbovap trap 2(#)	0.03	<0.001
Hexane 3 (#)	0.07	<0.001
ACN (J/K/L)	32.44	0.031
Ethofumesate	1.24	0.001
NC 9607	2.84	0.003
NC 20645	24.87	0.024
Polar fraction	2.72	0.003
Unknown	0.77	0.001
Solids (R1)*	66.00	0.063
MeOH (B)	4.76	0.005
Solids (R2)	61.23	0.058
Soxhlet extraction of R2		
MeOH (C)	5.29	0.005
Solids (R3)	55.95	0.053
MeOH extraction		
MeOH combined (B+C)	10.05	0.010
Hexane 4(#)	0.17	<0.001
MeOH (M)	9.88	0.009
MeOH (N)	4.78	0.005
Hexane (O)(#)	5.10	0.005
Enzymatic incubation of R3		
EtOH (D)	12.67	0.012
Hexane 5 (#)	0.22	<0.001

Extract	Liver	
<b>TRR [mg/kg]</b>	<b>0.095</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Turbovap trap 3 (#)	0.02	<0.001
EtOH (P)	12.43	0.012
Aqueous (Q)	12.43	0.012
Aqueous (R)	12.35	0.012
Hexane 6(#)	0.08	<0.001
Solids (R4)	43.27	0.041
Enzymatic incubation of R4		
EtOH (E)	14.20	0.013
EtOH (S)	14.13	0.013
Hexane 7(#)	0.07	<0.001
Solids (R5)	29.07	0.028
Exhaustive extraction of R5 (mild acid)		
Mild acid extract (F)	13.06	0.012
Acid extract (T)	13.03	0.012
Turbovap trap 4 (#)	0.03	<0.001
Solids (R6)	16.01	0.015
Exhaustive extraction of R5 (mild alkali)		
Mild acid extract (G) (#)	4.71	0.004
Solids (R7) (#)	11.30	0.011
Total identified	28.95	0.028
Total characterised	59.74	0.057
Total analysed	76.73	0.073
Solids	11.30	0.011
Accountability	100.00	0.095

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to figure 3 (extraction procedure) of the original report.

**Table 7.2.2-10 Radioactive residues of parent compound and metabolites in skin (pooled sample) sampled six hours after final dosage**

Extract	Skin	
<b>TRR [mg/kg]</b>	<b>0.020</b>	
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>
Conventional extraction		
Hexane (A)*	44.44	0.009
Hexane (G) (#)	0.87	<0.001
ACN (F)	43.56	0.009
Solids (R1)	55.56	0.011
ACN (B)*	27.23	0.005
ACN extraction		
ACN combined (F+B) (H)	70.79	0.014
ACN (I) reduced	69.87	0.014
ACN (L) reduced 2	69.87	0.014
Ethofumesate	42.75	0.009
NC 9607	8.72	0.002
NC 20645	6.58	0.001
polar material	6.77	0.001
unknown 1	2.25	<0.001
unknown 2	2.80	0.001
Turbovap trap (J) (#)	0.07	<0.001
Hexane (K) (#)	0.85	<0.001
Solids (R2)	28.34	0.006
MeOH extraction		
MeOH (C)*	9.09	0.002
Solids (R3)*	19.25	0.004



Extract	Skin	
TRR [mg/kg]	0.020	
Compound (ethofumesate)	% TRR	mg/kg
MeOH soxhlet extraction of R3		
MeOH (D)	6.67	0.001
Solids (R4)	12.55	0.003
Hexane (E) (#)	0.03	<0.001
Combined MeOH (C + D) (M)	15.76	0.003
MeOH (N)	9.14	0.002
Hexane (O)	6.62	0.001
Total identified	58.04	0.012
Total characterised	29.41	0.006
Total analysed	79.01	0.016
Solids	12.55	0.003
Accountability	100.00	0.020

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to figure 17 (extraction procedure) of the original report.

**Table 7.2.2-11 Radioactive residues of parent compound and metabolites in muscle (pooled sample) sampled six hours after final dosage**

Extract	Muscle	
TRR [mg/kg]	0.007	
Compound (ethofumesate)	% TRR	mg/kg
Conventional extraction		
ACN (A)*	80.00	0.006
Hexane (E) (#)	0.22	<0.001
Turbopap trap	1.18	<0.001
ACN (D)	78.60	0.006
Hexane (F) (#)	0.34	0.000
ACN (G)	78.27	0.005
Hexane (#)	0.33	0.000
ACN (H/I)	77.94	0.005
Ethofumesate	6.35	<0.001
NC 9607	5.23	<0.001
NC 20645	54.98	0.004
NC 8493	1.20	<0.001
unknown	10.18	0.001
Solids (R2)	20.00	0.001
MeOH (B)*	3.79	<0.001
Solids (R3)*	16.21	0.001
Soxhlet extraction of R3		
MeOH (C) (#)	5.44	<0.001
Solids (R4)	10.76	0.001
Total identified	67.75	0.005
Total characterised	21.48	0.001
Total analysed	77.94	0.005
Solids	10.76	0.001
Accountability	100.00	0.007

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to figure 21 (extraction procedure) of the original report.

#### IV. Conclusion

In egg yolks and whites, residue levels of ethofumesate were detectable 24 hours after the initial dose administration, with residue levels in egg yolks continuing to rise to reach a plateau by day 8 of dosing at a concentration of  $0.019 \pm 0.001$  mg/kg. The residue level in egg whites was an order of magnitude lower, with a maximum concentration of 0.002 mg/kg seen on day 5 of dosing. In undeveloped eggs (eggs of ovary and oviduct), the mean concentration of ethofumesate-derived residue was  $0.024 \pm 0.003$  mg/kg.

Residue levels of ethofumesate and/or its metabolites in the edible tissues of the hen were low, with the highest concentration seen in the liver ( $0.095 \pm 0.034$  mg/kg). Residues in skin and abdominal fat were lower at  $0.020 \pm 0.006$  mg/kg and  $0.019 \pm 0.003$  mg/kg respectively. Subcutaneous fat levels were also low, at  $0.016 \pm 0.003$  mg/kg. Skeletal muscle levels were the lowest of the edible tissues at  $0.007 \pm 0.005$  mg/kg. Thus transfer of radioactivity into edible tissues is very low.

Following administration of the first dose of ethofumesate, elimination of the radioactivity was rapid with >80% of the recovered radioactivity excreted within twenty-four hours.

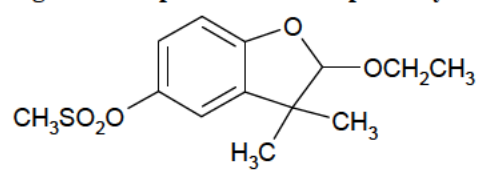
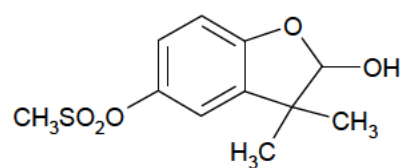
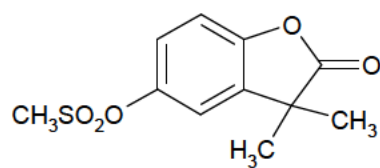
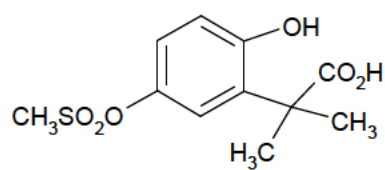
Ethofumesate was present in all tissues and was the major residue identified in egg yolk, fat and skin. NC 20645 (the carboxy analogue of NC 9607) was the major residue identified in the muscle and liver and was also present in the skin and egg yolk. NC 9607 (the lactone) was present in all tissues; the hydroxy derivative NC 8493 was present at low levels in the muscle. A polar fraction (probably containing conjugates of the known metabolites) and some unidentified metabolites were also determined at very low levels.

	Liver	Muscle	Abdominal Fat	Skin	Egg yolk
TRR [mg/kg]	0.095	0.007	0.019	0.020	0.019
% extracted	88.70	89.24	88.14	87.45	70.30
% analyzed	76.73	77.49	70.58	79.01	62.69
% identified	28.95	67.75	66.91	58.04	30.00
Ethofumesate	1.24	6.35	53.28	42.75	13.89
NC 8493	-	1.20	-	-	-
NC 20645	24.87	54.98	-	6.58	6.89
NC 9607	2.84	5.23	13.63	8.72	9.22
% characterized	59.74	7.64	21.23	29.41	40.30
% bound residues	11.30	23.02	11.86	12.55	4.73

Based on the metabolites identified in edible tissues, the following metabolic routes were deduced:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can undergo oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 can open to form the carboxy analogue NC 20645.

A proposed metabolic pathway is given in Figure 2.

**Figure 2 Proposed metabolic pathway for ethofumesate in poultry****Ethofumesate****NC 8493****NC 9607****NC 20645**

### B.7.2.2.2. Lactating ruminants

Ethofumesate is authorized for use on beet crops. Since beet roots and tops, as well as sugar beet by-products may be fed to ruminants, metabolism studies were conducted in sheep and lactating cow. These studies were submitted and evaluated during the Annex I inclusion process and were considered acceptable. Therefore, no additional data was considered necessary.

However to increase the readability and comprehensibility of the present dossier section, main data and results (on the basis of normalized recoveries) of the most recent cow study are summarized in the following paragraph. The first study in cow is only briefly summarized since it is in good agreement with the more recent study, but was conducted with a lower dose rate. The study in sheep was not further considered because the substance was only administered once to the animal.

The studies in cow showed that the metabolic pathway of ruminants and poultry are very similar. Ethofumesate should be considered to be not fat soluble due to a log POW < 3.

#### B.7.2.2.2.1. Metabolism in lactating ruminants (sheep) 1

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

Report:	KCA 6.2.3 /01 [REDACTED];1976;M-155235-01
Title:	THE METABOLISM OF <sup>14</sup> C-ETHOFUMESATE IN THE SHEEP
Report No:	A82958
Document No:	M-155235-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

The metabolism of <sup>14</sup>C-ethofumesate in the sheep has been studied by giving a 40 kg ewe a single 8.64 mg dose of <sup>14</sup>C-ethofumesate (0.2 mg/kg bw). The ewe excreted 70.7% of the radioactivity in the urine and 6.4% in the faeces within 24 hours. One major metabolite was found in urine and was characterized as the open ring form of NC 9607. This comprises >90% of the activity found in urine.

Small amounts of NC 9607 and NC 8493 were also excreted as conjugates. All tissue residue levels were below the LOD (0.01 ppm) at slaughter, 96 h after dosing.

Since the animal was dosed only once, the study does not fulfil the current requirements as outlined in the OECD Guideline 503 (Metabolism in Livestock, 2007), and will not be considered further.

#### B.7.2.2.2.2. Metabolism in lactating ruminants (cow) 2

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

Report:	KCA 6.2.3 /02 [REDACTED];1992;M-155245-01
Title:	THE METABOLISM OF <sup>14</sup> C-ETHOFUMESATE IN THE COW
Report No:	A82968
Document No(s):	Report includes Trial Nos.: SMS 296/920441 TOX 90541 M-155245-01-1
Guidelines:	USEPA (=EPA): Subdiv. O, 171-4; Deviation not specified
GLP/GEP:	yes

### I. Summary

The metabolism of ethofumesate was investigated in a 550-600 kg lactating Friesian cow fed with 200 mg [phenyl-UL-<sup>14</sup>C]-C-ethofumesate/day for seven consecutive days. The dose was equivalent to 13 mg/kg in the diet and was administered to the cow orally once daily in the morning, after milking. The cow was sacrificed 23 hours after the final dose and the following organs and tissues were retained for analysis: fat (subcutaneous, omental and perirenal), kidney, samples of muscle (foreleg and rump) and liver. Milk was collected each morning and afternoon during the study. Urine samples were collected pre-dosing (day -1) and on days one and seven following dosing. Blood samples were collected immediately prior to administration of the daily dose and prior to sacrifice and were separated into blood and plasma by centrifugation.

#### Recovery and Elimination of Radioactivity

Urine samples taken on day 1 and day 7 of the dose period suggested that urinary excretion was a major route for the excretion of administered radioactivity. The total radioactivity in the dissected organs and tissues represented less than 0.1% of the total dose administered. Residues in milk were negligible and were also far below 0.1% of the dose. Since neither urine nor faeces were collected quantitatively during the experimental phase, no radioactive balance can be established.

#### Total Radioactive Residues in Milk, Organs and Tissues

In organs and tissues, residues above 0.01 mg/kg were only detected in liver (0.027 mg/kg) and kidney (0.122 mg/kg). The distribution of the radioactive residues in the different matrices analysed are given in the table below.

**Table 7.2.2-12 Residues in milk, blood, plasma, fat (subcutaneous, omental and perirenal), kidney as well as muscle (leg and rump) and liver following oral administration once daily of <sup>14</sup>C-ethofumesate at a mean daily dose rate of 200 mg per day for seven consecutive days**

Sample	TRR (mg a.s. equivalents/L) or (mg a.s. equivalents/kg)	Transfer (mg/kg in tissue/ mg/kg in dry feed)
Milk (plateau level)	0.003	<0.001
Blood (plateau level)	0.009	<0.001
Plasma (plateau level)	0.013	0.001
Subcutaneous fat	<0.009	<0.001
Omental fat	0.010	<0.001
Perirenal fat	<0.010	<0.001
Kidney	0.122	0.009
Leg muscle	<0.003	<0.001
Rump muscle	<0.004	<0.001
Liver	0.027	0.002

The concentration of radioactivity was generally very low in milk (<0.01 mg/kg). The time course in the evening and morning milk pool samples showed a diurnal pattern. The radioactive residues increased slightly during the eight hour period after each administration followed by a decrease to a low level of about 0.001-0.002 mg/kg measured prior to the next dosing. The mean daily concentrations ranged between <0.002 mg/kg and 0.003 mg/kg. A stable plateau level of about 0.003 mg/kg was reached at approx. 96 hours after the first administration.

**Table 7.2.2-13 Time course of total radioactivity in milk following oral administration once daily of <sup>14</sup>C-ethofumesate at a daily dose rate of 200 mg per day for seven consecutive days**

Time after the first admin. [h]	TRR (mg a.s. equivalents/L)		
	Afternoon collection	Morning collection	Mean daily conc.
0-24	0.003	<0.002	<0.002
24-48	0.003	0.001	0.002
48-72	0.004	0.002	0.002
72-96	0.004	0.001	0.002
96-120	0.004	0.002	0.003
120-144	0.004	0.002	0.003
144-168	0.005	0.002	0.003

For blood and plasma highest residues of 0.009 and 0.013 mg a.s. equivalents/L, respectively were found in the samples. A plateau level was reached by day 2 (48 hours) after the initial dosing.

**Table 7.2.2-14 Time course of total radioactivity in blood and plasma following oral administration once daily of <sup>14</sup>C-ethofumesate at a mean daily dose rate of 200 mg per day for seven consecutive days**

Time (hours)	TRR in Blood (mg a.s. equivalents/L)	TRR in Plasma (mg a.s. equivalents/kg)
0*	<0.006	<0.005
24	<0.006	0.006
48	0.009	0.011
72	0.009	0.011
96	0.009	0.011
120	0.008	0.010
144	0.009	0.013
168	0.008	0.011

\*pre-dose on day of administration

### Metabolism

Since liver and kidney were the only organs showing radioactive residues above 0.01 mg/kg, extraction and characterization of residues was performed in these samples. The total radioactive residue level in liver was 0.027 mg/kg. Extraction with methanol released 72.2% of the TRR. Subsequent HPLC analysis showed that parent ethofumesate was the main compound, followed by metabolite NC 20645. Several minor metabolites were also detected, but could not be identified due to their low concentration in the extract.

The total radioactive residue level in kidneys was 0.122 mg/kg. The following characterization by ethyl acetate extraction showed that 90% of the extracted radioactivity was associated with the presence of NC 20645. Metabolite NC 20645 was also the predominant metabolite detected in urine. The distribution of the radioactivity in liver, kidney and urine is summarized in the following tables.

**Table 7.2.2-15 Radioactive residues of parent compound and metabolites in liver sampled 23 hours after final dosage**

Extract	Liver	
TRR [mg/kg]	0.027	
Compound (ethofumesate)	% of TRR	mg/kg
MeOH extraction		
Solids	27.8	0.008
MeOH	72.2	0.019
Ethofumesate	17.7	0.005
NC 20645	12.6	0.003
Unknown LC1	4.8	0.001
Unknown LC2	7.6	0.002
Background radioactivity (no discrete peaks)	6.8	0.002
Losses (not recovered after analysis)	22.6	0.006

Extract	Liver	
TRR [mg/kg]	0.027	
Compound (ethofumesate)	% of TRR	mg/kg
Total identified	30.3	0.008
Total characterised	41.8	0.011
Total analysed	72.2	0.019
Solids	27.8	0.008
Accountability	100.0	0.027

**Table 7.2.2-16 Radioactive residues of parent compound and metabolites in kidney sampled 23 hours after final dosage**

Extract	Kidney	
TRR [mg/kg]	0.122	
Compound (ethofumesate)	% of TRR	mg/kg
Ethyl acetate extraction		
Ethyl acetate	91.6	0.112
NC 20645	90.0	0.110
Unknown	1.6	0.002
Solids	8.4	0.010
Total identified	90.0	0.110
Total characterised	1.6	0.002
Total analysed	91.6	0.112
Solids	8.4	0.010
Accountability	100.00	0.122

**Table 7.2.2-17 Radioactive residues of parent compound and metabolites in urine sampled on day 1 and day 7**

Urine day 1		
TRR [mg/L]	20.5	
Compound (ethofumesate)	% of TRR	mg/L
NC 20645	96.9	19.9
Unknown	3.1	0.6
Urine day 7		
TRR [mg/L]	19.5	
Compound (ethofumesate)	% of TRR	mg/L
NC 20645	94.4	18.4
Unknown	5.6	1.1

#### B.7.2.2.2.3. Metabolism in lactating ruminants (cow) 3

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

Report:	KCA 6.2.3 /03 [REDACTED] 1999;M-185993-01
Title:	Metabolism, distribution and nature of the residues in milk and edible tissues Ethofumesate ruminant Code: AE B049913
Report No:	C003362
Document No(s):	Report includes Trial Nos.: TOX97226 M-185993-01-1
Guidelines:	EU (=EEC): 91/414/EEC; USEPA (=EPA): OPPTS 860. 1300; Deviation not specified
GLP/GEP:	no

### I. Summary

A lactating dairy cow was orally dosed twice daily for four consecutive days with [<sup>14</sup>C-benzene]-ethofumesate showing a radiopurity of >99% and a specific activity of 4.5 µCi/mg. The mean daily dose was 2.94 g (equivalent to 5.0 mg/kg body weight). This dose rate was equivalent to an exposure of 274 mg/kg in the diet.

Urine and faeces were collected daily and milk was collected twice daily. Samples of blood were taken at 0.5, 1, 2, 3, 4, 5, 6 and 8 hours post-initial dose and thereafter at each milking time. At sacrifice (96 hours after initial dose and approximately 16 hours after final dose) liver, kidney, heart, renal fat, subcutaneous fat, omental fat, muscle (psoas, loin and hindquarter), rumen and abomasal fluid, gastro-intestinal contents and bile were sampled and the radioactivity quantified. Identification of the metabolite residues was carried out in milk and edible tissues containing residues higher than 0.01 mg/kg, namely in liver, kidney, psoas muscle, omental fat, subcutaneous fat and renal fat. The metabolic profile of urine was also determined.

The radioactive content of the liquid samples and the extracts was determined by LCS. Extracts were analysed by TLC (normal phase) and/or HPLC (reversed phase) for metabolite identification. In addition, mass spectrometry identification was also obtained.

Following dosing of  $^{14}\text{C}$ -ethofumesate for four consecutive days, residues were detectable in all edible tissues between 0.033 and 1.863 mg/kg. The highest residue was detected in kidney as metabolizing organ, the lowest residue was detected in muscle. In most tissues the major component seen was unchanged parent compound followed by metabolites NC 20645, NC 8493 and NC 9607 which were detected in smaller quantities. In the kidney, the major metabolite seen was the highly water soluble NC 20645 which was readily excreted. Residue levels in milk reached a maximum of 0.134 mg/kg at 32 hours post-administration of the initial dose. The major compound identified was parent compound followed by metabolite NC 20645. Other metabolites identified were NC 8493 and NC 9607 each accounted for less than 10% of the residue.

In blood and plasma residues reached a maximum (0.477 and 0.602 mg/kg, respectively) 32 hours after the first dosing indicating a fast absorption and distribution. Excretion via urine was the major elimination pathway for ethofumesate. Transfer of radioactive residues in edible tissues and milk was low.

## II. Materials and Methods

### A. Materials

#### 1. Test Material

IUPAC Name	2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate
Code name	AE B049913
Common name	Ethofumesate
Empirical formula	$\text{C}_{13}\text{H}_{18}\text{O}_5\text{S}$
Molar mass	286.3 g/mol
Labelling	$^{14}\text{C}$ phenyl ring
Specific radioactivity used for administration	8.584 MBq/mg = 232 $\mu\text{Ci}$ /mg (delivered sample before radiodilution) 0.148 MBq/mg = 4.0 $\mu\text{Ci}$ /mg (sample after radiodilution)
Radiochemical purity	> 99 % (HPLC)
Dose level	twice daily, four consecutive days; 2.94 g/day = 5.0 mg/kg bw equivalent to 274 mg/kg in the diet
Vehicle	gelatine capsule

#### 2. Test Animal

Species	Bos taurus
Strain	British Friesian
Breeding facility	Dennis Clowes & Sons, Deans Farm, Gawsworth, Macclesfield, Cheshire, SK11 9QL
Sex and numbers involved	1 female animal
Body weight	588 kg
Identification	Ear tag LP75-25M
Housing	Metabolism crate with fluorescent lighting was used with a photoperiod appropriate to the season and to which the animal had become accustomed



	Temperature and relative humidity were measured throughout the study (temperature: 14.5-17°C; relative humidity: 35-59%)
Feed and water	The cow was fed ad libitum with hay. During the test period, the average feed consumption was 10.722 kg DM/day. Water was provided ad libitum.

## B. Study Design

### Dosing

A dairy cow was orally dosed twice daily for four consecutive days with  $^{14}\text{C}$ -ethofumesate, which showed a radiopurity of >99% and a specific activity of 4.5  $\mu\text{Ci}/\text{mg}$ .

The radiolabelled test compound was delivered with a specific radioactivity of 8.584 MBq/mg. It was diluted with the non-radiolabelled test compound to a specific radioactivity of 0.148 MBq/mg. Radio-diluted ethofumesate was weighed directly into gelatine capsules which were sealed and stored at 4°C until dosing. The purity and stability of the dose were determined using HPLC.

The cow was dosed orally twice daily for four consecutive days. The mean daily dose was 2.94 g (equivalent to 5.0 mg/kg bw/day). This dose rate is equivalent to an exposure of 274 mg/kg in the diet.

### Sampling urine and faeces, milk, blood and plasma during the in-life phase

Urine and faeces were collected at approx. 24 hours after the initial dose, and thereafter at approx. 24-hour intervals for four days.

Milk was collected at two milking times each day, the yields were recorded and aliquots taken for assay using fresh milk. Blood samples were taken at 0.5, 1, 2, 3, 4, 6 and 8 hours post initial dose and thereafter at each milking time point. Plasma was obtained by centrifugation. All samples were stored deep-frozen until analysis.

### Sacrifice and dissection of organs and tissues

Approx. 16 hours after administration of the final dose, the cow was stunned by captive bolt, pithed and exsanguinated. The following tissues were collected or sampled: Liver, kidneys, heart, lungs, muscle from three sites (psoas, hindquarter and loin), fat from three sites (renal, subcutaneous and omental), bile, rumen and abomasal fluid, and gastro-intestinal contents. All samples were stored deep-frozen until analysis.

### Sample preparation

Faeces: Faeces were stirred (approx. 10 min) followed by dilution with water and then homogenised. Aliquots were diluted again and solubilised with SHT (mixture of 10 N NaOH, Triton X-450, methanol and water). After solubilisation, the samples were acidified with glacial acetic acid prior to the addition of scintillant for determination of radioactivity by liquid scintillation counting.

Urine and bile: Aliquots of urine and bile were mixed with scintillation cocktail and the radioactivity determined by liquid scintillation counting.

Whole blood, rumen and abomasal fluid: Aliquots of whole blood, rumen and abomasal fluid were combusted in the Packard Oxidiser and  $^{14}\text{CO}_2$  trapped in Carbosorb/Permafluor E+ for scintillation counting.

Plasma and milk: Aliquots of plasma or milk were mixed with scintillation cocktail prior to determination of radioactivity by liquid scintillation counting.

Muscle: The muscle samples (psoas, loin and hindquarter) were minced and aliquots were solubilised in SHT overnight at approx. 40°C and acidified prior to addition of scintillation cocktail for determination of radioactivity.

Renal, subcutaneous and omental fat: aliquots of fats were solubilised with SHT and water overnight at approx. 60°C, then acidified prior to addition of scintillation cocktail for determination of radioactivity.

Liver, heart and kidney: Aliquots of 10% aqueous homogenate were solubilised with SHT and water overnight at approx. 40°C, then acidified prior to addition of scintillation cocktail for determination of radioactivity.

All sample handling for determination of radioactive content was initially completed within 4 weeks of dose termination.

### Radioactivity measurement

Samples of whole blood, rumen and abomasal fluid were combusted in an oxygen atmosphere using an oxidiser. The released  $^{14}\text{CO}_2$  was trapped in Carbosorb/Permafluor E+ and the radioactivity was determined by LSC.

All other sample materials were solubilised (if necessary) and the radioactivity was measured by liquid scintillation counting with automatic external standard quench correction.

### Metabolite analysis

Initial analysis involved extraction of the pooled aliquots of each tissue with a series of organic solvents. The radioactivity in all extracts was determined by LSC and the metabolite profile of each extract was determined by TLC analysis (2 different normal phase systems). Reversed phase HPLC analysis was conducted to confirm the metabolite assignment with an independent chromatographic method and for quantification purposes. Urine was analysed with an isocratic HPLC system. Mass spectrometric determination of compounds in the isocratic mode was done using the positive and negative ionisation mode.

Isolation and identification of the metabolite residues was triggered in edible tissues where the total radioactive residue was  $\geq 0.01$  mg/kg. For edible tissues with residues above this trigger value, organic extraction was performed and the extracts were quantified. Where the radioactivity in the extract was between 0.01 and 0.05 mg/kg, the extract was characterised. Attempts were made to identify the nature of each of the residue present in the extract at levels higher than 0.05 mg/kg.

## III. Results and Discussion

### A. Recovery and Elimination of Radioactivity

The total radioactive residues in milk, urine, blood, plasma, subcutaneous, omental and renal fat of cow after administration of an average daily dose of 2.94 g/day representing an intake in the diet of 274 mg/kg for 4 consecutive days is presented in Table 6.2.3-7.

**Table 7.2.2-18 Total radioactive residues in samples collected following oral administration twice daily of  $^{14}\text{C}$ -ethofumesate at a mean daily dose rate of 5.0 mg/kg body weight for four consecutive days**

Sample	TRR [mg/kg]	Transfer
Milk ( $\Sigma$ 8-95 h samples)	0.591	0.002
Urine ( $\Sigma$ 1-4 day samples)	371.26	n.c.
Blood (4 day sample)	0.145	n.c.
Plasma (4 day sample)	0.173	n.c.
Subcutaneous fat	0.548	0.002
Omental fat	0.539	0.002
Renal fat	0.528	0.002
Kidney	1.863	0.007
Hind quarters muscle	0.030	<0.001
Psoas muscle	0.033	<0.001
Loin muscle	0.029	<0.001

Sample	TRR [mg/kg]	Transfer
Heart muscle	0.062	<0.001
Liver	0.661	0.002

n.c. not calculated

The recovery of the administered dose in the excreta and tissues of the cow was quantified. The mean daily recovery of [ $^{14}\text{C}$ ]-Ethofumesate and its metabolites in faeces and urine was  $21.43 \pm 7.27\%$  and  $39.52 \pm 21.62\%$  respectively. In addition a further 2.17% was recovered in the digestive tract contents (including rumen, omental and abomasum). In total less than 1% of the administered dose was recovered in the milk and tissues.

#### B. Levels and time course of total radioactive residues in milk, urine, blood and plasma

In milk, residue levels raised to 0.134 mg/kg at 32 hours post the initial dose and then dropped to between 0.05 and 0.09 mg/kg for the remainder of the dosing period (32 – 96 hours).

**Table 7.2.2-19 Time course of total radioactivity in milk following oral administration of twice daily of  $^{14}\text{C}$ -ethofumesate at a mean daily dose rate of 5.0 mg/kg body weight for four consecutive days**

Time (hours)	Concentration (mg a.s. equiv./kg)
Pre-dose	-
-2	-
8	0.047
24	0.089
32	0.134
48	0.048
56	0.089
72	0.045
80	0.093
96	0.046

For urine a maximum level of 132.45 mg/kg was observed in the day 2 sample.

**Table 7.2.2-20 Time course of total radioactivity in urine following oral administration of twice daily of  $^{14}\text{C}$ -ethofumesate at a mean daily dose rate of 5.0 mg/kg body weight for four consecutive days**

Time (day)	Concentration (mg a.s. equiv./kg)
1	21.24
2	132.45
3	109.74
4	107.83

For blood and plasma highest residues were found in the samples taken 32 h after the first dosing (0.477 and 0.602 mg/kg respectively).

**Table 7.2.2-21 Time course of total radioactivity in blood and following oral administration of twice daily of  $^{14}\text{C}$ -ethofumesate at a mean daily dose rate of 5.0 mg/kg body weight for four consecutive days**

Time (hours)	Concentration (mg a.s. equiv./kg)	
	Blood	Plasma
Pre-dose	0.006	<0.001
0.5	0.006	<0.001
1	0.012	0.009
2	0.023	0.026
3	0.039	0.045
4	0.051	0.063
6	0.072	0.091
8	0.116	0.153
24	0.206	0.252
32	0.477	0.602

Time (hours)	Concentration (mg a.s. equiv./kg)	
	Blood	Plasma
48	0.162	0.200
56	0.289	0.360
72	0.149	0.174
80	0.304	0.376
96	0.145	0.173

### C. Extraction Efficiency of Residues from Milk, Urine and Tissues

#### Milk:

An aliquot of milk (32 h sample) was suspended in acetone and stirred continuously overnight at room temperature followed by centrifugation at 3000 rpm for 30 min and chilling in a freezer. The remaining residue was resuspended and extracted in acetone yielding a second acetone extract.

The residue remainder was air dried and quantified. As it contained only residues of 0.006 mg/kg, it was not analysed further. The acetone extracts were combined and reduced by turbovap. The resulting extract was acidified, applied to a C18 Bond Elut cartridge, washed with acidified water and eluted with acetonitrile followed by methanol. The acetonitrile eluate was concentrated resulting in a biphasic sample which was separated into upper and lower phase.

#### Urine:

Aliquots of urine from 24, 48, 72 and 96 hours were taken and analysed directly by HPLC.

#### Subcutaneous fat:

Subcutaneous fat was extracted in hexane by continuous shaking overnight in a water bath at approx. 37°C followed by filtration. The remaining solids after hexane extraction were extracted with acetonitrile followed by extraction with methanol. The remaining solids were air dried and analysed for  $^{14}\text{C}$  residues which were found to be only 0.005 mg/kg requiring no further analysis.

The hexane extract was back extracted twice with acetonitrile. All of the acetonitrile extracts were combined one after another and were reduced in volume by Turbovap.

#### Omental and renal fat:

Omental and renal fat was extracted in hexane by continuous shaking overnight in a water bath at approx. 37°C. The hexane extract was obtained following filtration. The remaining solids after hexane extraction were extracted with acetonitrile followed by extraction with methanol. The remaining solids were air dried and analysed for  $^{14}\text{C}$  residues which were found to be only 0.007 mg/kg for omental fat and 0.020 mg/kg for renal fat requiring no further analysis.

The hexane extract was back extracted with acetonitrile in case of omental fat, for renal fat the hexane extract was applied to a Si BE cartridge and eluted with acetonitrile. All of the acetonitrile extracts were combined and reduced in volume.

#### Kidney:

A homogenised sample of kidney was suspended in ethyl acetate and extracted by continuous stirring overnight at room temperature. The ethyl acetate extract was obtained following centrifugation and the residue was resuspended in ethyl acetate and the extraction repeated. In total three extractions were performed and the

extracts were pooled. The remaining solids were air dried.  $^{14}\text{C}$  residues were found to be 0.140 mg/kg which was below the trigger value of 10% of the total radioactive residue requiring further analysis.

The pooled ethyl acetate extract was reduced in volume by Turbovap, back washed with distilled water twice. The resulting ethyl acetate extract was reduced in volume.

#### **Psoas muscle:**

Psoas muscle was homogenised and extracted in acetonitrile with continuous stirring overnight at room temperature. A second extraction with acetonitrile was done. The remaining solids contained only 0.008 mg/kg and were not analysed further.

The combined acetonitrile extracts were reduced in volume by Turbovap and cleaned up by back-extraction with hexane. The resulting acetonitrile fraction was reduced in volume resulting in the separation of an oily phase and reduced further in a second step.

No identification of residues was performed in loin and hindquarter and heart muscle.

#### **Liver:**

A homogenised sample of liver was suspended in acetone and extracted by homogenisation for 15 minutes with the air homogeniser. The extraction was repeated twice and the three extracts were combined and reduced in volume.

The remaining solids were extracted in acetonitrile by continuous stirring overnight at room temperature, followed by an aqueous extraction (overnight at room temperature). The extract was cleaned on a C18 Bond Elut cartridge which was washed with acidified water and eluted with acetonitrile. The remaining solids after organic extraction were air dried and quantified.

#### **D. Distribution of parent compound and metabolites in organs and tissues, milk and urine**

The distribution of the parent compound and metabolites in milk, fat (subcutaneous, omental and renal), kidney, psoas muscle and liver is summarised in Table 7.2.2-22 to Table 7.2.2-28. Presented results were corrected for procedural losses during the solvent extractions to present worst-case residue conditions. Therefore, deviations to the data given in the original report may occur.

#### **Metabolites in milk**

Residue levels in milk reached a maximum of 0.134 mg/kg at 32 hours post-administration of the initial dose. Overall 95.3% of the total  $^{14}\text{C}$ -residue in milk was organo-extractable. The parent compound accounted for 55.2% (0.074 mg/kg) of the identified residue, and NC 20645 for 14.0% (0.019 mg/kg). The other metabolites identified were NC 8493 and NC 9607 each accounted for less than 10% of the residue, which equates to residues  $\leq 0.01$  mg/kg (2.5% and 8.9%, respectively).

#### **Urine**

Ethofumesate-derived residues in urine rose during the study to approximately 132.34 mg/kg urine.

Urine was found to contain a single major component, the free acid NC 20645 (carboxy derivative of the lactone NC 9607), formed by oxidation and ring opening of the parent compound ethofumesate. This metabolite is highly water soluble and therefore readily excreted in the urine.

### Metabolites in fat

In subcutaneous fat there were found residue of 0.548 mg/kg. Overall 99.1% of the total  $^{14}\text{C}$ -residue in subcutaneous fat was organo-extractable. Ethofumesate accounted for more than 90% of the identified residue (87.1% of TRR, 0.477 mg/kg). The metabolites NC 8493, NC 20645 and NC 9607 were found at comparable residue levels and accounted in total for further 7.3% of the total residue (0.040 mg/kg).

A residue level of 0.539 mg/kg was determined in omental fat. 98.7% of the total  $^{14}\text{C}$  residue was organo-extractable. The major component identified was ethofumesate (84.7%, 0.456 mg/kg). In addition, NC 20645 (3.7%, 0.020 mg/kg) was the only compound identified, while also an unknown compound (4.7%; 0.025 mg/kg) was detected.

In renal fat the level of residues was 0.528 mg/kg. 96.3% of the total  $^{14}\text{C}$ -residue was organo-extractable. Also here the major component was identified as ethofumesate (89.1%, 0.470 mg/kg). Only minor amounts of the metabolites NC 8493, NC 20645 and NC 9607 were found accounting for a total of 4.91% of the total residue (0.026 mg/kg). A very minor unknown metabolite (4.9%, 0.007 mg/kg) was also detected.

### Metabolites in kidney

In kidney high residues of ethofumesate-related residues were present (1.863 mg/kg). Overall 92.5% of the total  $^{14}\text{C}$ -residue in kidney was extractable by organic solvents. Ethofumesate was only present at 2.3% (0.044 mg/kg). The main metabolite seen was the polar carboxylic acid NC20645 formed from ring oxidation and ring-opening of ethofumesate (79.8%; 1.486 mg/kg), which was the predominant metabolite in urine. A further metabolite identified was NC 9607 (9.1%; 0.170 mg/kg), the precursor of metabolite NC 20645. Only minor amounts of NC 8493 were present (0.2%; 0.004 mg/kg).

### Metabolites in psoas muscle

The level of ethofumesate-derived residue in muscle was 0.033 mg/kg of tissue. 77.0% of the  $^{14}\text{C}$ -residues was organo-extractable. The major components present were identified as the parent compound, accounting for 45.1% of the residue (0.015 mg/kg) and NC 9607 accounting for 13.9% (0.005 mg/kg). The metabolites NC 8493, NC 20645 as well as an unknown compound were present at similar residue levels (4.8-5.5%).

### Metabolites in liver

A total of 0.661 mg/kg were determined in liver. 78.7% of the total  $^{14}\text{C}$ -residue in liver was extractable by organic solvents. The major component identified was ethofumesate, accounting for 38.9% of the residue (0.257 mg/kg). NC 9607 and NC 20645 accounted for 10.7% and 10.6%, respectively (0.071 and 0.070 mg/kg), in addition to two unknown minor metabolites (in total. 0.052 mg/kg) and some polar material (0.043 mg/kg).

The distribution of the parent compound and metabolites in milk, subcutaneous, omental and renal fat, kidney, psoas muscle and liver is summarized in the following tables.

**Table 7.2.2-22 Radioactive residues of parent compound and metabolites in milk sampled 32 hours after final dosage**

Extract	Milk (32 h sample)	
TRR [mg/kg]	0.134	
Compound (ethofumesate)	% TRR	mg/kg
Conventional extraction		
Acetone (A)*	84.39	0.113
Solids I (R1)*	15.61	0.021

Extract	Milk (32 h sample)	
<b>TRR [mg/kg]</b>	<b>0.134</b>	
Compound (ethofumesate)	% TRR	mg/kg
Final solids 2 (R2) (#)	4.73	0.006
Acetone (B)	10.88	0.015
Acetone (A + B) (C)	95.27	0.128
Turbovap trap (#)	0.24	0.000
Reduced extract (D)	95.05	0.127
Non-retained (E) ( #)	0.86	0.001
Water wash (F) (#)	<0.01	<0.001
MeOH (H) (#)	2.47	0.003
ACN (G)	91.67	0.123
Lower phase (J) (#)	5.47	0.007
Turbovap trap (#)	0.09	<0.001
Upper phase (I)	86.12	0.115
Ethofumesate	55.17	0.074
NC 9607	8.93	0.012
NC 8493	2.47	0.003
NC 20645	13.97	0.019
Unknown	5.55	0.007
Total identified	80.53	0.108
Total characterised	14.74	0.020
Total analysed	86.12	0.115
Solids	4.73	0.006
Accountability	100.00	0.134

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 3 (extraction procedure) of the original report.

**Table 7.2.2-23 Radioactive residues of parent compound and metabolites in subcutaneous fat sampled 16 hours after final dosage**

Extract	Subcutaneous fat	
<b>TRR [mg/kg]</b>	<b>0.548</b>	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Hexane (A)*	67.91	0.372
Solids (R1)	32.09	0.176
ACN (B)*	28.45	0.156
Solids (R2)	3.64	0.020
MeOH (C)*	2.79	0.015
Final solids (R3)* (#)	0.86	0.005
Hexane extraction		
Hexane (A)	67.91	0.372
Hexane (D)	5.49	0.030
Hexane (F) (#)	1.35	0.007
ACN (E)	62.41	0.342
ACN (G)	4.15	0.023
Concentration of ACN extract		
ACN (E + B) (H)	90.86	0.498
Turbovap trap (J) (#)	0.07	0.000
ACN (I)	90.79	0.498
ACN (G + I) (K)	94.94	0.520
Turbovap trap (#)	0.05	<0.001
Final extract (L)	94.40	0.517
Ethofumesate	87.07	0.477
NC 9607	2.37	0.013
NC 8493	2.79	0.015

Extract	Subcutaneous fat	
TRR [mg/kg]	0.548	
Compound (ethofumesate)	% of TRR	mg/kg
NC 20645	2.16	0.012
Total identified	94.40	0.517
Total characterised	4.75	0.026
Total analysed	94.40	0.517
Solids	0.86	0.005
Accountability	100.00	0.548

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 6 (extraction procedure) of the original report.

**Table 7.2.2-24 Radioactive residues of parent compound and metabolites in omental fat sampled 16 hours after final dosage**

Extract	Omental fat	
TRR [mg/kg]	0.539	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Hexane (A)*	54.88	0.296
Solids (R1)	45.12	0.243
ACN (B)*	38.34	0.207
Solids (R2)	6.78	0.037
MeOH (C)*	5.52	0.030
Final solids (R3)* (#)	1.26	0.007
Hexane extraction		
Hexane (A)	54.88	0.296
Hexane (D) (#)	0.16	0.001
ACN (E)	54.72	0.295
ACN (E + B) (F)	93.06	0.502
Turbovap trap (G) (#)	<0.01	<0.001
ACN (H)	93.06	0.502
Reduced extract (I)	93.06	0.502
Ethofumesate	84.66	0.456
NC 20645	3.67	0.020
Unknown	4.73	0.025
Total identified	88.33	0.476
Total characterised	10.41	0.056
Total analysed	93.06	0.502
Solids	1.26	0.007
Accountability	100.00	0.539

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 9 (extraction procedure) of the original report.

**Table 7.2.2-25 Radioactive residues of parent compound and metabolites in renal fat sampled 16 hours after final dosage**

Extract	Renal fat	
TRR [mg/kg]	0.528	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Hexane (A)*	34.26	0.181
Solids (R1)	65.74	0.347
ACN (B)*	52.62	0.278
Solids (R2)	13.13	0.069
Solids (R3)*	3.74	0.020
MeOH (C)*	9.39	0.050



Extract	Renal fat	
TRR [mg/kg]	0.528	
Compound (ethofumesate)	% of TRR	mg/kg
Turbovap (I) (#)	<0.01	<0.001
MeOH (K/L)	9.39	0.050
Ethofumesate	8.10	0.043
Unknown	1.29	0.007
Hexane extraction		
Hexane (A)	34.26	0.181
Hexane (D) (#)	0.16	0.001
ACN (E )	34.09	0.180
Hexane (F) (#)	0.75	0.004
ACN (G)	33.34	0.176
ACN (G + B) (H)	85.96	0.454
Turbovap trap (J) (#)	0.07	<0.001
ACN (I)	85.89	0.454
Ethofumesate	80.97	0.428
NC 9607	1.51	0.008
NC 8493	2.24	0.012
NC 20645	1.17	0.006
Total identified	93.99	0.496
Total characterised	2.27	0.012
Total analysed	95.28	0.503
Solids	3.74	0.020
Accountability	100.00	0.528

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 12 (extraction procedure) of the original report.

**Table 7.2.2-26 Radioactive residues of parent compound and metabolites in kidney sampled 16 hours after final dosage**

Extract	Kidney	
TRR [mg/kg]	1.863	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Solids (R1)*	7.52	0.140
Pooled ethyl acetate extract (A)*	92.48	1.723
Turbovap trap (B) (#)	0.04	0.001
Ethyl acetate concentrate (C )	92.44	1.722
Ethyl acetate (D)	87.26	1.626
Ethofumesate	2.16	0.040
NC 9607	8.49	0.158
NC 20645	76.61	1.427
Water washes (E)	5.18	0.097
C18 Clean-up of water washes		
Non-retained fraction (F) (#)	<0.01	<0.001
Acid wash (G) (#)	0.40	0.007
MeOH (I) (#)	0.57	0.011
ACN eluate (H/J)	4.21	0.078
Ethofumesate	0.17	0.003
NC 9607	0.65	0.012
NC8493	0.22	0.004
NC 20645	3.17	0.059
Total identified	91.47	1.704
Total characterised	1.01	0.019
Total analysed	91.47	1.704
Solids	7.52	0.140
Accountability	100.00	1.863

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis  
The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 18 (extraction procedure) of the original report.

**Table 7.2.2-27 Radioactive residues of parent compound and metabolites in psoas muscle sampled 16 hours after final dosage**

Extract	Psoas muscle	
TRR [mg/kg]	0.033	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Solids (R1)	32.58	0.011
Solids (R2)* (#)	23.02	0.008
ACN (B)*	9.56	0.003
ACN (A)*	67.42	0.022
ACN (A + B) (C)	76.97	0.025
Turbovap trap 1 (#)	<0.01	<0.001
ACN (D)	76.97	0.025
Hexane (F) (#)	0.82	<0.001
ACN (E)	76.16	0.025
Turbovap trap 2 (#)	<0.01	<0.001
Oily phase (#)	1.67	0.001
ACN (G/H)	74.50	0.025
Ethofumesate	45.11	0.015
NC 9607	13.87	0.005
NC 8493	4.83	0.002
NC 20645	5.52	0.002
Unknown	5.17	0.002
Total identified	69.34	0.023
Total characterised	7.64	0.003
Total analysed	74.50	0.025
Solids	23.02	0.008
Accountability	100.00	0.033

\* radioactivity in extracts and solids were added to calculate the TRR of the sample

(#) sample below the level requiring further analysis

The recoveries presented here were normalized to 100% recovery.

For details of the extraction procedure please refer to Figure 22 (extraction procedure) of the original report.

**Table 7.2.2-28 Radioactive residues of parent compound and metabolites in liver sampled 16 hours after final dosage**

Extract	Liver	
TRR [mg/kg]	0.661	
Compound (ethofumesate)	% of TRR	mg/kg
Conventional extraction		
Solids (R1)	51.16	0.338
Solids (R2)	41.21	0.272
Acetone (C)*	3.34	0.022
Solids (R3)	37.87	0.250
ACN (H)*	0.33	0.002
Solids (R4)	37.54	0.248
Solids (R5)*	21.26	0.141
Aqueous fraction (I)*	16.28	0.108
C18 clean-up of aqueous phase		
Non-retained fraction (J)	0.18	0.001
Acid water wash (K) (#)	<0.01	<0.001
ACN (M) (#)	1.62	0.011
ACN (L/N)	14.47	0.096
Ethofumesate	0.88	0.006
NC 9607	2.51	0.017

Extract	Liver	
TRR [mg/kg]	0.661	
Compound (ethofumesate)	% of TRR	mg/kg
NC 20645	3.69	0.024
Unknown polar	6.53	0.043
Unknown	0.85	0.006
Acetone (B)*	9.95	0.066
Acetone (A)*	48.84	0.323
Acetone extraction		
Acetone (A + B + C) (D)	62.13	0.411
Turbovap trap (F) (#)	2.08	0.014
Reduced extract (E )/ACN (G)	60.05	0.397
Ethofumesate	38.00	0.251
NC 9607	8.15	0.054
NC 20645	6.91	0.046
Unknown 1	3.82	0.025
Unknown 2	3.17	0.021
Total identified	60.15	0.398
Total characterised	18.59	0.123
Total analysed	74.52	0.493
Solids	21.26	0.141
Accountability	100.00	0.661

#### IV. Conclusion

Following dosing of [ $^{14}\text{C}$ -benzene]-ethofumesate at a dose rate of 2.94 g per day (equivalent to 274 mg/kg in the diet) for four consecutive days, the mean combined daily recovery for urine and faeces was  $60.95 \pm 20.66\%$ . Elimination occurred predominantly via the urine. The single component identified in the urine was the water soluble carboxylic acid NC 20645.

The tissue residues of ethofumesate ranged from 0.033 mg/kg in the muscle to 1.863 mg/kg in the kidney. In most tissues the major component seen was unchanged parent compound with the metabolites NC 20645, NC 8493 and NC 9607 detected in smaller quantities. However in the kidney, the major metabolite seen was the highly water soluble metabolite NC 20645 which was readily excreted. Residue levels in milk reached a maximum of 0.134 mg/kg at 32 hours post-administration of the initial dose. The main compound identified was parent compound followed by NC 20645. The other metabolites identified were NC 8493 and NC 9607 each accounted for less than 10% of the residue. In blood and plasma residues reached a maximum (0.477 and 0.602 mg/kg, respectively) at 32 hours h after the first dosing. Overall, the transfer of ethofumesate related residues in tissues and milk was low. Highest radioactivity concentrations were detected in the metabolising organs kidney and liver.

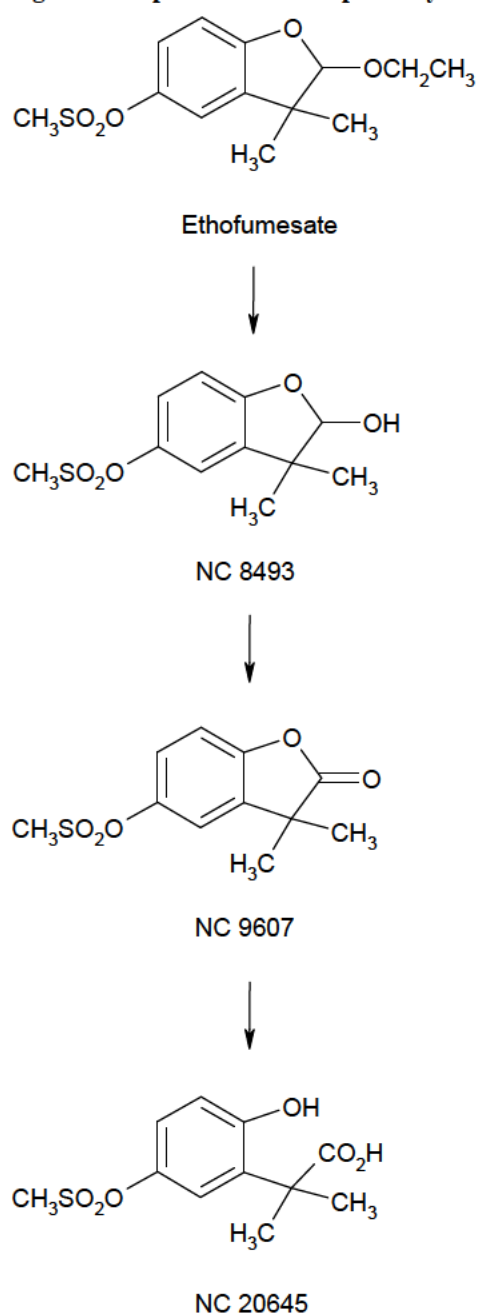
	Liver	Kidney	Muscle	Omental fat	Renal fat	Subcut. fat	Milk
TRR [mg/kg]	0.661	1.863	0.033	0.539	0.528	0.548	0.134
% extracted	78.74	92.48	76.98	98.74	96.26	99.14	95.27
% analyzed	74.52	91.47	74.50	93.06	95.28	94.40	86.12
% identified	60.15	91.47	69.34	88.33	93.99	94.40	80.53
Ethofumesate	38.88	2.34	45.11	84.66	89.08	87.07	55.17
NC 8493	-	0.22	4.83	-	2.24	2.79	2.47
NC 20645	10.61	79.77	5.52	3.67	1.17	2.16	13.97
NC 9607	10.67	9.14	13.87	-	1.51	2.37	8.93
% characterized	18.59	1.01	7.64	10.41	2.27	4.75	14.74
% bound residues	21.26	7.52	23.02	1.26	3.74	0.86	4.73

Based on the metabolites identified in edible tissues and milk, the following metabolic routes were proposed:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can undergo oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 can open to form the carboxy analogue NC 20645.

A proposed metabolic pathway is given in Figure 3.

**Figure 3 Proposed metabolic pathway for ethofumesate in ruminants**



### B.7.2.2.3. Pigs

Since the metabolism in the rat and in the cow was very similar, no pig metabolism study was conducted; the pattern of metabolites was in good agreement with the rat metabolism studies.

### B.7.2.2.4. Fish

Root and tuber crops are usually only used in small quantities e.g. as binders to increase the water stability of diets and therefore not considered as a significant part of the diet. Furthermore, ethofumesate is not considered to accumulate since the log POW of ethofumesate is 2.7, i.e. < 3. Therefore no metabolism studies on fish are deemed to be necessary.

In addition, no international agreed guidelines are available for conducting studies on metabolism in fish.

## B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

### B.7.3.1. Suitability of Analytical Methods for Analysis of Ethofumesate and its Relevant Metabolites according to the proposed Residue Definition

The proposed residue definition is sum of ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645), and its conjugate.

According to the plant metabolism, ethofumesate will degrade to 2-keto ethofumesate (NC9607). Furtheron the ring of 2-keto ethofumesate (NC9607) will be opened by hydrolysis. The open ring form of 2-keto ethofumesate is NC20645. This metabolite undergoes conjugation with the phenyl-OH.

Hence, a conjugated form of 2-keto ethofumesate is always a conjugate of NC20645. Therefore methods, which analyse for conjugated 2-keto ethofumesate, measure in fact the conjugated form of NC20645.

The methods used for residue determination in sugar and fodder beet specimens presented within the submitted dossiers cover this residue definition as outlined in the following.

#### B.7.3.1.1. Transformation via acidic hydrolysis

This study shows that conjugated as well as free NC 20645 will be transformed to and quantified as NC 9607 when using acidic conditions (e.g. acidic hydrolysis step).

Report:	KCA 6.3.1/15, Tandy, R. (2012b)
Title:	Validation of the analytical method A0019 to confirm the conversion of NC 20645 to NC 9607 in sugar beet roots and tops and wheat grain and straw
Document No:	S11-03715
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes

## I. Materials and Methods

Untreated control specimens of wheat straw, wheat grain and sugar beet root and tops were extracted with method A0019 which was used in the studies by Tandy (2012a), Perny, (2002), Perny (2003) and Tandy (2013). After extraction of the samples with a dichloromethane/methanol solvent mixture, water was added to the

solvent mixture and separated from organic phase in a separating funnel. This was combined with residual filter cake and fortified with NC20645 prior to acidic hydrolysis, which converts any NC20645 residues to NC9607 (2-keto Ethofumesate). Acetone was added and the hydrolysate was extracted with dichloromethane. After solvent concentration, residues of 2-keto Ethofumesate were determined by GC-MS. The limit of quantification for NC20645 (quantified as 2-keto Ethofumesate) in wheat straw and grain, and sugar beet tops and roots was set at 0.05 mg/kg. The fortification with NC20645 was done at LOQ (0.05 mg/kg) and at 10x LOQ (0.5 mg/kg). Three replicates were carried out for each matrix and each fortification level.

## II. Results and Discussion

The recoveries of NC20645 quantified as 2-keto ethofumesate (NC9607) ranged between 77% and 107% for all matrices and all fortification levels. The mean recovery for wheat straw was 93% with a standard deviation of 2.6% at LOQ and 96% with a standard deviation of 3.7% at 10x LOQ. The mean recovery for wheat grain was 99% with a standard deviation of 6.7% at LOQ and 97% with a standard deviation of 3.8% at 10x LOQ. The mean recovery for sugar beet roots was 82% with a standard deviation of 2.3% at LOQ and 80% with a standard deviation of 4.4% at 10x LOQ. The mean recovery for sugar beet tops was 101% with a standard deviation of 1.5% at LOQ and 94% with a standard deviation of 3.5% at 10x LOQ.

## III. Conclusion

This study confirms the conversion of NC 20645 to NC 9607 in sugar beet roots and tops by the acid hydrolysis step during the analytical method A0019.

The acidic hydrolysis step will liberate NC 20645 from its conjugate and then convert it into NC 9607. The free form of NC 20645 can undergo the same conversion into NC 9607 in case it ends up in the extraction fraction that undergoes the acidic hydrolysis.

The non-conjugated open-ring form of 2-keto-ethofumesate (NC 20645) is considered to be analysed with this method. The open and closed forms of 2-keto Ethofumesate (NC 20645 and NC 9607) are convertible into each other depending on the concentration of H<sup>+</sup> ions. To generate the open ring form of 2-keto ethofumesate (NC 20645) and to keep this form stable, alkaline conditions have to be kept.

This is also detailed in the method description for testing the storage stability of NC 20645. Please see chapter B.7.1.1.2 and the original study report KCA 6.1/01, Hamberger, R. (2013).

In the process of extraction the aqueous phase was made alkaline resulting in a conversion of NC 9607 into NC 20645. During the further procedure, the pH was adjusted to neutral conditions and acidic hydrolysis was carried out. By this method, the metabolite NC 20645 was converted to NC 9607 and finally detected as NC 9607. In the extraction method used in the studies Tandy (2012a and 2013) and Perny (2002 and 2003), the pH was set to acidic conditions for hydrolysis resulting in a conversion of NC 20645 into NC 9067. Since NC 9607 was quantified in the end, NC 20645 was determined as well.

### B.7.3.1.2. Transformation of free NC 20645 to NC 9607 via GC/MS

Free NC 20645 will be transformed to and quantified as NC 9607 when analysis is done by GC/MS.

Even if the non-conjugated open-ring form of 2-keto-ethofumesate (NC 20645) would not end up in the fraction that undergoes the acidic hydrolysis step or acidic conditions during extraction then the analytical method used

in the studies of Tandy (2012a), Perny, (2002), Perny (2003) and Tandy (2013) are still considered to capture the concentrations of non-conjugated NC 20645: All final samples were measured by GC/MS.

NC 20645 is converted into NC 9607 during GC/MS analysis as proved by the report 12A04042-01-VMWA.

This report is summarised in detail in the section for analytical methods, Section 4 (KCA 4.2/08, Hamberger (2012c)):

NC 9607 is the intra molecular ester of NC 20645. The loss of water is considered to be preferred at higher temperatures (more entropy) and reduced pressure (less water for a back reaction to NC 20645). By GC/MS, NC9607 was detected with three characteristic fragment ions with  $m/z = 256$  (quantification),  $m/z = 149$  and 150 (confirmation). Due to the transformation of NC 20645 to NC 9607 under GC/MS conditions, NC 20645 was detected as NC 9607 with its three characteristic fragment ions with  $m/z = 256$  (quantification),  $m/z = 149$  and 150 (confirmation). A recovery experiment with surface water fortified with both metabolites (NC 9607 and NC 20645) at 0.05 mg/kg resulted in recoveries of 75-90%, which fulfils the criteria of SANCO/825/00 rev. 8.1.

Hence, an analytical method in which Ethofumesate and NC 9607 were analysed with GC/MS is considered to capture non-conjugated NC 20645 as well.

This holds also true for older studies, which have already been reviewed on EU level if GC/MS methods were applied for quantification. Studies that have used GC/MS are considered to have measured unintentionally NC20645 as part of the results for NC 9607.

The studies from the Monograph of Ethofumesate (September 1998) and the Addendum thereof (December 2000) can therefore be used.

In studies of Huaulmé (2013a and 2013b) and Chevallier (2012), crop specimens were analysed for residues of Ethofumesate and its metabolites NC 8493 (free and conjugated), NC 9607 and NC 20645 (free and conjugated). This was proved by fortification with NC 8493, NC 9607 and NC 20645 prior to extraction. Both methods include an acidic hydrolysis step and the final residues were measured by GC/MS. Hence, the analysis of Ethofumesate residues included in the residue definition is covered. The final determination of the residues was carried out by GC/MS.

The analytical methods used in studies of Waalkens & Hamberger (2005a, b, c, d, e, f) and the analytical method in Anspach (2001) is identical to the method used in studies of Huaulmé (2013a and 2013b) and Chevallier (2012) including alkaline extraction conditions and acidic hydrolysis of conjugates among others. Hence, these methods cover -in addition to Ethofumesate and NC 9607- the analysis of NC 20645 (free and conjugated) as well.

Regarding extraction efficiency, residues in crucial commodities, i.e. leaves with tops/leaves with collar and roots of sugar and fodder beets at harvest were negligible in all residue trials covering the intended GAP. Since no residues at or above the respective LOQ were measured in crucial commodities in the residue trials covering the intended GAP, a verification of extraction procedures is not required according to SANCO/825/00 rev. 8.1 (Point 2.13).

The transformation of free NC 20645 to NC 9607 was also addressed by a new study which confirms the conversion of non-conjugated NC 20645 into NC 9607 in sugar beet matrices during the whole analytical method A0019 (Weir, 2014). This study is summarised and discussed in detail below.

Report:	KCA 6.3.1/16, Weir, A. (2014)
Title:	Method Modification and Validation of an Analytical Method for the Determination of

Document No:	Ethofumesate and its Metabolites NC 20645 and NC 9607 in Sugarbeet Roots and Tops S13-03837
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4
GLP:	Yes

## I. Material and methods

Untreated control specimens of sugar beet root and tops were fortified with Ethofumesate, NC 9607, and free NC 20645, respectively. For each analyte/matrix, five recoveries at the limit of quantification (LOQ) of 0.01 mg/kg and five recoveries at 10 times of the LOQ (0.1 mg/kg) were determined along with two control specimens and one reagent blank. Please note, that a fortification with conjugated NC 20645 is not possible. Validation for this conjugated form is usually done by spiking with the non-conjugated form prior to the acidic hydrolysis step. The fortified specimens were extracted with method A0019 which was used in the studies described above (Tandy, 2012a, Perny, 2002, Perny, 2003, Tandy, 2013). To track the fate of non-conjugated NC 20645, recoveries fortified with NC 26045 were taken within the different steps of the extraction procedure. Fortified sugar beet root and top specimens were double extracted with methanol/dichloromethane. After filtration, the organic filtrates containing Ethofumesate, NC 9607 and the free form of NC 20645 were combined, whereas the filter cake containing conjugated NC 20645 was retained. The organic filtrates were cleaned up by liquid-liquid partitioning with water and methanol/dichloromethane (10/90, v/v). During this partitioning the analytes separated. Ethofumesate and NC 9607 remained in the organic phase, whereas non-conjugated NC 20645 partitioned into the aqueous phase. The aqueous extract was combined with the retained filter cake. The organic extracts were evaporated and re-dissolved in dichloromethane prior to GC-MS analysis. To the combined filter cake and the aqueous extract water/methanol and hexane were added. The solution was heated in a water bath for 60 minutes. Afterwards, the extract was refluxed for 75 minutes under acidic conditions. During this step NC 20645 is converted to NC 9607. After clean-up by liquid-liquid partitioning, filtration and concentration, the extracts were re-dissolved in dichloromethane and analysed by GC-MS.

## II. Results and discussion

The recoveries of Ethofumesate, NC 9607 and free NC20645 (after acidic hydrolysis) in sugar beet roots and tops ranged between 60% and 120% (with a relative standard deviation lower than 30%) at 0.01 mg/kg and between 70% and 120% (with a relative standard deviation lower than 20%) at 0.10 mg/kg for each analyte and matrix. Three ion masses for each analyte have been determined. Non-conjugated NC 20645 was not found in the organic phase after extraction, but in the aqueous phase which underwent acidic hydrolysis. Conjugated NC 20645 will enter this same step. The reported conversion of the non-conjugated NC 20645 into NC 9607 demonstrates the efficacy of the acidic hydrolysis. A separate fortification with non-conjugated NC 20645 prior to acidic hydrolysis, as is usually done for conjugated NC 20645, is therefore redundant. Hence, the validation of the method covers both free and conjugated NC 20645. Linear regression curves were generated for all transitions and in all cases the coefficient of determination ( $r^2$ ) was found to be greater than 0.990 and the correlation coefficient ( $r$ ) greater than 0.995. The LOQ of this method was herein lowered to 0.01 mg/kg for each analyte and matrix.



## I. Conclusion

As a conclusion, older residue trials which have already been reviewed on EU level can be used to support the current submission, if ethofumesate and NC 9607 have been analysed by using acidic conditions and GC/MS analysis, since these methods would have analysed unintentionally free and conjugated NC 20645 as well as part of the analysis of NC 9607. The new study confirms the conversion of non-conjugated NC 20645 to NC 9607 in sugar beet roots and tops by the whole analytical method A0019.

### B.7.3.2. Sugar beet (representative use)

The representative use supported in the AIR 3 process is the use in sugar beet (including the root crops fodder beet and beetroot).

The supported good agricultural practice (GAP) for the application of Ethofumesate in/on sugar and fodder beets in Europe is summarized below:

**Table 7.3.2-1 Use pattern (GAP) for the application of Ethofumesate in/on sugar and fodder beets in Europe**

Formulation	Region	Application timing	Max. rate of application (L/ha [prod.])	Max. a.s. rate of application (kg/ha)	Max. no. of appls.	PHI (days)
Ethofumesate 500 SC (500 g/L ethofumesate)	EU-N EU-S	BBCH 16-18	2.0	1.0**	3	n.a.†
Ethofol 500SC (500g/L ethofumesate)	EU-N EU-S	Pre emergence	2.0	1.0**	1	n.a.†
Ethofol 500SC (500g/L ethofumesate)	EU-N EU-S	Post emergence until BBCH 18	0.66	0.33**	3*	n.a.†

EU-N = northern EU residue region, EU-S = southern EU residue region

\* Splitting application with a maximum total rate of 1 kg a.s./ha per season. The maximum application rate per treatment is 0.33 kg a.s./ha. The critical GAP therefore is 3 applications of 0.33 kg a.s./ha. More applications (max. 6) at a lower application rate are possible, but they do not represent the critical GAP.

\*\* The maximum amount of active substance must not exceed 1.0 kg/ha every 3 years

† n.a. = not applicable since PHI is covered by the normal vegetation period between last application and harvest

*These studies were submitted and evaluated for the first inclusion of ethofumesate on Annex I:*

The following table summarizes all supervised residue trials in beet crops (sugar beet, fodder beet including mangold and beetroot) - which have already been submitted for the Annex I inclusion of ethofumesate - and have been conducted at the critical total application rate of 1.0 kg ethofumesate/ha ( $\pm 25\%$ ). The product was applied either as a pre-emergence or post-emergence spray.

**Table 7.3.2-2 Summary of residue trials conducted with the active substance ethofumesate in/on root crops in Europe**

Study ID		Crop	Formulation	Region	Application timing	Max. a.s. <sup>1</sup> rate per season (kg/ha)
1	A82993	fodder beet	Nortron / Trammat	N-EU	pre-emergence	1.0
2	A82996	fodder beet	Nortron / Trammat	N-EU	post-emergence	1.0
3	A83007	beetroot	Nortron / Trammat	N-EU	pre- or post-emergence	1.0
4	A83020	fodder beet	Nortron / Trammat	N-EU	pre- & post-emergence	1.0
		pre- or post-emergence			1.0	
		pre- & post-emergence			1.0	
5	A82998	sugar beet	Nortron / Trammat	N-EU	pre-emergence	1.0
6	A83019	sugar beet	Nortron / Trammat	N-EU	pre- or post-emergence	1.0
7	A83035	sugar beet	Nortron / Trammat SC Nortron / Trammat EC	N-EU	post-emergence	1.2

8	A83058	sugar beet	ETO & chloridazon SC	N-EU	pre-emergence	1.0
9	A83064	sugar beet	ETO & chloridazon SC	N-EU	pre-emergence	1.02
10	A83071	sugar beet	ETO & phenmedipham EC	N-EU	post-emergence	1.13
11	A62042	sugar beet	Betanal Progress OF	N-EU	post-emergence	0.82
12	A83005	sugar beet	Nortron / Trammat	S-EU	pre-emergence	1.0
13	A83290	sugar beet	Nortron / Trammat	S-EU	post-emergence	0.8

1 a.s. = ethofumesate

Report:	KCA 6.3.5 /01;Crofts, M.;1975;M-155284-01
Title:	RESIDUES IN FODDER BEET AND RED BEET FROM 1974 APPLICATIONS OF NORTRON IN THE UK
Report No:	A83007
Document No:	M-155284-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /02;Crofts, M.; Whiteoak, R. J.;1976;M-155297-01
Title:	RESIDUES IN MANGOLDS, FODDER BEET AND RED BEET FROM 1975 AND 1976 APPLICATIONS OF NORTRON IN THE UK (AND 1 RED BEET TRIAL IN SWEDEN)
Report No:	A83020
Document No:	M-155297-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /03;Crofts, M.; Whiteoak, R. J.;1977;M-155299-01
Title:	RESIDUES IN RED BEET ROOTS FROM 1976 TRIALS WITH NORTRON IN AUSTRALIA
Report No:	A83022
Document No:	M-155299-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /04;Crofts, M.;1978;M-155313-01
Title:	HARVEST RESIDUES IN RED BEET FROM NORTRON TRIALS IN THE USA (NEW YORK, TEXAS AND WISCONSIN) IN 1976/77
Report No:	A83036
Document No:	M-155313-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /05;Crofts, M.;1978;M-155316-01
Title:	HARVEST RESIDUES IN RED BEET FROM A NORTRON TRIAL IN CANADA IN 1977
Report No:	A83039
Document No:	M-155316-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /06;Wrede, A.;1995;M-155393-01
Title:	Residues in red beet after application of Betanal progress in France 1993
Report No:	A83118
Document No(s):	Report includes Trial Nos.: PF-R 93 098 M-155393-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes

Report:	KCA 6.3.5 /07;Crofts, M.; Whiteoak, R. J.;1973;M-155252-01
Title:	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM 1972 TRIALS WITH NORTRON IN THE UK

Report No:	A82975
Document No:	M-155252-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /08;Crofts, M.; Whiteoak, R. J.;1973;M-155253-01
Title:	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM FRENCH TRIALS WITH NORTRON IN 1972
Report No:	A82976
Document No:	M-155253-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /09;Crofts, M.; Whiteoak, R. J.;1973;M-155254-01
Title:	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM DANISH TRIALS WITH NORTRON IN 1972
Report No:	A82977
Document No:	M-155254-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /10;Crofts, M.; Whiteoak, R. J.;1973;M-155255-01
Title:	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM AUSTRIAN TRIALS WITH NORTRON IN 1972
Report No:	A82978
Document No:	M-155255-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /11;Crofts, M.; Whiteoak, R. J.;1973;M-155256-01
Title:	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM 1972 TRIALS WITH NORTRON IN YUGOSLAVIA
Report No:	A82979
Document No:	M-155256-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /12;Whiteoak, R. J.; Crofts, M.; Harris, R. J.;1973;M-155257-01
Title:	RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM 1972 TRIALS WITH NORTRON IN W. GERMANY (UPDATED)
Report No:	A82980
Document No:	M-155257-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /13;Whiteoak, R. J.;1973;M-155259-01
Title:	RESIDUE DECLINE STUDIES IN COLORADO (USA) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON IN 1972
Report No:	A82982
Document No:	M-155259-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /14;Whiteoak, R. J.; Crofts, M.;1974;M-155260-01
Title:	RESIDUE DECLINE STUDIES IN MICHIGAN (USA) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON IN 1972
Report No:	A82983
Document No:	M-155260-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /15;Crofts, M.; Whiteoak, R. J.;1974;M-155263-01
Title:	NORTON RESIDUE IN HARVEST SUGAR BEET FROM NINE REGIONS OF THE USA IN 1972
Report No:	A82986
Document No:	M-155263-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /16;Crofts, M.; Whiteoak, R. J.;1974;M-155267-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM 1973 PRE-EMERGENCE APPLICATIONS OF NORTON (TRAMAT) IN ITALY
Report No:	A82990
Document No:	M-155267-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /17;Crofts, M.; Whiteoak, R. J.;1974;M-155269-01
Title:	RESIDUE DECLINE STUDY IN THE UK (1973) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTON
Report No:	A82992
Document No:	M-155269-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /18;Crofts, M.; Whiteoak, R. J.;1974;M-155270-01
Title:	HARVEST RESIDUES IN FODDER BEET FROM 1973 PRE-EMERGENCE APPLICATION OF NORTON (TRAMAT) IN W. GERMANY
Report No:	A82993
Document No:	M-155270-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /19;Crofts, M.; Whiteoak, R. J.;1974;M-155273-01
Title:	HARVEST RESIDUES IN FODDER BEET FROM 1972 AND 1973 POST-EMERGENCE APPLICATIONS OF NORTON (TRAMAT) IN W. GERMANY
Report No:	A82996
Document No:	M-155273-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /20;Crofts, M.; Whiteoak, R. J.;1974;M-155274-01
Title:	RESIDUE DECLINE STUDY IN THE UK (1973) WITH SUGAR BEET TREATED POST-EMERGENCE WITH NORTON
Report No:	A82997
Document No:	M-155274-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /21;Crofts, M.; Whiteoak, R. J.;1974;M-155275-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM 1973 PRE-EMERGENCE APPLICATIONS OF NORTON (TRAMAT) IN W. GERMANY
Report No:	A82998
Document No:	M-155275-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /22;Crofts, M.; Whiteoak, R. J.;1974;M-155559-01
Title:	HARVEST RESIDUES IN SUGAR BEET AND SOIL FROM 1973 POST-EMERGENCE

Report No:	APPLICATIONS OF NORTRON (TRAMAT) IN ITALY A83290
Document No:	M-155559-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /23;Crofts, M.; Whiteoak, R. J.;1974;M-155276-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM 1973 POST-EMERGENCE APPLICATIONS OF NORTRON IN THE UK
Report No:	A82999
Document No:	M-155276-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /24;Crofts, M.;1975;M-155281-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM 1974 PRE-EMERGENCE APPLICATIONS OF NORTRON IN CANADA
Report No:	A83004
Document No:	M-155281-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /25;Crofts, M.;1975;M-155282-01
Title:	DECLINE IN RESIDUES IN SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON (TRAMAT) IN ITALY (1974)
Report No:	A83005
Document No:	M-155282-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /26;Crofts, M.;1975;M-155283-01
Title:	DECLINE OF RESIDUES IN SUGAR BEET TREATED POST-EMERGENCE WITH NORTRON (TRAMAT) IN ITALY (1974)
Report No:	A83006
Document No:	M-155283-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /27;Crofts, M.; Whiteoak, R. J.;1976;M-155289-01
Title:	NORTRON RESIDUES IN MATURE SUGAR BEET FOLLOWING POST-EMERGENCE APPLICATIONS AS A TANK MIX WITH DESMEDIPHAM IN THE USA
Report No:	A83012
Document No:	M-155289-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /28;Crofts, M.; Harris, R. J.; Wilkie, P. M.;1976;M-155290-01
Title:	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON 20 EC OR TCA OR A TANK MIX OF BOTH COMPONENTS IN THE USA
Report No:	A83013
Document No:	M-155290-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /29;Crofts, M.; Harris, R. J.; Wilkie, P. M.;1976;M-155293-01
Title:	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON OR PYRAMIN OR A TANK MIX OR BOTH COMPONENTS IN THE USA IN 1975
Report No:	A83016
Document No:	M-155293-01-1

Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /30;Crofts, M.;1976;M-155294-01
Title:	NORTON AND RO-NEET RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE-EMERGENCE APPLICATION AND TANK MIX IN THE USA IN 1974
Report No:	A83017
Document No:	M-155294-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /31;Crofts, M.;1976;M-155296-01
Title:	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED WITH AN SC OR AN EC FORMULATION OF NORTON IN UK, 1975
Report No:	A83019
Document No:	M-155296-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /32;Crofts, M.;1978;M-155311-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM PRE- EMERGENCE APPLICATIONS OF TRAMAT (NORTON) SC FORMULATION IN W. GERMANY IN 1976.
Report No:	A83034
Document No:	M-155311-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /33;Crofts, M.;1978;M-155312-01
Title:	HARVEST RESIDUES IN SUGAR BEET FROM 1977 TRIALS WITH TRAMAT (NORTON) SC AND EC FORMULATIONS IN W. GERMANY
Report No:	A83035
Document No:	M-155312-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /34;Harris, R. J.; Reary, J. B.;1979;M-155322-01
Title:	RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE-EMERGENCE APPLICATIONS OF SEPARATE OR TANK-MIX FORMULATIONS OF ETHOFUMESATE AND CHLORIDAZON IN MICHIGAN AND OHIO 1978
Report No:	A83045
Document No:	M-155322-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /35;Browne, P. M.; Reary, J. B.;1979;M-155323-01
Title:	ETHOFUMESATE RESIDUES IN MATURE SUGAR BEET TREATED POST EMERGENCE IN MIXTURES WITH PHENMEDIPHAM AND/OR DESMEDIPHAM IN USA 1977
Report No:	A83046
Document No:	M-155323-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /36;Reary, J. B.;1980;M-155326-01
Title:	RESIDUES IN MATURE SUGAR BEET TREATED POST-EMERGENCE WITH MIXTURES OF ETHOFUMESATE AND/OR PHENMEDIPHAM AND DESMEDIPHAM (COMMERCIAL EC FORMULATIONS) IN USA 1979
Report No:	A83049
Document No:	M-155326-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

Report:	KCA 6.3.5 /37;Reary, J. B.;1980;M-155327-01
Title:	RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE AND POST-EMERGENCE APPLICATION OF ETHOFUMESATE (20 EC) IN CALIFORNIA 1977
Report No:	A83050
Document No:	M-155327-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /38;Browne, P. M.; Reary, J. B.;1980;M-155330-01
Title:	RESIDUES IN SUGAR BEET TREATED PRE- EMERGENCE WITH A SUSPENSION CONCENTRATE FORMULATION (50 SC) OF ETHOFUMESATE IN WEST GERMANY
Report No:	A83053
Document No:	M-155330-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /39;Reary, J. B.;1980;M-155328-01
Title:	RESIDUES IN MATURE SUGAR BEET TREATED PRE-EMERGENCE WITH MIXTURES OF ETHOFUMESATE AND/OR PEBULATE OR CYCLOATE (COMMERCIAL EC FORMULATIONS) IN CALIFORNIA 1979
Report No:	A83051
Document No:	M-155328-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /40;Housden, M. C.; Reary, J. B.;1981;M-155334-01
Title:	Residues of Ethofumestae and metabolites in sugar beet treated pre--emergence with a one-pack mixture of Ethofumestae and Lenacil in West Germany 1980
Report No:	A83057
Document No:	M-155334-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /41;Reary, J. B.;1981;M-155335-01
Title:	Residues of Ethofumesate and metabolites in sugar beet treated pre-emergence with a one-pack mixture of Ethofumesate and Chloridazon in West Germany 1980
Report No:	A83058
Document No:	M-155335-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /42;Reary, J. B.;1981;M-155336-01
Title:	Residues in sugar beet treated post-emergence with a suspension concentrate formulation (50 SC) of Ethofumesate in West Germany 1980
Report No:	A83059
Document No:	M-155336-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /43;Haldeman, J. K.; Ford, J. J.;1982;M-164269-01
Title:	ANTOR AND NORTRON HERBICIDE RESIDUES IN SUGAR BEETS FROM TREATED PLOTS
Report No:	A89134
Document No:	M-164269-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /44;Cron, J. H.;1982;M-155341-01

Title:	Residues of Ethofumesate and metabolites in sugar beet treated pre-emergence with a one-pack mixture of Ethofumesate and Chloridazon in West Germany 1981
Report No:	A83064
Document No:	M-155341-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /45;Haldeman, J. K.;1982;M-155343-01
Title:	NORTRON HERBICIDE RESIDUES IN SUGAR BEETS TREATED PRE- AND POST-PLANTING
Report No:	A83066
Document No:	M-155343-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /46;Cron, J. H.;1982;M-155342-01
Title:	Residues of Ethofumesate and metabolites in sugar/fodder beet treated post-emergence with Ethofumesate (50 SC) in West Germany 1981
Report No:	A83065
Document No:	M-155342-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /47;Haldeman, J. K.;1982;M-155344-01
Title:	ETHOFUMESATE RESIDUES IN SUGAR BEETS FROM TWO CALIFORNIA LOCATIONS
Report No:	A83067
Document No:	M-155344-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /48;Ford, J. J.;1983;M-164271-01
Title:	DIETHATYL ETHYL (ANTOR HERBICIDE) AND ETHOFUMESATE (NORTRON HERBICIDE) RESIDUES IN 6-MONTH SUGAR BEETS FROM CALIFORNIA
Report No:	A89135
Document No(s):	Report includes Trial Nos.: H41/3/81 H79/3/3 M-164271-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /49;Lee, G. E.; Weishedel, B. C.;1984;M-155346-01
Title:	ETHOFUMESATE (NORTON HERBICIDE) RESIDUES IN SUGAR BEETS FROM QUEBEC AND MANITOBA
Report No:	A83069
Document No:	M-155346-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /50;Manley, J. D.; Snowdon, P. J.;1984;M-155349-01
Title:	Residues of Ethofumesate and major metabolites in sugarbeet treated in West Germany 1982 and 1983, with a Co-formulation of Ethofumesate and Phenmedipham
Report No:	A83072
Document No(s):	Report includes Trial Nos.: 041/03/080 M-155349-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /51;Snowdon, P. J.;1985;M-155348-01



Title:	Residues of Ethofumesate and major metabolites in sugarbeet treated in France 1984 with Ethofumesate and Phenmedipham as either a Co-formulation of a Tank-mix
Report No:	A83071
Document No(s):	Report includes Trial Nos.: 041/03/082 M-155348-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /52;Manley, J. D.; Snowden, P. J.;1986;M-155353-01
Title:	Residues of Ethofumesate and major metabolites in sugar beet treated in the Federal Republic of Germany, 1983 with a Co-formulation of Ethofumesate and Phenmedipham
Report No:	A83077
Document No(s):	Report includes Trial Nos.: 041/03/080 M-155353-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /53;Manley, J. D.; Snowden, P. J.;1986;M-155354-01
Title:	Residues of Ethofumesate and major metabolites in sugarbeet treated in the Federal Republic of Germany 1985 with a Co-formulation of Ethofumesate and Phenmedipham
Report No:	A83078
Document No(s):	Report includes Trial Nos.: 041/03/085 M-155354-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.3.5 /54;Banwell, M.; Bright, J. H. M.;1990;M-155764-02; Amended: 1990-11-01
Title:	Residues of Ethofumesate and its major metabolites in sugar beet following multiple post-emergence application of an EC Co-formulation with Penmedipham and Desmedipham in Denmark 1989 (2nd Edition)
Report No:	A83095
Document No(s):	Report includes Trial Nos.: 041/03/116 M-155764-02-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 6.3.5 /55;Straszewski, A.;1993;M-155390-01
Title:	Ethofumesate: SC (CQ 1273/01): Residues of Ethofumesate and its major metabolite in sugar beets France 1992
Report No:	A83115
Document No:	M-155390-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	KCA 6.3.5 /56;Wrede, A.;1995;M-145562-01
Title:	Residues in sugar beet after application of Betanalarprol in France 1993
Report No:	A62042
Document No:	M-145562-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes

## I. Material and Methods

Over several growing seasons (1973 – 1991), 37 residue trials were conducted in the northern European residue region on sugar beets, fodder beets (including mangold) and beetroots, 6 residue trials were conducted in the southern European residue region between 1973 and 1974.

The trials were designed as harvest or decline trials. Root and leaf samples were collected and analysed for parent compound ethofumesate and metabolite NC 9607 before and after an acidic hydrolysis step. The hydrolysis step was conducted to cleave the conjugate of metabolites NC 20645 and to convert the exocon to the common moiety NC 9607.

The acidic conditions were nearly identical in all residue analytical methods applied (6 M HCl at 100°C for 60 to 75 min.) and were in line with the conditions needed to determine all constituents of the residue definition, including the conjugated form of metabolite NC 20645. The distribution of the relevant residue detected in the supervised field trials is shown in Table 7.3.2-3 (roots/body) and Table 7.3.2-4 (tops/leaves).

In some studies, analysis of the transient metabolite NC 8493 and its conjugates (which are found only in immature plant matrices) was also included.

The conjugates of metabolites NC 20645 or NC 8493 were analysed in a separate step after acidic hydrolysis. The acidic treatment released the exocons NC 8493 and NC 20645. Under the acidic conditions of the hydrolysis, the free carboxylic acid NC 20645 was immediately converted to NC 9607 due to an intramolecular ring closure, as postulated in the plant metabolism studies with incurred <sup>14</sup>C residues.

(The plant metabolism studies showed that the acidic treatment effectively hydrolyses the conjugated residue and transforms the released exocon into the analytical target NC 9607 when the sample is boiled gently with 6 M HCl for 6 h at 95°C; cleavage of the conjugate was also proven when applying less harsh conditions: 3 M HCl for 1 h at 60 °C). Direct GC-FPD analysis (sulfur mode) of the formed NC 9607 is feasible, whereas NC 8493 has to be acetylated for subsequent GC analysis.

Recovery experiments were conducted alongside the analysis of the field samples. In the absence of conjugated NC 20645 for spiking purposes (as the endocon of the conjugate was never elucidated), the addition of metabolite NC 9607 simulated recoveries of the conjugate, when considered in conjunction with plant metabolism studies with samples containing incurred <sup>14</sup>C residues. The hydrolysis/transformation conditions chosen (treatment with 6 M HCl at 100°C for 75-150 min) were very similar to those applied in the plant metabolism studies (6 M HCl at 95°C for 360 min).

In addition, a conversion experiment was conducted which confirmed the extensive transformation of NC 20645 into NC 9607 when applying the weakest acidic conditions of all residue analytical methods used (6 M HCl at 100°C for 60 min.). In sugar beet leaves and roots, ≥85% of NC 20645 was converted into the analytical target NC 9607.

**Table 7.3.2-3 Distribution of residues in beet roots after application of ethofumesate according to the intended EU GAP (“representative use”)**

Intended EU GAI ( Representative use )							
Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in roots [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
EU-N							
A82993 <sup>a</sup>	1 x 1.0	155	<0.02	<0.02	<0.02	<0.06	0.02

Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in roots [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
(fodder beet)	1 x 1.0	163	<0.02	<0.02	<0.02	<0.06	
A82996 <sup>a</sup> (fodder beet)	1 x 1.0, Po	104	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.0, Po	118	<0.02	<0.02	<0.02	<0.06	
A83007 <sup>a</sup> (beetroot)	1 x 1.0, Po	116	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.0	144	<0.02	<0.02	<0.02	<0.06	
A83020 <sup>b</sup> (fodder beet)	1 x 1.0, Po	119	0.05	<0.02	<0.02	0.09	0.02
(beetroot)	1 x 1.0, Po	132	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	100	0.03	<0.02	<0.02	0.07	
	1 x 1.0, Po	90	<0.02	<0.02	<0.02	<0.06	
(mangold = fodder beet)	1 x 1.0	127	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0, Po	119	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0, Po	132	<0.02	<0.02	<0.02	<0.06	
A82998 <sup>a</sup> (sugar beet)	1 x 1.0	189	<0.02	<0.02	0.03 <sup>1</sup>	0.07	0.02
	1 x 1.0	180	0.02	<0.02	<0.02	0.06	
	1 x 1.0	187	<0.02	<0.02	<0.02	<0.06	
A83019 <sup>b</sup> (sugar beet; SC formulation)	1 x 1.0	158	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.0	157	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	136	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	138	<0.02	<0.02	0.02	0.06	
A83035 <sup>b</sup> (sugar beet)	1 x 1.2, Po	146	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.2, Po	119	<0.02	<0.02	<0.02	<0.06	
	1 x 1.2, Po	131	<0.02	<0.02	<0.02	<0.06	
	1 x 1.2, Po	135	<0.02	<0.02	<0.02	<0.06	
A83058 <sup>b</sup> (sugar beet)	1 x 1.0*	93	<0.02	<0.02	<0.02	<0.06	0.02
		148	<0.02	<0.02	<0.02	<0.06	
		178	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	136	<0.02	<0.02	<0.02	<0.06	
		156	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	139	<0.02	<0.02	<0.02	<0.06	
		167	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	181	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	73	<0.02	<0.02	0.02	0.06	
		148	<0.02	<0.02	<0.02	<0.06	
		175	<0.02	<0.02	<0.02	<0.06	
A83064 <sup>b</sup> (sugar beet)	1 x 1.02*	132	<0.02	<0.02	<0.02	<0.06	0.02
		160	<0.02	<0.02	<0.02	<0.06	
		193	<0.02	<0.02	<0.02	<0.06	
	1 x 1.02*	70	<0.02	<0.02	<0.02	<0.06	
		116	<0.02	<0.02	<0.02	<0.06	
		148	<0.02	<0.02	<0.02	<0.06	
		185	<0.02	<0.02	<0.02	<0.06	
	1 x 1.02*	119	<0.02	<0.02	<0.02	<0.06	
		148	<0.02	<0.02	<0.02	<0.06	
		175	<0.02	<0.02	<0.02	<0.06	
A83071 <sup>c</sup> (sugar beet)	1 x 1.13, Po	137	<0.03	<0.03	<0.03	<0.10	0.05
	1 x 1.13, Po	149	<0.03	<0.03	<0.03	<0.10	

Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in roots [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
A62042 <sup>d</sup> (sugar beet)	1 x 0.83, Po	139	<0.01	- <sup>††</sup>	<0.05 <sup>‡</sup>	<0.06	0.05
	1 x 0.83, Po	144	<0.01	- <sup>††</sup>	<0.05 <sup>‡</sup>	<0.06	
	1 x 0.83, Po	141	<0.01	- <sup>††</sup>	<0.05 <sup>‡</sup>	<0.06	
EU-S							
A83005 <sup>a</sup> (sugar beet)	1 x 1.0*	75	<0.02	0.05 <sup>1</sup>	0.05 <sup>2</sup>	0.12	0.02
		105	0.03	<0.02	<0.02	0.07	
		137	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	86	<0.02	<0.02	<0.02	<0.06	
		118	<0.02	<0.02	<0.02	<0.06	
		150	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	64	<0.02	<0.02	0.04	0.08	
		93	<0.02	<0.02	<0.02	<0.06	
		125	<0.02	<0.02	<0.02	<0.06	
A83290 <sup>a</sup> (sugar beet)	1 x 0.8	118, Po	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 0.8	111, Po	<0.02	<0.02	<0.02	<0.06	
	1 x 0.8	107, Po	<0.02	<0.02	<0.02	<0.06	

PO post-emergence use

† in the residue reports the conjugate of NC 20645 was described as “conjugated form of NC 9607” since the conjugate is cleaved by an acidic hydrolysis step and the exocon is converted into NC 9607 in the methods applied. Extraction and analysis of metabolite NC 9607 (“free form”) and the conjugate of NC 20645 (“conjugated form of NC9607”) was done in separate steps.

†† the extracts containing free NC 9607 and NC 9607 from the conversion of the conjugate of NC 20645 were combined for analysis

‡ no detectable peak = <0.01 mg/kg (in metabolite equivalents), thus <0.05 mg/kg (in a.s. equivalents)

1 control sample contained residues of 0.03 mg/kg

2 control sample contained residues of 0.02 mg/kg

n.a. = not analysed

\* decline trials (last sampling = mature crop)

a analysed according to method RESID/73/18/1 (cf. KCA 4.1.2/28)

b analysed according to method RESID/73/18/2 (cf. KCA 4.1.2/29)

c analysed according to method RESID/84/42 (cf. KCA 4.1.2/30)

d analysed according to method AL081/96-0 (cf. KCA 4.1.2/10)

**Table 7.3.2-4 Distribution of residues in beet tops (leaves) after application of ethofumesate according to the intended EU GAP**

Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in tops [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
EU-N							
A82993a (fodder beet)	1 x 1.0	155	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.0	163	0.03 <sup>1</sup>	<0.02	<0.02	0.07	
A82996a (fodder beet)	1 x 1.0, Po	104	0.03	<0.02	<0.02	<b>0.07</b>	0.02
	1 x 1.0, Po	118	<0.02	<0.02	<0.02	<b>&lt;0.06</b>	
A83007a (beetroot)	1 x 1.0, Po	116	n.a.	n.a.	n.a.	<b>n.a</b>	
	1 x 1.0	144	n.a.	n.a.	n.a	n.a	
A83020b (fodder beet) (beetroot)  (mangold = fodder beet)	1 x 1.0, Po	119	<0.02	<0.02	<0.02	<b>&lt;0.06</b>	0.02
	1 x 1.0, Po	132	0.02	<0.02	<0.02	<b>0.06</b>	
	1 x 1.0	100	0.02	<0.02	0.05	0.09	
	1 x 1.0, Po	90	n.a.	n.a	n.a.	<b>n.a</b>	
	1 x 1.0	127	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0, Po	119	<0.02	<0.02	<0.02	<b>&lt;0.06</b>	
	1 x 1.0, Po	132	<0.02	<0.02	<0.02	<b>&lt;0.06</b>	
A82998a	1 x 1.0	189	<0.02	<0.02	0.10 <sup>1</sup>	0.14	0.02

Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in tops [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
(sugar beet)	1 x 1.0	180	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	187	0.04	<0.02	<0.02	0.08	
A83019b (sugar beet)	1 x 1.0	158	<0.02	<0.02	0.02	0.06	0.02
	1 x 1.0	157	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0	136	<0.02	<0.02	0.02	0.06	
	1 x 1.0	138	<0.02	<0.02	0.03	0.07	
A83035b (sugar beet)	1 x 1.2, Po	146	<0.02	<0.02	<0.02	<0.06	0.02
	1 x 1.2, Po	119	<0.02	<0.02	<0.02	<0.06	
	1 x 1.2, Po	131	<0.02	<0.02	<0.02	<0.06	
	1 x 1.2, Po	135	<0.02	<0.02	<0.02	<0.06	
A83058b (sugar beet)	1 x 1.0*	93	<0.02	<0.02	0.03 <sup>1</sup>	0.07	0.02
		148	<0.02	<0.02	0.03 <sup>1</sup>	0.07	
		178	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	136	<0.02	<0.02	0.02	0.06	
		156	<0.02	<0.02	0.02	0.06	
	1 x 1.0*	139	<0.02	<0.02	0.02	0.06	
		167	<0.02	<0.02	0.04	0.08	
	1 x 1.0	181	<0.02	<0.02	0.02	0.06	
	1 x 1.0*	73	<0.02	<0.02	0.05	0.09	
		148	<0.02	<0.02	0.02 <sup>2</sup>	0.06	
		175	<0.02	<0.02	<0.02	<0.06	
A83064b (sugar beet)	1 x 1.02*	132	<0.02	<0.02	0.06	0.10	0.02
		160	<0.02	<0.02	0.04	0.08	
		193	<0.02	<0.02	0.04	0.08	
	1 x 1.02*	70	<0.02	<0.02	0.03	0.07	
		116	<0.02	<0.02	0.02	0.06	
		148	<0.02	<0.02	0.02	0.06	
		185	<0.02	<0.02	<0.02	<0.06	
	1 x 1.02*	119	<0.02	<0.02	<0.02	<0.06	
		148	<0.02	<0.02	<0.02	<0.06	
		175	<0.02	<0.02	<0.02	<0.06	
A83071c (sugar beet)	1 x 1.13, Po	137	<0.03	<0.03	<0.06	<0.12	0.05
	1 x 1.13, Po	149	<0.03	<0.03	<0.03	<0.10	
A62042d (sugar beet)	1 x 0.83, Po	139	<0.01	-††	<0.05‡	<0.06	0.05
	1 x 0.83, Po	144	<0.01	-††	<0.05‡	<0.06	
	1 x 0.83, Po	141	<0.01	-††	<0.05‡	<0.06	
EU-S							
A83005a (sugar beet)	1 x 1.0*	75	<0.02	<0.02	0.03	0.07	0.02
		105	0.02	0.02	0.06	0.10	
		137	<0.02	<0.02	0.03	0.07	
	1 x 1.0*	86	<0.02	<0.02	0.03	0.07	
		118	<0.02	<0.02	<0.02	<0.06	
		150	<0.02	<0.02	<0.02	<0.06	
	1 x 1.0*	64	<0.02	0.04	0.05	0.11	
		93	<0.02	<0.02	<0.02	<0.06	
		125	<0.02	<0.02	<0.02	<0.06	

Study ID	Seasonal appl. rate [kg a.s./ha]	PHI	Residues in tops [mg/kg]				LOQ (individual analytes)
			a.s.	NC 9607	Conj. of NC 20645 <sup>†</sup>	Total residue	
A83290a (sugar beet)	1 x 0.8, Po	118	n.a.	n.a.	n.a.	n.a.	0.02
	1 x 0.8, Po	111	n.a.	n.a.	n.a.	n.a.	
	1 x 0.8, Po	107	<0.02	<0.02	0.02	0.06	

PO post-emergence use

<sup>†</sup> in the residue reports the conjugate of NC 20645 was described as “conjugated form of NC 9607” since the conjugate is cleaved by an acidic hydrolysis step and the exocon is converted into NC 9607 in the methods applied. Extraction and analysis of metabolite NC 9607 (“free form”) and the conjugate of NC 20645 (“conjugated form of NC9607”) was done in separate steps.

1 control sample contained residues of 0.02 mg/kg

2 control sample contained residues of 0.04 mg/kg

3 control sample contained residues of 0.07 mg/kg

n.a. = not analysed

\* decline trials (last sampling = mature crop)

a analysed according to method RESID/73/18/1 (cf. KCA 4.1.2/28)

b analysed according to method RESID/73/18/2 (cf. KCA 4.1.2/29)

c analysed according to method RESID/84/42 (cf. KCA 4.1.2/30)

d analysed according to method AL081/96-0 (cf. KCA 4.1.2/10)

## II. Conclusion

In 1972 – 1993 residue studies showed total residue levels in mature roots (root/body) below 0.10 mg/kg, independent if the product was applied as pre- or post-emergence spray. One sample from S-EU showed a total residue of 0.12 mg/kg, however the respective control sample showed already residues of NC 9607 (0.03 mg/kg) and of the conjugate of NC 20645 (0.02 mg/kg). In addition, this sample was collected 75 days after application and did not represent a fully mature sugar beet.

Mature sugar beet roots collected from the same trial 137 days after application showed no ethofumesate related residues above the LOQ of 0.02 mg/kg.

The post emergence use with an application rate at approximately 1 kg a.s./ha was considered as the worst-case use regarding the magnitude of residues in mature sugar-, and fodder beet.

In the northern European climatic zone 17 post-emergence trials show total residues of ethofumesate and its metabolites in mature sugar, and fodder beet roots as follows: <0.1 (2x), <0.06 (14x), 0.09 mg/kg.

In the southern European climatic zone 3 post-emergence trials show total residues of ethofumesate and its metabolites in mature sugar-, and fodder beet roots as follows: <0.06 mg/kg (3x).

Sugar beet tops (leaves) have not been analysed in all trials. The studies in NEU and SEU indicated very low residues. The total residue levels ranged from <0.06 mg/kg to 0.14 mg/kg. Again, the post emergence use with an application rate at approximately 1 kg a.s./ha was considered as the worst-case use regarding the magnitude of residues in mature sugar-, and fodder beet tops.

In the northern European climatic zone 15 post-emergence trials show total residues of ethofumesate and its metabolites in mature sugar-, and fodder beet tops as follows: <0.12, <0.10, 0.07, 0.06, <0.06 (11x) mg/kg.

In the southern European climatic zone in 1 post-emergence trial the total residue of ethofumesate and its metabolites in mature sugar beet leaves was 0.06 mg/kg.

The current EU MRL for sugar beets is 0.5 mg/kg and respects the fact that the analysis of ethofumesate related residues is difficult as shown in the method validation of the different methods. Recoveries of the metabolites showed often a high variability and in some cases the recoveries of the metabolites were <70%. The difficult analysis can be explained by the high amount of interfering matrix compounds (e.g. sugar and starch).

New data for Annex I Renewal (AIR):**B.7.3.2.1. Residue trials in Sugar beet (Bayer CropScience) – SEU**

Additional supervised residue trials, conducted in southern Europe, are presented to support the use in beets (sugar beets, fodder beets and beetroots) and confirm the current MRLs in *Beta vulgaris*.

**Southern European residue region**

Report:	KCA 6.3.5 /57; Helgers, A.; 1997; M-165366-02; Amended: 1997-02-27
Title:	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
Report No:	A89772
Document No:	M-165366-02-1
Guidelines:	Deviation not specified
GLP/GEP:	yes

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Report:	KCA 6.3.5 /58; Schulte, G.; 2013; M-444836-02; Amended: 2013-07-09
Title:	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
Report No:	10-2109
Document No(s):	Report includes Trial Nos.: 10-2109-01 10-2109-02 10-2109-03 10-2109-04 M-444836-02-1
Guidelines:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) US EPA OCSPP Guideline No. 860.1500.SUPP; Except for the soil characterization, the weather data recording, the irrigation recording, the pesticide history, the cultural practices and the applications for maintenance (if relevant) which were not conducted under GLP.
GLP/GEP:	yes

**I. Materials and Methods**

Four independent residue trials were performed in sugar beets in Italy (SEU), testing two different Ethofumesate & Lenacil formulations (420 SC and 420 WP) in parallel, containing 300 g/L (300 g/kg) ethofumesate and 120 g/L (120 g/kg) lenacil. One application was made before emergence of the sugar beet at a nominal rate of 3.5 L/ha or 3.5 kg/ha, corresponding to 1.05 kg ethofumesate/ha and 420 g lenacil/ha.; the water rate was 300 L/ha, reflecting local practice in the trial region.

Additional four independent trials were performed in Italy (2), Spain (1) and Greece (1), using the representative formulation Ethofumesate SC 500, containing 500 g/L ethofumesate. A total of 2 L product per hectare was applied post-emergence (at BBCH 17 to 18 = 7 to 8 leaves unfolded) which is equivalent to 1.0 kg ethofumesate/ha. The water rate was 300 L/ha. The applications in all studies were at the representative application rates for EU approval.

Samples of sugar beets were taken at maturity (BBCH code 49 = beet root has reached harvestable size) or at the day of application and at maturity. Laboratory samples of sugar beet roots and sugar beet tops (leaves) were prepared within 24 hours after sampling.

**Table 7.3.2-5 Application scenario in residue trials conducted in/sugar beet after spraying with Ethofumesate & Lenacil (420 SC or 42 WP) or Ethofumesate SC 500 (southern EU residue region)**

Study No. Trial No. Plot No. GLP Year	Crop Variety	Country	Application					DALT
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	
Ethofumesate & Lenacil SC 420								
A89772 ER95ECS446 ITA0001 ITA0001-P2 GLP: yes 1995	Beet, sugar Monodoro	Italy 40128 Bologna Europe, South	420 SC	1	1.05	0.35	03	170
A89772 ER95ECS446 ITA0002 ITA0002-P2 GLP: yes 1995	Beet, sugar Adrienne	Italy 40052 Baricella Europe, South	420 SC	1	1.05	0.35	05	165
A89772 ER95ECS446 ITA0003 ITA0003-P2 GLP: yes 1995	Beet, sugar Break	Italy 40012 Caldera di Reno Europe, South	420 SC	1	1.05	0.35	03	163
A89772 ER95ECS446 ITA0004 ITA0004-P2 GLP: yes 1995	Beet, sugar Adige	Italy 44020 Gallo Europe, South	420 SC	1	1.05	0.35	05	133
Ethofumesate & Lenacil WP 42								
A89772 ER95ECS446 ITA0001 ITA0001-P2 GLP: yes 1995	Beet, sugar Monodoro	Italy 40128 Bologna Europe, South	42 WP	1	1.05	0.35	03	170
A89772 ER95ECS446 ITA0002 ITA0002-P2 GLP: yes 1995	Beet, sugar Adrienne	Italy 40052 Baricella Europe, South	42 WP	1	1.05	0.35	05	165
A89772 ER95ECS446 ITA0003 ITA0003-P2 GLP: yes 1995	Beet, sugar Break	Italy 40012 Caldera di Reno Europe, South	42 WP	1	1.05	0.35	03	163
A89772 ER95ECS446 ITA0004 ITA0004-P2 GLP: yes 1995	Beet, sugar Adige	Italy 44020 Gallo Europe, South	42 WP	1	1.05	0.35	05	133
Ethofumesate 500 SC								
10-2109	Beet, sugar	Spain	500	1	1.0	0.333	17	155



Study No. Trial No. Plot No. GLP Year	Crop Variety	Country	Application					DALT
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	
			SC					
10-2109-01 10-2109-01-T GLP: yes 2011	Sanlucar	E-41730 Las Cabezas Europe, South	SC					
10-2109 10-2109-02 10-2109-02-T GLP: yes 2010	Beet, sugar Leila	Italy I-44012 Settepolesini Europe, South	500 SC	1	1.0	0.333	18	116
10-2109 10-2109-03 10-2109-03-T GLP: yes 2010	Beet, sugar Houston	Italy I-40128 Bologna Europe, South	500 SC	1	1.0	0.333	18	84
10-2109 10-2109-04 10-2109-04-T GLP: yes 2010	Beet, sugar Greta	Greece GR-50100 Drepano Europe, South	500 SC	1	1.0	0.333	17	116

The samples were analysed for ethofumesate related residues (parent ethofumesate, metabolite NC 9607 and free and conjugated metabolite NC 20645) using either method RESID/96/21 (cf. KCA 4.1.2/08) or method 00955/M002 (cf. KCA 4.1.2/26). In both methods the conjugate of metabolite NC 20645 is converted to the common moiety NC 9607, which is the analytical target besides parent ethofumesate. In both methods the ethofumesate related residues are extracted in a two-part process and quantified using GC-MSD. In the first step, ethofumesate is separated from the more polar metabolites by extraction with n-hexane or a mixture of ethyl acetate/n-hexane. The metabolites remain in the aqueous phase. In the second step, the aqueous phase (containing the metabolites) and the solids from the first extraction step (containing bound residues) are hydrolysed with concentrated hydrochloric acid for 60 to 90 minutes to liberate bound residues and to cleave the conjugate of metabolite NC 20645. Under the acidic conditions, the common moiety NC 9607 is formed from the exocon NC 20645 by intramolecular condensation. After partition of the common moiety NC 9607 into diethyl ether or a mixture of ethyl acetate/n-hexane, the extracts are subjected to a clean-up step and analysed by GC-MSD.

The respective LOQs for ethofumesate and the common moiety NC 9607 were 0.05 mg/kg (in analyte equivalents) in method RESID/96/21 or 0.01 mg/kg (in analyte equivalents) in method 00955/M002. Residue values were not corrected for recoveries.

## II. Results and discussion

Concurrent recoveries of ethofumesate and the common moiety NC 9607 were obtained from samples of sugar beet roots and sugar beet tops (leaf with root collar) at levels between 0.01 mg/kg and 0.1 mg/kg (expressed in analyte equivalents) and additionally of immature whole plants with roots at levels between 0.01 mg/kg and 34 mg/kg for ethofumesate and at levels between 0.01 mg/kg and 0.9 mg/kg for NC 9607 (expressed in analyte equivalents) in study 10-2109. The sample materials chosen served to represent all relevant sample materials collected in these trials. Mean recoveries for sugar beet roots and tops were all within acceptable ranges (74-

104%, RSDs 5.6-11.8%, n=1-3), except for one single recovery determined for NC 9607 in sugar beet body at a spiking level of 0.01 mg/kg (61%). This recovery was accepted since it was only an individual value and the corresponding recoveries determined during method validation were all within the acceptable range of 70-110% (cf. KCA 4.1.2/26). For the matrix “whole plant with roots” also some individual recoveries were below 70%, however since this matrix is neither a food nor a feed commodity and the samples were analysed for information purposes only, the somewhat lower recoveries were deemed acceptable. Details of the concurrent recovery data are shown in Table 7.3.2-7.

All trial data are summarized below and in greater detail at the end of this document (Annex I).

Samples of immature whole plants with roots, sampled at the day of application, yielded ethofumesate residues ranging from 12 to 34 mg a.s./kg (median value 25 mg/kg) and NC 9607 residues ranging from 0.05 to 0.93 mg NC 9607/kg (median value 0.16 mg NC 9607/kg), equating to 0.05 to 0.84 mg a.s./kg (median value 0.14 mg a.s./kg). The residues of both analytes declined to levels below the limit of quantification in mature roots at harvest. In study M-165366-02-1 (cf. KCA 6.1.3/57), relevant residues of ethofumesate were determined in sugar beet root and sugar beet top (root collar with leaves) samples taken 133 to 170 days subsequent to the application. Ethofumesate, as well as NC 9607 residues were below the limit of quantification of 0.05 mg/kg in roots and leaves independent from the formulation used. Thus the “total ethofumesate residue” was always below 0.1 mg/kg (< 0.095 mg/kg).

In study M-444836-01-1 (cf. KCA 6.1.3/58), ethofumesate, as well as NC 9607 residues were below the limit of quantification of 0.01 mg/kg in roots and leaves sampled at maturity, 84 to 155 days after the application. The “total ethofumesate residue” was always below 0.02 mg/kg, when expressed in analyte equivalents or in a.s. equivalents (<0.02 mg/kg).

In the following table only those trials were summarised which were conducted according to the worst case use parameters (1 kg a.s./ha and BBCH 16-18).

**Table 7.3.2-6 Results of residue trials conducted in/on sugar beet after spraying with Ethofumesate & Lenacil (420 SC or 42 WP) or Ethofumesate SC 500 (southern EU residue region)**

Study No. Trial No. Plot No. GLP Year	Portion analysed	DALT (days)	Residues (mg/kg)			
			Ethofumesate (a.s.)	NC 9607 (analyte equiv.)	NC 9607 (a.s. equiv.)	total residue calculated
Ethofumesate 500 SC						
10-2109 10-2109-01 10-2109-01-T GLP: yes 2011	whole plant with root	0	25	0.17	0.19	25.2
	body	155	<0.01	<0.01	<0.011	<0.02
	leaf with root collar	155	<0.01	<0.01	<0.011	<0.02
10-2109 10-2109-02 10-2109-02-T GLP: yes 2010	whole plant with root	0	25	0.14	0.156	25.2
	body	116	<0.01	<0.01	<0.011	<0.02
	leaf with root collar	116	<0.01	<0.01	<0.011	<0.02

Study No. Trial No. Plot No. GLP Year	Portion analysed	DALT (days)	Residues (mg/kg)			
			Ethofumesate (a.s.)	NC 9607 (analyte equiv.)	NC 9607 (a.s. equiv.)	total residue calculated
10-2109 10-2109-03 10-2109-03-T GLP: yes 2010	whole plant with root	0	12	0.05	0.056	12.1
	body	84	<0.01	<0.01	<0.011	<0.02
	leaf with root collar	84	<0.01	<0.01	<0.011	<0.02
10-2109 10-2109-04 10-2109-04-T GLP: yes 2010	whole plant with root	0	34	0.93	1.04	35.0
	body	116	<0.01	<0.01	<0.011	<0.02
	leaf with root collar	116	<0.01	<0.01	<0.011	<0.02

Table 7.3.2-7 Concurrent recovery data for ethofumesate and NC 9607 in/on sugar beet matrices

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
Ethofumesate & Lenacil SC 420 and WP 42										
A89772 ER95ECS446 ITA0001 to ITA0004 ITA0001-P2 to ITA0004-P2 GLP: yes 1995	Beet, sugar	body	ethofumesate	2	0.05	91; 76	76	91	84	-
				3	0.10	107; 97; 107	97	107	104	5.6
				5	overall		76	107	96	13.5
			NC 9607	2	0.05	83; 64	64	83	74	-
				1	0.10	84			84	-
				3	overall		64	84	77	14.6
		leaf with root collar	ethofumesate	1	0.1	77			77	-
				1	overall				77	-
			NC 9607	1	0.1	103			103	-
				1	overall				103	-
Ethofumesate SC 500										
10-2109 10-2109-01 to 10-2109-04 10-2109-01-T to 10-2109-04-T GLP: yes 2011	Beet, sugar	whole plant with root	ethofumesate	3	0.01	63; 67; 57	57	67	62	8.1
				3	0.10	83; 76; 73	73	83	77	6.6
				1	34	84			84	-
				7	overall		57	84	72	14.1
			NC 9607	3	0.01	71; 86; 70	70	86	76	11.8
		3		0.10	78; 68; 62	62	78	69	11.7	
		1		0.90	81			81		
			7	overall		62	86	74	11.3	
		body	ethofumesate	1	0.01	80			80	-
				1	overall				80	-
			NC 9607	1	0.01	61			61	-
				1	overall				61	-
		leaf with root collar	ethofumesate	1	0.01	90	90	90	90	-
				1	0.10	86			86	-
			NC 9607	2	overall		86	90	88	-
				1	0.01	103	103	103	103	-
				1	0.10	87			87	-

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
				2	overall		87	103	95	-

#### B.7.3.2.2. Residue trials in Sugar beet (UPL) - NEU + SEU

The following studies were submitted by United Phosphorus Ltd to support the use of ethofumesate on sugar beets. The studies were performed to cover the proposed residue definition (sum of ethofumesate, NC 9607, NC 20645 and its conjugates).

Some of the analytical methods used in the new residue trials presented below proved the analysis of conjugated NC 20645 by using NC 20645 as reference standards. Moreover, a study is summarised therein, showing that conjugated as well as free NC 20645 will be transformed to and quantified as NC 9607 when using acidic conditions (e.g. acidic hydrolysis step) and NC 20645 will be transformed to and quantified as NC 9607 when analysis is done by GC/MS. A new method validation study on an existing method for the determination of Ethofumesate and its metabolites NC 20645 and NC 9607 in sugar beet roots and tops is summarised herein which corroborates these conclusions. Hence, older residue trials which have already been reviewed on EU level can be used to support the current submission, if ethofumesate and NC 9607 have been analysed by using acidic conditions and GC/MS analysis, since these methods would have analysed unintentionally free and conjugated NC 20645 as well as part of the analysis of NC 9607.

Report:	KCA 6.3.1/01, Tandy, R. (2012a)
Title:	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
Document No:	S09-01656
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/02, Perny, A. (2002)
Title:	Residue study in sugar beets following treatments with a formulated product containing Ethofumesate 128 g/l, Phenmedipham 62 g/l and Desmedipham 16 g/l on sugar beet fields under field conditions in France and in the Netherlands in 2000
Document No:	R A0015
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/03, Perny, A. (2003)
Title:	Residue study in sugar beets following treatments with a formulated product containing Ethofumesate 128 g/l, Phenmedipham 62 g/l and Desmedipham 16 g/l on sugar beet fields under field conditions in France and in The Netherlands in 2001
Document No:	R A1114
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part

GLP:	A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5) Yes
Report: Title:	KCA 6.3.1/04, Huauilmé, J.-M. (2013a) 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
Document No:	BPL12/436/GC
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report: Title:	KCA 6.3.1/05, Chevallier, E. (2012) 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
Document No:	BPL11/380/GC
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report: Title:	KCA 6.3.1/06, Waalkens, W.M. and Hamberger, R. (2005a) Determination of the decline of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l EC, Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2003
Document No:	R03-16-NF-08
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report: Title:	KCA 6.3.1/07, Waalkens, W.M. and Hamberger, R. (2005b) Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l EC, Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2003
Document No:	R03-16-NF-09
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report: Title:	KCA 6.3.1/08, Waalkens, W.M. and Hamberger, R. (2005c) Determination of the decline of the residues of phenmedipham, MHPC, methylaniline, desmedipham, EHPC, aniline, ethofumesate, 2-keto-ethofumesate in/on sugar beet plants and roots after foliar applications of phenmedipham 157 g/l SE and ethofumesate/phenmedipham/desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and in northern France, 2004
Document No:	R04-16-NF-08

Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/09, Waalkens, W.M. and Hamberger, R. (2005d)
Title:	Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l EC, Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2004
Document No:	R04-16-NF-09
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/10, Anspach, T. (2001)
Title:	Magnitude of the residue of Phenmedipham, Desmedipham, Ethofumesate and its metabolite 2-oxo-Ethofumesate in sugar beets (roots and leaves/tops) after the application of Betasana Trio under filed conditions in Germany, 2000
Document No:	ADN-0004
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/11, Tandy, R. (2013)
Title:	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
Document No:	S10-00258
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes
Report:	KCA 6.3.1/12, Waalkens, W.M. and Hamberger, R. (2005e)
Title:	Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in northern Spain, 2003
Document No:	R03-16-SP-06
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes

Report:	KCA 6.3.1/13, Waalkens, W.M. and Hamberger, R. (2005f)
Title:	Determination of the decline of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on fodder beet plants and roots after foliar applications of Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to fodder beets in southern France, 2003
Document No:	R03-16-FR-07
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes

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Report:	KCA 6.3.1/14, Huauclm�, J.-M. (2013b)
Title:	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
Document No:	BPL12/435/GC
Guidelines:	According to Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market General recommendation for the design, preparation and realisation of residue trials (7029/VI/95 rev. 5)
GLP:	Yes

## I. Materials and Methods

In the growing seasons from 2000 to 2012, 37 trials (49 plots) have been carried out in Northern Europe and 9 trials (9 plots) have been carried out in Southern Europe in several countries of the EU (UK, NL, BE, DE, N-FR, S-FR, ES, IT). Ethofumesate was applied either once or twice or three times (a summary of the application scenarios is listed in the table below.

**Table 7.3.2-8 Application scenario in residue trials conducted in/sugar beet after spraying with ethofumesate (northern and southern EU residue region)**

Study No. Trial No. Plot No. GLP Year	Crop Variety	Country	Application			DALT
			No	g/ha (a.s.)	GS	
Northern Europe						
S09-01656 S09-01656-01 (2012a)	Sugar beet, BEAVA, Opta	NG32 1PN Harston, Notts, UK	1	1031	BBCH 10-12	146
			1	1078	BBCH 18	111
			3	224 245 225	BBCH 14 BBCH 17 BBCH 18	111
S09-01656 S09-01656-02 (2012)	Sugar beet, BEAVA, Bullfinch	C07 6PE Colchester, Essex, UK	1	1050	BBCH 12-13	188
			1	1022	BBCH 18	162
			3	184 232 229	BBCH 14 BBCH 16 BBCH 18	162
S09-01656 S09-01656-03 (2012)	Sugar beet, BEAVA, Bobcat	YO42 4RU North Yorkshire, UK	1	1000	BBCH 07	149
			1	1067	BBCH 18	149
			3	190 238 245	BBCH 15 BBCH 16 BBCH 18	69
S09-01656 S09-01656-04	Sugar beet, BEAVA, Ace	YO8 5LF Osgodby		983	BBCH 07	145
				967	BBCH 18	102

Study No. Trial No. Plot No. GLP Year (2012)	Crop Variety	Country	Application			DALT
			No	g/ha (a.s.)	GS	
		North Yorkshire, UK		196 215 215	BBCH 15 BBCH 16 BBCH 18	102
R A0015 A0015 ND1 (2002)	Sugar beet, Somate	60400 Appilly, NORTHERN FRANCE	2	372.3 369.1	BBCH 16 BBCH 33-39	175
R A0015 A0015 AN1 (2002)	Sugar beet, Rifle	67160 Seebach, NORTHERN FRANCE	2	387.0 383.3	BBCH 16 BBCH 16-18	164
R A0015 A0015 HL1 (2002)	Sugar beets, Lonera	6085NR Horn, THE NETHERLANDS	2	394.6 365.3	BBCH 16-18 BBCH 19	164
R A0015 A0015 HL2 (2002)	Sugar beets, Aresto	6201AB Mechelen THE NETHERLANDS	2	399.4 394.0	BBCH 14 BBCH 16-17	127
R A1114 R A1114 BP1 (2003)	Sugar beets, Sterna	45170 Attray NORTHERN FRANCE	2	381 400	BBCH 16 BBCH 31	103
R A1114 (2003)	Sugar beets, Rifle	67160 Seebach, NORTHERN FRANCE	2	376 397	BBCH 16 BBCH 16-18	110
R A1114 A1114 HL1 (2003)	Sugar beets, Toledo	6281AB Mechelen THE NETHERLANDS	2	402 364	BBCH 16 BBCH 18	134
R A1114 A1114 HL2 (2003)	Sugar beets, Hiteatia	6085NR Horn THE NETHERLANDS	2	386 394	BBCH 16 BBCH 18-19	119
BPL 12/436/GC BPL12/436/GC-01-NL (2013)	Sugar Beet, Shakira	5973 AC Lottum Limburg – NL	1	1021	BBCH 18	113
BPL 12/436/GC BPL12/436/GC-02-NL (2013)	Sugar beet, Coyote	6599 CJ Ven- Zelderheide Limburg – NL	1	975	BBCH 18	116
BPL 12/436/GC BPL12/436/GC-03-BE (2013)	Sugar beet, Rubens	3470 Kortenaken Brabant – BELGIUM	1	1046	BBCH 18	113
BPL 12/436/GC BPL12/436/GC-04-BE 2013	Sugar beet, Candama	3890 Kortijns Limburg – BELGIUM	1	1002	BBCH 18	113
BPL 11/380/GC BPL11/380/GC-01-NL, Plot T3 2012	Sugar beet, Arrival	5973 RC Lottum Limburg– The NETHERLANDS	1	1032	BBCH 14	124
BPL 11/380/GC BPL11/380/GC-02-NL, Plot T3 2012	Sugar beet, Coyote	6595 CJ Ottersum Limburg– The NETHERLANDS	1	1014	BBCH 14	125
BPL 11/380/GC BPL11/380/GC-03-BE, Plot T3 2012	Sugar beet, Rubens	3473 Waanrode Brabant – BELGIUM	1	1024	BBCH 14	136
BPL 11/380/GC BPL11/380/GC-04-BE, Plot T3 2012	Sugar beet, Bernadette	3870 Opheers Limburg– BELGIUM	1	1004	BBCH 14	136



Study No. Trial No. Plot No. GLP Year	Crop Variety	Country	Application			DALT
			No	g/ha (a.s.)	GS	
R03-16-NF-08 R03-168-01 2005	Sugar beets, Cyntia	Elst, NL	2	261.23 531.81	BBCH 12 BBCH 14	173
R03-16-NF-08 R03-168-02 2005	Sugar beets, Guépard	Le Gault Saint Denis N-FRANCE	2	278.9 536.7	BBCH 12 BBCH 14	120
R03-16-NF-09 R03-169-01 2005	Sugar beets, Santesse	Angeren THE NETHERLANDS	2	275.99 527.33	BBCH 12 BBCH 14	142
R03-16-NF-09 R03-169-02 2005	Sugar beets, Baccara	Esbarres NORTHERN FRANCE	2	265.4 548.1	BBCH 12 BBCH 16	146
R04-16-NF-08 R04-168-01 2005	Sugar beets, Shakira	Valburg NL	2	258.85 522.40	BBCH 12 BBCH 14-15	134
R04-16-NF-08 R04-168-02 2005	Sugar beets, Monarch	Inchy en Artois N-FRANCE	2	280.79 596.84	BBCH 12 BBCH 14	166
R04-16-NF-09 R04-169-01 2005	Sugar beets, Pursan	Angeren THE NETHERLANDS	2	264.60 536.34	BBCH 12 BBCH 14-15	135
R04-16-NF-09 R04-169-02 2005	Sugar beets, Crocodile	Houdilcourt NORTHERN FRANCE	2	283.01 545.26	BBCH 12 BBCH 14	155
AND-0004 FR 20/00/50 2001	Sugar beet, Fox	02692 Gnaschwitz GERMANY	3	229 292 289	BBCH 12 BBCH 14 BBCH 18	92
AND-0004 FR 20/00/70 2001	Sugar beet, Ascona	04668 Motterwitz	3	230 294 297	BBCH 12 BBCH 16 BBCH 18	138
AND-0004 AR 0003 2001	Sugar beet, Helix	Velen-Ramsdorf, Westphalia GERMANY	3	230 286 286	BBCH 12 BBCH 16 BBCH 19	121
AND-0004 AC/00/59 2001	Sugar beet, Ascona	16833 Lentzke GERMANY	3	231 294 292	BBCH 12 BBCH 14-16 BBCH 16-18	107
S10-00258 S10-00258-01 2013	Sugar Beet BEAVA Carissima	PE6 OSY, Thorney, Cambridgeshire, UK	1	1040	0	153
			1	1070	14-18	97
			3	200 388 400	BBCH 14-18	97
S10-00258 S10-00258-02 2013	Sugar Beet BEAVA bobcat	PE9 4BE, Little Casterton, Cambridgeshire, UK	1	1015	0	189
			1	1100	16	134
			3	200 452 408	18	134
S10-00258 S10-00258-03 2013	Sugar Beet BEAVA Saracen	C07 8SD, Little Bentley, Essex, UK	1	1000	BBCH 5	206
			1	996	BBCH 18	155
			3	202 416 404	BBCH 18	155
S10-00258 S10-00258-04 2013	Sugar Beet BEAVA	IP21 4BQ, Thrandeston, Cambridgeshire, UK	1	1015	5	204
			1	1010	18	153

Study No. Trial No. Plot No. GLP Year	Crop Variety	Country	Application			DALT
			No	g/ha (a.s.)	GS	
			3	196 396 404	18	
S10-00258 S10-00258-09 2013	Sugar Beet BEAVA Bobcat	PE9 4BE, Little Casterton, Cambridgeshire, UK	1 3	1020 206 432 412	18 18	105 105
Southern Europe						
R03-16-SP-06 R03-166-01 2005	Sugar beets, Alama	Rodezno SPAIN		274.3 562.4	BBCH 12 BBCH 14	165
R03-16-FR-07 R03-167-01 2005	Fodder beets, Boléro	Niort SOUTHERN FRANCE		260.0 524.8	BBCH 12 BBCH 14	146
BPL12/435/GC BPL12/435/GC-01-SP 2013	Sugar Beet, Isabella KWS	01213-Lantarón Alava, SPAIN		937	BBCH 18	128
BPL12/435/GC BPL12/435/GC-02-SP 2013	Sugar Beet, Sonja	01428-Iruna de Oca Alava SPAIN		1009	BBCH 18	128
BPL12/435/GC BPL12/435/GC-03-IT 2013	Sugar Beet, Nektarine	35040-Merlara, Veneto ITALY		974	BBCH 18	108
BPL12/435/GC BPL12/435/GC-04-IT 2013	Sugar Beet, Montana	37055-Ronco all'Àdige, Verona ITALY		979	BBCH 18	108
S10-00258 S10-00258-05 2013	Sugar Beet BEAVA Rizor	40054, Budrio, Bologna ITALY		1035	00-07	137
S10-00258 S10-00258-06 2013	Sugar Beet BEAVA, Pauletta	48017, Conselice, Ravenna, ITALY		915	0	132
S10-00258 S10-00258-07 2013	Sugar Beet BEAVA Noelia	42212, Covarrubias, Soria SPAIN		1000	0	185
S10-00258 S10-00258-08 2013	Sugar Beet BEAVA Emestina	42392, Velamazán, Soria, SPAIN		1050	0	189
S10-00258 S10-00258-10 2013	Sugar Beet BEAVA Newton	Lebrija, SPAIN		1040	0	71

In those trials in which Ethofumesate was applied once, the application rate ranged between 915 g a.s./ha and 1100 g a.s./ha. These application rates are within the 25% deviation of the intended critical application rate of 1000 g a.s./ha. The application was done at a BBCH between 0 and 18.

The critical GAP is covered by the post-emergence application (up to BBCH 18) at a critical rate of 1000 g a.s./ha. Hence, those trials in which ethofumesate was applied post-emergence at 1000 g a.s./ha constitute a worst-case scenario and were further considered in the evaluation.

The notifier also submitted residue data representing the split application (2 and 3 applications). As these trials are considered as less critical, the results of these trials were reported in Annex I as additional information only.

Samples of whole plants with roots, leaves with tops were sampled for analysis. The crucial commodities for human consumption and livestock feeding, i.e. roots at harvest and leaves with tops at harvest, were taken between 87 days and 220 days after last application. Samples were stored deep frozen until analysis. The maximum storage period of sugar and fodder beet leaves and roots at harvest from sampling until extraction was 364 days. The storage period of ethofumesate and its relevant metabolites in sugar beet leaves and roots is covered by available storage stability data.

#### **Analysis of samples and validation of methods**

Specimens of leaves with tops/leaves with collar and roots were analysed for Ethofumesate and its metabolites taken into account the proposed residue definition: sum of Ethofumesate, 2-keto-ethofumesate (NC 9607), opening-2-keto-ethofumesate (NC 20645) and its conjugate. The methods are detailed below. The suitability of the respective method to cover the proposed residue definition is given as well at the end of this subsection.

A new method validation study is summarised below (Weir, 2014) which shows that the method A0019 fulfils the criteria of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 for the analysis of Ethofumesate, NC 9607, and free and conjugated NC 20645 in sugar beet roots and tops as well. The new limit of quantification of this method is now 0.01 mg/kg for each analyte and matrix. This new study furthermore shows that free NC 20645 will enter the acidic hydrolysis step of the method and hence will be converted into NC 9607. This means that the older studies that have been analysed according to this method will have covered not only the conjugated NC 20645, but also the free form of NC 20645 (both forms measured as 2-keto ethofumesate = NC 9607). These older studies therefore also cover the new residues definition.

**KCA 6.3.1/01 (Tandy, R. (2012a), S09-01656), KCA 6.3.1/02 (Perny, A. (2002), R A0015), KCA 6.3.1/03 (Perny, A. (2003), R A1114) and KCA 6.3.1/11 (Tandy, R. (2013), S10-00258):** Crop specimens were analysed for residues of Ethofumesate and 2-keto ethofumesate (free and conjugated form) using the method as detailed in ANADIAG S.A. study report number A0019 “Validation of the Method of Analysis of the residues of Ethofumesate and its metabolite 2-keto Ethofumesate (free and conjugated form) in Sugar Beets”. This report is attached to the report of Tandy, R. (2012a). The method was validated by 5 recoveries at LOQ and 5 recoveries at 10x LOQ for sugar beets leaves and roots for Ethofumesate and 2-keto-ethofumesate (extraction procedure for free analyte and extraction procedure for conjugated analyte). Recoveries were well in the range of 70-110%, the relative standard deviation was well below 20%. The method fulfils therefore the criteria of SANCO/3029/99 rev.4.

Free residues were extracted with dichloromethane/methanol and the conjugated residues were extracted from residual filter cake by extraction with aqueous methanol followed by acidic hydrolysis to yield free metabolites. Extracts were purified by liquid partitioning. After concentration to low volume, quantification was carried out with gas chromatography with mass selective detection. The limit of quantification for Ethofumesate and 2-keto Ethofumesate in sugar beet whole plant, leaves with tops and roots was 0.05 mg/kg.

Procedural recoveries were done concurrently with leaves with tops/leaves with collar samples at 0.05 mg/kg-1.0 mg/kg resulting in an overall mean recovery of 82-105.7% for Ethofumesate, 83-96.3% for “free”-2-keto ethofumesate and 74.1-102% for “conjugated”-2-keto Ethofumesate. Procedural recoveries were done concurrently with root samples at 0.05 mg/kg-10.0 mg/kg resulting in an overall mean recovery 83-92.6% for Ethofumesate, 82-99.0% for “free”-2-keto ethofumesate and 74.7-101% for “conjugated”-2-keto Ethofumesate.

Procedural recoveries were done concurrently with whole plant with roots samples at 0.05 mg/kg-25 mg/kg resulting in an overall mean recovery of 82-90.2% for Ethofumesate, 78.6-87% for “free”-2-keto ethofumesate and 73.2-96% for “conjugated”-2-keto ethofumesate. These studies cover the proposed residues definition.

**KCA 6.3.1/04 (Hualmé, J.-M. (2013a), BPL12/436/GC), KCA 6.3.1/05 (Chevallier, E. (2012), BPL11/380/GC), KCA 6.3.1/14 (Hualmé, J.-M. (2013b), BPL12/435/GC):** Crop specimens were analysed for residues of Ethofumesate and its metabolites NC8493 (free and conjugated), NC9607 and NC20645 (free and conjugated) using the validated method 11A04042-01-VMSB. Metabolites NC20645 and NC9607 were both measured as NC9607. The validation of this method is detailed in the CA 4.2. The validation was done according to SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. Please refer to the section for analytical methods. This method is in analogy to the method described in “De Bredelaar BV” studies presented herein (R03-16-NF-08 (20031150/03-R(V)SB), R03-16-NF-9 (20031150/04-RSB), R04-16-NF-08 (20031150/05-RSB), R04-16-NF-09 (20031150/06-RSB), R03-16-SP-06 (20031150/01-RSB), R03-16-FR-07 (20031150/02-RSB)) and the method described in KCA 6.3.1/10 (Anspach, T. (2001), ADN0004).

Sugar beet specimens (whole young plants with root, leaves with 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 NC 9607 into its open form (opening of the cyclic ester, this is metabolite NC 20645). In the following extraction step with hexane, only Ethofumesate was extracted, whereas the metabolites NC 8493, NC 9607 and NC 20645 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 metabolites were converted into its free form at this step. Finally, the hydrolysed sample matrix cleaned up by SPE. 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 clean-up was done. Analysis was performed using capillary gas chromatography with mass selective detection (GCMSD). The metabolite NC 20645 (free and conjugated) is converted to NC 9607 within the analytical method and was finally detected as NC 9607. In the three different matrices of sugar beet (whole plants with root, leaves with top and roots), the limit of quantification (LOQ) was 0.01 mg/kg for Ethofumesate, for its metabolite NC8493 and for the sum of its metabolites NC 20645 and NC 9607. Metabolites NC20645 and NC9607 were both measured as NC 9607. The limit of detection (LOD) was 0.003 mg/kg for Ethofumesate and for its metabolites.

Procedural recoveries were done concurrently with leaves with tops samples at 0.005 mg/kg-0.01 mg/kg resulting in an overall mean recovery of 87-92% for Ethofumesate, 86-92% for sum of NC 9607 and NC 20645 and 91-92% for NC 8493. Procedural recoveries were done concurrently with root samples at 0.005 mg/kg-0.01 mg/kg resulting in an overall mean recovery of 90-99% for Ethofumesate, 86-91% for sum of NC 9607 and NC 20645 and 88-91% for NC 8493. Procedural recoveries were done concurrently with whole plant with roots samples at 0.005 mg/kg-5 mg/kg resulting in an overall mean recovery of 78-104% for Ethofumesate, 83-94% for sum of NC 9607 and NC 20645 and 86-103% for NC 8493.

These studies cover the new residues definition.

**KCA 6.3.1/06 (Waalkens and Hamberger (2005a), R03-16-NF-08), KCA 6.3.1/07 (Waalkens and Hamberger (2005b), R03-16-NF-09), KCA 6.3.1/08 (Waalkens and Hamberger (2005c), R04-16-NF-08), KCA 6.3.1/09 (Waalkens and Hamberger (2005d), R04-16-NF-09), KCA 6.3.1/12 (Waalkens and Hamberger (2005e), R03-16-SP-06), KCA 6.3.1/13 (Waalkens and Hamberger (2005f), R03-16-FR-07):**

Crop specimens were analysed for residues of Ethofumesate and its metabolite 2-keto ethofumesate (NC 9607, free and conjugated with the conjugated form being in fact conjugated NC 20645) using the method validated in 20031150/01-RVSB, 20031150/02-RVSB, 20031150/03-RVSB, 20031150/04-RVSB. The validation of the method is attached to the report R03-16-NF-08, R03-16-NF-09, R03-16-SP-06 and R03-16-FR-07. The method was validated according to SANCO/825/00 rev. 6 and rev.7. At least 5 recoveries were carried out at the LOQ of 0.02 mg/kg in each matrix and for each analyte and at least 5 recoveries were carried out at 10x LOQ in each matrix and for each analyte. The single recoveries ranged between 70-110% for Ethofumesate and NC9607 (2-keto Ethofumesate) in each matrix. The relative standard deviation was <20%. Hence, the method fulfils the requirements of SANCO/3029/99 rev.4. The limit of quantification is 0.02 mg/kg for each analyte and each matrix. The limit of detection is 0.006 mg/kg for each analyte and each matrix.

The method for extraction and analysis of Ethofumesate and its metabolite 2-keto ethofumesate (NC 9607) is identical to the method used in studies KCA 6.3.1/04 (Hualmé, J.-M. (2013a), BPL12/436/GC), KCA 6.3.1/05 (Chevallier, E. (2012), BPL11/380/GC), KCA 6.3.1/14 (Hualmé, J.-M. (2013b), BPL12/435/GC) including alkaline extraction conditions and acidic hydrolysis of conjugates among others. This method is detailed above. Hence, this method covers -in addition to Ethofumesate and NC 9607- the analysis of NC 20645 (free and conjugated) as well. W.M. Waalkens and R. Hamberger have therefore measured NC 20645 (free and conjugated) as 2-keto-ethofumesate (NC 9607) during the analysis of the trial samples. These studies cover therefore the new residues definition.

The overall mean recovery for both plants and roots was 90% for Ethofumesate and 80-85% for NC 9607 (2-keto Ethofumesate).

**KCA 6.3.1/10 (Anspach, T. (2001), ADN0004):** Crop specimens were analysed for residues of Ethofumesate and its metabolite 2-keto ethofumesate (NC9607) using the analytical test method L-15.045. This method was validated according to SANCO/825/00 rev.6 within the study. Five recoveries were carried out at LOQ (0.02 mg/kg) and 5 recoveries were carried out at 10x LOQ for each analyte and each matrix. Single recovery values are within the range of 70-110%. The overall mean recovery is 98% for Ethofumesate in sugar beet leaves with top and 93% in sugar beet roots and 82% for NC9607 in sugar beet leaves with tops and 80% in sugar beet roots. The relative standard deviation is < 20%. Hence, this method fulfils the requirements of SANCO/3029/99 rev.4. The method for extraction and analysis of Ethofumesate and its metabolite 2-keto ethofumesate (NC9607) is identical to the method used in studies KCA 6.3.1/04 (Hualmé, J.-M. (2013a), BPL12/436/GC), KCA 6.3.1/05 (Chevallier, E. (2012), BPL11/380/GC), KCA 6.3.1/14 (Hualmé, J.-M. (2013b), BPL12/435/GC) including alkaline extraction conditions and acidic hydrolysis of conjugates among others. This method is detailed above. Hence, this method covers -in addition to Ethofumesate and NC 9607- the analysis of NC 20645 (free and conjugated) as well. T. Anspach has therefore measured NC 20645 (free and conjugated) as 2-keto-ethofumesate (NC 9607) during the analysis of the trial samples. This study covers therefore the new residues definition.

The overall mean of the procedural recoveries was 91% for Ethofumesate in sugar beet leaves with tops and sugar beet roots and 84% for NC9607 in sugar beet leaves with tops and 78% for NC9607 in sugar beet roots.

## II. Results and Discussion

For the evaluation of the representative use only those residue trials were taken into account, which represents the worst case scenario with respect to application rate and growth stage at the last treatment. All trial data are summarized below in Table 7.3.2-9 and in greater detail at the end of this document (Annex 1).

Directly after the application ethofumesate residues in whole plant samples were found between 6.8 and 67.8 mg/kg. Residues of the main metabolite NC 9607 were found from <0.05 to 2.2 mg/kg.

In some residue trials the NC 9607 and its conjugates were analysed in separate analytical procedures. The residues of all analytes declined to levels below the limit of quantification in mature roots at harvest. Thus the “total ethofumesate residue” was always below 0.16 mg/kg.

Ethofumesate related residues in green material (whole plant and leaves with tops) declined from 7.20 – 68.2 mg/kg to 0.18 – <0.02 mg/kg at maturity. The residue results are summarised in Table 7.3.2-9. At maturity the predominant residues were found to be NC 20645conjugates.

**Table 7.3.2-9 Results of residue trials conducted in/on sugar beet after spray application in northern and southern Europe**

Study No. Trial No. Plot No. Location Year	Portion analysed	PHI (days)	Residues (mg/kg)					
			Ethofume- sate (a.s.)	NC 9607	NC 9607 (a.s. equiv.)	NC 20645- conj	NC 20645- conj (a.s. equiv.)	total residue
Northern Europe								
S09-01656 S09-01656-01 UK, 2009	Leaves with top	90	<0.05	<0.05	<0.056	0.06	0.067	0.173
	Roots	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with top	111	<0.05	<0.05	<0.056	0.06	0.07	<u>0.18</u>
	Roots	111	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S09-01656 S09-01656-02 UK, 2009	Leaves with top	90	<0.05	<0.05	<0.056	0.25	0.279	0.385
	Roots	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with top	162	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	162	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S09-01656 S09-01656-03 UK, 2009	Leaves with top	121	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	121	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with top	149	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	149	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S09-01656 S09-01656-04 UK, 2009	Whole plant	0	19.18	<0.05	<0.056	0.61	0.68	19.9
	Whole plant	8	0.82	<0.05	<0.056	3.40	3.80	4.7
	Whole plant	21	<0.05	<0.05	<0.056	0.87	0.97	1.1
	Leaves with top	89	<0.05	<0.05	<0.056	0.15	0.17	0.27
	Roots	89	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with top	102	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	102	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
BPL 12/436/GC BPL12/436/GC- 01-NL NL, 2012	Leaves with top	91	<0.01	<0.01	<0.01	1)	1)	<0.02
	Roots	91	<0.01	<0.01	<0.01			<0.02
	Leaves with top	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 12/436/GC BPL12/436/GC- 02-NL NL, 2012	Whole plant	0	16.8	0.12	0.13	1)	1)	16.93
	Whole plant	8	0.31	0.25	0.28			0.59
	Whole plant	29	<0.01	0.03	0.03			0.04
	Leaves with top	90	<0.01	<0.01	<0.01			<0.02
	Roots	90	<0.01	<0.01	<0.01			<0.02
	Leaves with top	116	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	116	<0.01	<0.01	<0.01			<u>&lt;0.02</u>

Study No. Trial No. Plot No. Location Year	Portion analysed	PHI (days)	Residues (mg/kg)					
			Ethofume- sate (a.s.)	NC 9607	NC 9607 (a.s. equiv.)	NC 20645- conj	NC 20645- conj (a.s. equiv.)	total residue
BPL 12/436/GC BPL12/436/GC- 03-BE BE, 2012	Leaves with top	92	<0.01	<0.01	<0.01	1)	1)	<0.02
	Roots	92	<0.01	<0.01	<0.01			<0.02
	Leaves with top	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 12/436/GC BPL12/436/GC- 04-BE BE, 2012	Whole plant	0	19.9	0.72	0.80	1)	1)	20.70
	Whole plant	7	1.12	0.44	0.49			1.61
	Whole plant	28	<0.01	0.03	0.03			0.04
	Leaves with top	92	<0.01	<0.01	<0.01			<0.02
	Roots	92	<0.01	<0.01	<0.01			<0.02
	Leaves with top	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	113	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 11/380/GC BPL11/380/GC- 01-NL, Plot T3 NL, 2011	Leaves with top	124	<0.01	<0.01	<0.01	1)	1)	<u>&lt;0.02</u>
	Root	124	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 11/380/GC BPL11/380/GC- 02-NL, Plot T3 NL, 2011	Whole plant	0	67.8	0.36	0.40	1)	1)	68.20
	Whole plant	7	1.43	0.29	0.32			1.75
	Whole plant	28	0.02	0.02	0.02			0.04
	Leaves with top	96	<0.01	<0.01	<0.01			<0.02
	Root	96	<0.01	<0.01	<0.01			<0.02
	Leaves with top	125	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Root	125	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 11/380/GC BPL11/380/GC- 03-BE, Plot T3 BE, 2011	Leaves with top	136	<0.01	<0.01	<0.01	1)	1)	<u>&lt;0.02</u>
	Root	136	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL 11/380/GC BPL11/380/GC- 04-BE, Plot T3 BE, 2011	Whole plant	0	49.0	1.92	2.145	1)	1)	51.15
	Whole plant	6	4.78	0.38	0.425			5.20
	Whole plant	26	0.03	0.04	0.045			0.07
	Leaves with top	87	<0.01	<0.01	<0.01			<0.02
	Root	87	<0.01	<0.01	<0.01			<0.02
	Leaves with top	136	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Root	136	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
S10-00258 S10-00258-01 UK, 2010	Whole plant with roots	0	25.20	<0.05	<0.056	0.73	0.82	26.07
	Whole plant with roots	6	6.23	<0.05	<0.056	3.90	4.36	10.64
	Whole plant with roots	20	0.08	<0.05	<0.056	1.32	1.48	1.61
	Leaves with tops	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with tops	97	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	97	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S10-00258 S10-00258-02 UK, 2010	Whole plant with roots	1	15.48	<0.05	<0.056	0.68	0.760	16.30
	Whole plant with roots	7	3.56	<0.05	<0.056	2.77	3.10	6.71
	Whole plant with roots	21	0.29	<0.05	<0.056	2.77	3.10	3.44
	Leaves with tops	91	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	91	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with tops	134	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	134	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S10-00258 S10-00258-03 UK, 2010	Leaves with tops	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with tops	155	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	155	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>

Study No. Trial No. Plot No. Location Year	Portion analysed	PHI (days)	Residues (mg/kg)					
			Ethofume- sate (a.s.)	NC 9607	NC 9607 (a.s. equiv.)	NC 20645- conj	NC 20645- conj (a.s. equiv.)	total residue
S10-00258 S10-00258-04 UK, 2010	Leaves with tops	91	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	91	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with tops	153	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	153	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
S10-00258 S10-00258-09 UK, 2010	Whole plant with roots	0	6.78	<0.05	<0.056	0.33	0.37	7.20
	Whole plant with roots	7	0.75	<0.05	<0.056	0.92	1.03	1.83
	Whole plant with roots	21	0.11	<0.05	<0.056	1.33	1.49	1.65
	Leaves with tops	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Roots	90	<0.05	<0.05	<0.056	<0.05	<0.056	<0.16
	Leaves with tops	105	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
	Roots	105	<0.05	<0.05	<0.056	<0.05	<0.056	<u>&lt;0.16</u>
<b>Southern Europe</b>								
BPL12/435/GC BPL12/435/GC-01-SP ES, 2012	Leaves with top	90	<0.01	0.01	0.01	1)	1)	0.02
	Roots	90	<0.01	<0.01	<0.01			<0.02
	Leaves with top	128	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	128	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL12/435/GC BPL12/435/GC-02-SP	Whole plants with root	0	22.4	0.40	0.45	1)	1)	22.85
	Whole plants with root	8	0.25	0.30	0.34			0.59
	Whole plants with root	32	<0.01	0.04	0.04			0.05
	Leaves with top	91	<0.01	0.03	0.03			0.04
	Roots	91	<0.01	<0.01	<0.01			<0.02
	Leaves with top	128	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
	Roots	128	<0.01	<0.01	<0.01			<u>&lt;0.02</u>
BPL12/435/GC BPL12/435/GC-03-IT IT, 2012	Leaves with top	91	<0.01	0.07	0.08	1)	1)	0.09
	Roots	91	<0.01	<0.01	<0.01			<0.02
	Leaves with top	108	<0.01	0.03	0.03			0.04
	Roots	108	<0.01	<0.01	<0.01			<0.02
BPL12/435/GC BPL12/435/GC-04-IT IT, 2012	Whole plants with root	0	15.7	0.22	0.25	1)	1)	15.95
	Whole plants with root	7	0.66	0.31	0.35			1.01
	Whole plants with root	32	<0.01	0.06	0.07			0.08
	Leaves with top	90	<0.01	0.04	0.04			0.05
	Roots	90	<0.01	<0.01	<0.01			<0.02
	Leaves with top	108	<0.01	0.12	0.13			<u>0.14</u>
	Roots	108	<0.01	<0.01	<0.01			<u>&lt;0.02</u>

1) this footnote refers to residue trials where the metabolites NC 9607 and its conjugates were analysed together as sum of NC 9607 and its conjugates as expressed as NC 9607.

### III. Overall conclusion

A number of additional residue trials were conducted to support the representative use on sugar beets. All trials were analysed according to the new residue definition including NC 9706 and NC 20645 conjugates.

Some residue trials were analysed for NC 20645 conjugates in an additional analytical procedure and so it was demonstrated, that at later growth stages NC 20645 conjugates were the predominant residues in sugar beet leaves and tops.

Considering the worst case application (application rate app. 1 kg a.s./ha at BBCH 14-18) no residue above the LOQ were conducted in sugar beet roots. In leaves and tops residues up to 0.18 mg/kg could be detected.



The results of the additional residue trials are in good accordance to the residue trials evaluated during the Annex I inclusion process.

#### B.7.4. FEEDING STUDIES

According to the data requirements for the dossiers to be submitted for the approval of active substances (Regulation (EU) 283/2013), feeding studies shall be provided where metabolism studies indicate that residues at levels of above 0.01 mg/kg may occur in edible animal tissue, milk, eggs or fish, taking into account the residue levels in potential feeding stuffs, obtained at the 1x dose rate, calculated on the dry weight basis.

According to the OECD guidance document on residues in livestock published on July 10<sup>th</sup> 2013 (ENV/JM/MONO(2013)8), fodder beets, sugar beet tops, dried pulp of beets, ensiled pulp of beets and molasses are the beet matrices fed to livestock. In addition, rotational crops, planted directly after crop failure (within 30 days after treatment of the failed beet crop) can show small ethofumesate related residues. These rotational crops can also contribute to the animal diet. The dietary burdens were calculated for different groups of livestock using the summarized residue values and the OECD calculator.

**Table 7.3.2-1 Ethofumesate related residues in animal feed items**

Crop	Residue [mg/kg] HR	STMR
<b>Primary crops</b>		
Sugar / fodder beet root	0.1	0.06
Sugar / fodder beet tops	0.18	0.06
<b>Processed commodities</b>		
Sugar beet, dried pulp		0.35 <sup>1</sup>
Sugar beet, ensiled pulp		0.06 <sup>1</sup>
molasses		1.27 <sup>2</sup>
<b>Rotational crops</b>		
Cereal, forage	0.03	0.03
Root crops, root	0.05	0.04

1 value estimated based on the residue in sugar beet root (dry matter (DM) = 15) and the DM of 88 for dried pulp and DM of 15 for ensiled pulp

2 median processing factor of 12.7 for molasses was applied (please refer to B 7.5.3)

**Table 7.3.2-2 Anticipated dietary burden for ethofumesate residues in livestock based on EU residue data (OECD calculator)**

	Dietary burden (mg/kg bw/day)	Dietary burden (mg/kg DM)
<b>Cattle</b>		
Beef	0.013	0.5
Dairy cattle	0.019	0.5
<b>Sheep</b>		
Ram/Ewe	0.015	0.4
Lamb	0.019	0.4
<b>Swine</b>		
Breeding	0.008	0.3
Finishing	0.007	0.2
<b>Poultry</b>		
Broiler	0.003	0.04
Layer	0.005	0.08
Turkey	0.003	0.04

**Table 7.3.2-3 Transfer factors determined in the cow metabolism study (cf. KCA 6.2.3 /03)**  
**(dietary burden in the metabolism study = 274 mg/kg dry feed, equivalent to ~ 5 mg/kg bw)**

Sample	Transfer of total residue
Milk (Σ 8-95 h samples)	0.002
Subcutaneous fat	0.002
Omental fat	0.002
Renal fat	0.002
Kidney	0.007
Hind quarters muscle	<0.001
Psoas muscle	<0.001
Loin muscle	<0.001
Heart muscle	<0.001
Liver	0.002

**Table 7.3.2-4 Transfer factors determined in the poultry metabolism study (cf. KCA 6.2.2 /02)**  
**(dietary burden in the metabolism study = 11 mg/kg dry feed, equivalent to ~ 0.8 mg/kg bw)**

Sample	Transfer of total residues
Egg yolk (steady state: day 8)	0.002
Egg white (steady state: day 6)	<0.001
Muscle	<0.001
Skin	0.002
Fat, abdominal	0.002
Fat, subcutaneous	0.001
Liver	0.008

Considering the transfer factors for the total radioactive residue in an animal matrix, as estimated in the livestock metabolism studies, and the corresponding maximum dietary burden of the animal, it can be concluded that the residues in all animal matrices will not exceed 0.01 mg/kg and therefore no feeding studies have to be conducted, neither in ruminants nor in poultry.

Nevertheless, in the scope of the original Annex II submission in 1996, a feeding study in poultry and two feeding studies in lactating cow were submitted and evaluated. All feeding studies were conducted in the US and did not completely follow the EU guidelines, however confirmed the low transfer of the ethofumesate related residues in edible matrices.

#### B.7.4.1. Poultry

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

Report:	KCA 6.4.1 /01; [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 6.4.1 /02; [REDACTED] 1999;M-185950-01
Title:	REVIEW OF ANIMAL METABOLISM DATA; MAXIMUM ESTIMATED DIETARY CONCENTRATION FOR POULTRY AND CATTLE; REBUTTAL FOR FURTHER ANIMAL FEEDING STUDIES ETHOFUMESATE CODE: AE B049913
Report No:	C003329
Document No:	M-185950-01-1
Guidelines:	Deviation not specified

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GLP/GEP: no

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### B.7.4.2. Ruminants

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

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Report:	KCA 6.4.2 /01; [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

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Report:	KCA 6.4.2 /02; [REDACTED] 1977;M-164398-01
Title:	RESIDUES IN MILK AND TISSUES FOLLOWING A 28-DAY FEEDING STUDY WITH ETHOFUMESATE IN DAIRY COWS - PART 2
Report No:	A89223
Document No:	M-164398-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

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Report:	KCA 6.4.2 /03; [REDACTED] 1994;M-237976-01
Title:	ETHOFUMESATE-DERIVED RESIDUES IN THE MEAT AND MILK OF DAIRY COWS: RESULTING FROM ORAL INGESTION OF ETHOFUMESATE
Report No:	B002201
Document No(s):	Report includes Trial Nos.: B93R04/05 M-237976-01-1
Guidelines:	USEPA (=EPA): 171-4(j); Deviation not specified
GLP/GEP:	no

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Report:	KCA 6.4.2 /04; [REDACTED] 1999;M-185950-01
Title:	REVIEW OF ANIMAL METABOLISM DATA; MAXIMUM ESTIMATED DIETARY CONCENTRATION FOR POULTRY AND CATTLE; REBUTTAL FOR FURTHER ANIMAL FEEDING STUDIES ETHOFUMESATE CODE: AE B049913
Report No:	C003329
Document No:	M-185950-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

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#### New data for AIR:

In the course of the evaluation of ethofumesate in the US, an additional feeding study with lactating cows was conducted. This study was done at three dose levels, the lowest at approx. 29 mg a.s./kg dry feed. However the dietary burden of ruminants is significantly lower in the EU (approx. 0.6 mg/kg dry feed instead of 29 mg/kg dry feed). Therefore, the study was not further considered in this report.

### B.7.4.3. Pigs

No feeding study in pigs is required because metabolic pathways in the rat, in ruminants and in poultry are similar (cf. CA 6.2.4).

#### B.7.4.4. Fish

No feeding study in fish is required because root and tuber crops are generally not fed to fish. They can be used in small quantities e.g. as binders to increase the water stability of diets but they will never represent a significant part of the diet.

#### B.7.5. EFFECTS OF PROCESSING

Numerous processing studies have been conducted to support the use of ethofumesate in sugar beet. Representative processing studies for sugar production were conducted with field samples collected from overdosed supervised residue trials.

According to Commission Regulation (EU) No 283/2013 the following data requirements are stated:

Studies on the nature of residues in processing shall be provided where residues in products of plant or animal origin subject to processing may occur at a level of or higher than 0.01 mg/kg (based on the residue definition for risk assessment for the raw commodity).

No studies shall, however, be required in the following cases:

- substances with a water solubility < 0.01 mg/L;
- only simple physical operations, not involving a change in temperature of the commodity are carried out, such as washing, trimming or pressing; or
- the distribution of residues between pulp and inedible peel is the only effect of processing.

The nature of residues was not investigated during Annex I inclusion and therefore a new study on the nature of residues in processing was submitted.

##### B.7.5.1. Nature of the residue

A high temperature hydrolysis was conducted with ethofumesate to evaluate if breakdown or reaction products arise from the parent compound in the raw agricultural commodities during processing.

Report:	KCA 6.5.1 /01;Miebach, D.; Bongartz, R.;2010;M-397800-01
Title:	Nature of the residues of ethofumesate in processed commodities - High temperature hydrolysis
Report No:	MEF-10/803
Document No:	M-397800-01-1
Guidelines:	EU 91/414/EEC as amended by 96/68/EC, Section 6.5.1.; OECD 507; Conditions of industrial processing for sugar productions were investigated in addition to the standard tests defined by OECD Guideline 507.
GLP/GEP:	yes

### I. Summary

The behaviour of ethofumesate was studied under conditions representative for processing. Due to the use of Ethofumesate in sugar beets, conditions of industrial processing for sugar production and refinement were investigated in addition to the standard tests defined by OECD Guideline 507. The additional hydrolysis experiment was performed at pH 11, 90°C for 30 min. This test is a simulation of the carbonation process used in the sugar production. The radiolabelled test compound [phenyl-UL-14C]-ethofumesate was used for the hydrolysis investigations.

One concentration (approx. 1.0 mg/L) of the analyte was prepared in sterilized buffered drinking water and incubated under three representative sets of hydrolysis conditions:

Pasteurisation: 90°C at pH 4 for 20 min

Baking, brewing, boiling: 100°C at pH 5 for 60 min

Sterilisation: 120°C at pH 6 for 20 min

Industrial extraction and purification: 90°C at pH 11 for 30 min

At test termination, the material balances in all tests were in the range of 99.9 to 100.6% of the applied radioactivity, indicating that no radioactivity and no volatile degradation products dissipated from the test system.

HPLC profiling of samples before and after processing proved that the test compound ethofumesate was stable under the test conditions. The test compound amounted to  $\geq 97.9\%$  in all test solutions before and after hydrolysis. Hydrolysis products were detected in a range between 0.7% and 2.1%. They were not further investigated, due to their low amount in the test solutions.

## II. Materials and Methods

### A. Materials

**Table 7.5.1-1 Test material**

	Position of radiolabel	Radiochemical purity (%)	Specific Activity (MBq/mg)
ethofumesate	[phenyl-UL- <sup>14</sup> C]	> 99% by HPLC > 99% by TLC	3.78

### B. Study Design

Experimental conditions: One sample of the test solution was prepared for each of the four tests. An appropriate amount of the stock solution was concentrated to dryness and the buffer solutions, prepared from drinking water and ready to use commercial buffer concentrates, were added to give a theoretical concentration of approx. 1 mg/L in the test solution. The pH value of all samples was measured and three aliquots of each test solution were subjected to LS-measurement to determine the actual radioactivity in the test solution before starting the treatment. A further aliquot from each sample was taken for chromatographic analysis of the zero-time purity.

The test compound was incubated in buffered drinking water at the following three representative sets of conditions to investigate the effects of hydrolysis as appropriate for the relevant processing operations:

pH	Temperature [°C]	Test period [min]	Process
4 ± 0.1	90 ± 5	20 + 1	pasteurisation
5 ± 0.1	100 ± 5	60 + 1	baking, brewing and boiling
6 ± 0.1	120 ± 5	20 + 1	sterilisation
11 ± 0.1	90 ± 5	30 + 1	industrial process of sugar production

The tests at 90°C and 100°C were carried out by using a dry block heater. The test at 120°C was performed in an autoclave. For the experiments at 90°C and 100°C, the actual temperature was recorded in a control vial filled with blank buffer solution. For the autoclave experiment at 120°C, the programmed figures were used as temperature data. The intended test durations listed in the table above do not include the time until reaching the test temperature or ambient temperature after test termination.

After the application procedure, the test vessels were closed with a septum and a crimp top and were subjected to the intended incubation conditions. Samples for hydrolysis were weighed before and after hydrolysis to correct for possible losses by evaporation of water.

Sampling: After termination of each test and cooling to room temperature, the pH value was measured. Three aliquots were again taken from each test solution for the determination of the radioactivity content by Liquid Scintillation Counting (LSC).

### C. Analytical Procedure

Processing: The radioactivity content of each test solution was determined by LSC before starting and after termination the hydrolysis. Aliquots of all samples were analysed by HPLC for detection of possible hydrolysis products.

Quantitation: The  $^{14}\text{C}$ -radioactivity of liquid samples was determined by liquid scintillation counting (LSC). Sample aliquots were generally measured in triplicate. To analyse the proportion of parent compound in the sample, the sample was analysed and quantified by LC-MS/MS.

Identification and characterisation: The identity of the test compound was confirmed by mass spectroscopy (LC-MS/MS). In the test solutions, parent compound was additionally identified by HPLC co-chromatography with a radiolabelled reference compound.

## III. Results and Discussion

pH, temperatures and test periods: The pH values of the test solutions were adjusted to pH 4, pH 5, pH 6 and pH 11 (each  $\pm 0.1$ ) and remained as required. The temperatures were in the ranges of  $90 \pm 5^\circ\text{C}$ ,  $100 \pm 5^\circ\text{C}$  and  $120 \pm 5^\circ\text{C}$  during the test periods.

Material balance: The applied radioactivity was defined as the amount of radioactivity measured in the samples taken at the beginning of the incubation period. Based on the results of LSC measurements immediately after test termination, a radioactivity balance was established for each experiment.

All material balances were in the range between 99.9 to 100.6%. The material balances demonstrate that no radioactivity dissipated from the test systems.

Test compound and hydrolysis products in test samples: The radiochemical purity of the test compound was checked in the stock solution by HPLC and amounted to 100%. Aliquots of all tests solutions were analysed by HPLC before and after hydrolysis. Chromatograms were nearly identical before and after hydrolysis. The test compound was found in amounts higher than 97.9% in any test solution after hydrolysis. The LOD was estimated as being approx. 0.7% of the total radioactivity.

## IV. Conclusions

The test compound ethofumesate was stable under all conditions of high temperature hydrolysis for simulation of food processing. No significant hydrolysis products of ethofumesate ( $\leq 2.1\%$ ) were detected above an estimated LOD of 0.7% of the total radioactivity.

### B.7.5.2. Distribution of the residue in peel and pulp

As the "representative use" crop supported in the dossier, sugar beet, is not peeled before use, no data was necessary to cover this point.

### B.7.5.3. Magnitude of residues in processed commodities

The total theoretical maximum daily intake (TMDI) of ethofumesate related residues is less than 10% of the ADI when calculating with the STMR values from the supervised field trials. As a conclusion, processing studies are generally not needed for crops treated with ethofumesate according to the intended use pattern. Nevertheless, several processing studies were conducted to support the Annex I inclusion of ethofumesate. These studies demonstrated that ethofumesate related residues were never present in refined sugar indicating that probable residues in the raw agricultural commodity are efficiently eliminated during processing. A concentration of the residue was detected in molasses (maximum and median processing factor was 24 and 12.7, respectively) and in thick juice (maximum and median processing factor was 6.5 and 4.7, respectively).

The studies were submitted and evaluated during the Annex I inclusion process and were considered acceptable. Therefore, no additional data was considered necessary.

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

Report:	KCA 6.5.3 /01;Whiteoak, R. J.; Crofts, M.;1973;M-155250-01
Title:	CONJUGATED RESIDUES IN FRACTIONS PROCESSED FROM SUGAR BEET TREATED WITH NORTON
Report No:	A82973
Document No:	M-155250-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.5.3 /02;Crofts, M.; Whiteoak, R. J.;1974;M-155262-01
Title:	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET
Report No:	A82985
Document No:	M-155262-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.5.3 /03;Crofts, M.; Whiteoak, R. J.;1975;M-155279-01
Title:	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET - ARTIFICIALLY HIGH RESIDUES IN BEET GROWN AND PROCESSED IN THE UNITED KINGDOM
Report No:	A83002
Document No:	M-155279-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no
Report:	KCA 6.5.3 /04;Crofts, M.; Whiteoak, R. J.;1975;M-155280-01
Title:	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET - ARTIFICIALLY HIGH RESIDUE IN BEET GROWN AND PROCESSED IN W. GERMANY
Report No:	A83003
Document No:	M-155280-01-1
Guidelines:	Deviation not specified
GLP/GEP:	no

## B.7.6. RESIDUES IN ROTATIONAL CROPS

The aerobic degradation of ethofumesate in soil was investigated in laboratory and field studies (see Section B.8). Ethofumesate is degraded rather slowly in soil through the action of soil micro flora via either desalkylation (NC 8493) followed by oxidation (NC 9607) and ring opening (NC 20645) or the loss of the methanesulfonate moiety to transient degradates (all metabolites <5%) which are rapidly converted to soil 'bound' residues (16 - 34%). Mineralization to CO<sub>2</sub> was also observed (6 - 13% CO<sub>2</sub> at 100 days).

### B.7.6.1. Metabolism in rotational crops

Since the exposure of succeeding crops to ethofumesate soil residues cannot be excluded, in the Monograph prepared in the framework of Directive 91/414/EEC the metabolism of ethofumesate was investigated in representative rotational crops (wheat, radish and cabbage) following soil application of [<sup>14</sup>C-benzene]-ethofumesate.

The main data and results (on the basis of normalized recoveries) are summarized as follows.

#### B.7.6.1.1. Confined rotational crop study (1)

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

Report:	KCA 6.6.1 /01;Carlton, R.; Cordell, P.;1993;M-155664-01
Title:	THE UPTAKE AND METABOLISM OF ETHOFUMESATE AND ITS SOIL METABOLITES IN A CONFINED ROTATIONAL CROP STUDY
Report No:	A83396
Document No(s):	Report includes Trial Nos.: 90B M-155664-01-1
Guidelines:	USEPA (=EPA): N-165-1;Deviation not specified
GLP/GEP:	yes

The metabolism of the herbicide ethofumesate was investigated in the representative rotational crops spring/winter wheat, radish and cabbage from three consecutive rotations. [<sup>14</sup>C-benzene]-ethofumesate was formulated as Nortron EC and used for one spray application onto the soil of planting pots. The actual application rate corresponded to 4.6 kg a.s./ha, which is approx. 4.5 times the anticipated maximum seasonal field rate of 1 kg a.s./ha. Approx. 3, 9 and 12 months after the application to soil radish, wheat and cabbage were sown. Re-sowing of wheat was necessary after 5 months due to crop failure at 3 months. The three sowing dates represented the three rotations. Crops were harvested at an immature growth stage and at maturity.

The TRR values for all mature RACs are given in the following table.

**Table 7.6.1-1 Total radioactivity residues in mature plant matrices of radish, wheat, and cabbage (results of one replicate are presented)**

Rotation	Sowing time (months)	Radish		Wheat			Cabbage
		Foliage	Root	Grain	Straw	Chaff	Foliage
		TRR [mg/kg]					
1st	3 or 5*	31.98	1.66	0.04	0.70	0.12	0.39**
2nd	9	5.14	0.40	0.017	0.16	0.032	1.88**
3rd	12	9.27	0.43	0.06	1.27	0.16	3.45

\* 3 months: radish and cabbage; 5 months: wheat

\*\* results are not conclusive: immature cabbage showed residues of 15.15 mg/kg and 8.21 mg/kg after the first and second rotation

The results given in this table are summarised from tables 12 and 14 of the original report.



Conventional extraction with acetonitrile and acetonitrile/water mixtures released between 44.1% (wheat grain, 3rd rotation) and 90.6% (mature radish foliage, 2nd rotation) of the TRR. To increase the extraction efficiency in wheat matrices an additional exhaustive extraction step with hydrochloric acid was applied. Finally, the extraction efficiency in all matrices was always above 80%.

Parent compound and metabolites in the extracts were analysed by HPLC and TLC. Identification was achieved by co-chromatography with reference compounds. Conjugates were identified on the basis of their exocons after an acidic hydrolysis step.

Parent ethofumesate was the most prominent compound in radish roots, but a minor compound in shoots and in the aerial plant parts of all other crops. The second major compound was metabolite NC 20645, free or in conjugated form. This metabolite and its conjugate were also the main residue in all aerial plant parts, followed by metabolite NC 9607. Metabolite NC 8493 was also detected as free metabolite or as conjugate.

The results are summarised in the following tables.

**Table 7.6.1-2 Distribution of radioactivity in samples grown in aged soil treated with  $^{14}\text{C}$ -ethofumesate; characterization before and after acidic hydrolysis (3 M HCl) (results after acidic hydrolysis are reported in brackets); (distribution of residue (%))**

	Radish foliage		Radish roots		cabbage	
Compound (ethofumesate)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	1x, maturity, 3 months		1x, maturity, 3 months		1x, maturity, 3 months	
TRR [mg a.s. equiv./kg] =	31.98		1.66		0.39	
Conventional extraction						
Combined extracts (ACN + ACN/H2O)	76.9	24.59	74.7	1.24	84.4	0.33
C18 eluent	76.4	24.43	74.2	1.23	83.5	0.33
Ethofumesate	2.6 (<0.1)	0.83 (<0.01)	28.6 (0.0)	0.47 (0.00)	2.9 (<0.1)	0.01 (<0.01)
NC 20645	30.1 (30.2)	9.63 (9.66)	16.2 (15.3)	0.27 (0.25)	24.7 (26.3)	0.10 (0.10)
NC 8493	1.2 (6.0)	0.38 (1.92)	1.5 (32.5)	0.02 (0.54)	0.9 (6.7)	<0.01 (0.03)
NC 9607	16.9 (34.6)	5.40 (11.07)	8.2 (17.5)	0.14 (0.29)	6.5 (41.3)	0.03 (0.16)
Unknowns (polar)	24.5 (3.6)	7.84 (1.15)	18.6 (7.1)	0.31 (0.12)	47.3 (6.5)	0.18 (0.03)
Remainder	1.1 (2.0)	0.35 (0.64)	1.1 (1.8)	0.02 (0.03)	1.2 (2.7)	<0.01 (0.01)
Ethyl acetate extract*	0.5	0.16	0.5 (0.5)	0.01 (0.01)	0.9	<0.01
H2O extract*					2.5	0.01
Remainder	0.5	0.16	0.5	0.01		
Solids	23.1	7.39	25.3	0.42	13.0	0.05
Total identified	50.8 (70.8)	16.3 (22.6)	54.5(65.3)	0.90 (1.08)	35.0 (74.3)	0.14 (0.29)
Total characterised	26.1 (6.1)	8.35 (1.95)	20.2 (9.4)	0.34 (0.16)	51.9 (12.6)	0.20 (0.05)
Total analysed	76.9	24.59	74.7	1.24	86.9	0.34
Solids	23.1	7.39	25.3	0.42	13.0	0.05
Accountability	100.0	31.98	100.0	1.66	100.0	0.39
	1x, maturity, 9 months		1x, maturity, 9 months		1x, maturity, 9 months	
TRR [mg a.s. equiv./kg] =	5.14		0.40		1.88	
Conventional extraction						
Combined extracts (ACN + ACN/H2O + H2O)	90.6	4.66	89.2	0.36	80.9	1.52
C18 eluent	90.4	4.65	88.4	0.35	80.2	1.51
Ethofumesate	1.0 (0.0)	0.05 (0.00)	60.2	0.24	1.4 (<0.1)	0.03 (<0.01)
NC 20645	35.0 (32.5)	1.80 (1.67)	10.2	0.04	32.7 (12.7)	0.61 (0.24)
NC 8493	0.0 (4.3)	0.00 (0.22)	0.0	0.00	- (3.8)	- (0.07)
NC 9607	23.2 (48.5)	1.19 (2.49)	8.1	0.03	5.0 (55.7)	0.09 (1.05)
Unknowns					- (3.3)	- (0.06)
Unknowns (polar)	29.4 (2.7)	1.51 (0.14)	9.4	0.04	40.0 (3.9)	0.75 (0.07)
Remainder	1.8 (2.4)	0.09 (0.12)	0.5	<0.01	1.1 (0.8)	0.02 (0.02)
Ethyl acetate extract*	0.2	0.01	0.8	<0.01	0.7	0.01

	Radish foliage		Radish roots		cabbage	
Compound (ethofumesate)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Remainder	0.2	0.01 ()	0.8	<0.01		
Solids	9.4	0.48 ()	10.9	0.04	10.7	0.20
Exhaustive extraction(2 M HCl)						
HCl extract					8.5	0.16
Ethofumesate					0.0	0.00
NC 20645					2.3	0.04
NC 8493					0.5	0.01
NC 9607					1.7	0.03
Unknown (polar)					3.3	0.06
Unknown (remainder)					0.7	0.01
Total identified	59.2 (85.3)	3.04 (4.38)	78.5	0.31	43.6 (76.7)	0.82 (1.44)
Total characterised	31.4 (5.3)	1.61 (0.27)	10.7	0.04	45.8 (12.7)	0.86 (0.24)
Total analysed	90.6	4.66	89.2	0.36	89.4	1.68
Solids	9.4	0.48	10.9	0.04	10.7	0.20
Accountability	100.0	5.14	100.0	0.40	100.0	1.88
	1x, maturity, 12 months		1x, maturity, 12 months		1x, maturity, 12 months	
Conventional extraction						
TRR [mg a.s. equiv./kg] =	9.27		0.43		3.45	
Combined extracts (ACN + ACN/H2O + H2O)	84.4	7.82	89.3	0.38	89.3	3.08
C18 eluent	83.0	7.69	88.9	0.38	88.4	3.05
Ethofumesate	1.0 (<0.1)	0.09 (<0.01)	56.0	0.24	0.9 (<0.1)	0.03 (<0.01)
NC 20645	19.8 (18.0)	1.84 (1.67)	10.5	0.05	18.4 (20.2)	0.63 (0.70)
NC 8493	0.0 (4.6)	0.00 (0.43)	0.0	0.00	<0.1 (9.2)	<0.01 (0.32)
NC 9607	26.5 (45.9)	2.46 (4.25)	6.8	0.03	1.7 (52.5)	0.06 (1.81)
Unknowns (polar)	34.2 (12.7)	3.17 (1.18)	14.8	0.06	65.2 (4.9)	2.25 (0.17)
Remainder	1.5(1.8)	0.14 (0.17)	0.8	<0.01	2.2 (1.6)	0.08 (0.06)
Ethyl acetate extract*	1.4	0.13	0.4	<0.01	0.9	0.03
Remainder	1.4	0.13	0.4	<0.01		
Exhaustive extraction (2 M HCl)						
HCl extract	12.5	1.16	6.0	0.03	4.4	0.15
Ethofumesate	<0.1 (<0.1)	<0.01 (<0.01)			<0.1	<0.01
NC 20645	4.5 (4.5)	0.42 (0.42)			0.9	0.03
NC 8493	0.0 (0.0)	0.00 (0.00)			0.2	0.01
NC 9607	5.5 (5.5)	0.51 (0.51)			0.6	0.02
Unknowns (polar)	2.0 (2.0)	0.19 (0.19)	6.0	0.03	2.5	0.09
Remainder	0.5 (0.5)	0.05 (0.05)			0.2	0.01
Solids	3.1 (3.1)	0.29 (0.29)	4.8	0.02	6.4	0.22 ()
Exhaustive extraction(2 M HCl)						
HCl extract					4.4	
Ethofumesate					<0.1	
NC 20645					0.9	
NC 8493					0.2	
NC 9607					0.6	
Unknown (polar)					2.5	
Unknown (remainder)					0.2	
Total identified	57.3 (78.5)	5.31 (7.28)	73.3	0.32	22.7 (83.6)	0.78 (2.88)
Total characterised	39.6 (18.4)	3.67 (1.71)	22.0	0.09	71.0 (10.1)	2.45 (0.35)
Total analysed	96.9	8.98	95.3	0.41	93.7	3.23
Solids	3.1	0.29	4.8	0.02	6.4	0.22
Accountability	100.0	9.27	100.0	0.43	100.0	3.45

\* ethyl acetate fraction was not subjected to acidic hydrolysis and was not analysed

**Table 7.6.1-3 Wheat grain: Distribution of radioactivity in samples grown in aged soil treated with <sup>14</sup>C-ethofumesate; characterization before and after acidic hydrolysis (6 M HCl) (distribution of residue (%))**

	1x, maturity, 12 months			
<b>TRR [mg a.s. equiv./kg] =</b>	<b>0.06</b>			
<b>Compound (ethofumesate)</b>	<b>% TRR</b>	<b>mg/kg</b>	<b>% TRR</b>	<b>mg/kg</b>
Conventional extraction				
Combined extracts (ACN + ACN/H <sub>2</sub> O)	44.1	0.03	42.1	0.03
C18 clean-up	before acidic hydrolysis		after acidic hydrolysis	
C18 eluent	42.1	0.03		
Ethofumesate	<0.1	<0.01	-	-
NC 20645	3.7	<0.01	-	-
NC 8493	<0.1	<0.01	-	-
NC 9607	<0.1	<0.01	-	-
Unknowns (polar)	33.2	0.02	-	-
Remainder	5.2	<0.01	-	-
Ethyl acetate extract*	2.0	<0.01	2.0	<0.01
Hydrolysis of C18 eluent (6 M HCl)				
Diethyl ether extract**	-	-	27.7	0.02
Ethofumesate	-	-	2.1	<0.01
NC 20645	-	-	5.8	<0.01
NC 8493	-	-	1.5	<0.01
NC 9607	-	-	14.5	0.01
Unknowns (polar)	-	-	0.7	<0.01
Remainder	-	-	3.1	<0.01
HCl extract***	-	-	14.4	0.01
Exhaustive extraction(4 M HCl)				
HCl extract	43.4	0.03	43.4	0.03
Unknown (polar)	43.4	0.03	43.4	0.03
Solids	12.4	0.01	12.4	0.01
Total identified	3.7	<0.01	23.9	0.01
Total characterised	83.8	0.05	63.6	0.04
Total analysed	87.5	0.05	87.5	0.05
Solids	12.4	0.01	12.4	0.01
Accountability	100.0	0.06	99.9	0.06

The results given in this table are summarised from tables 13, 21 and 22 of the original report.

\* ethyl acetate fraction generated during the clean-up of solvent extracts (not subjected to acidic hydrolysis and not analysed)

\*\* diethyl ether extract produced by back extraction of the acidic (6 M HCl) hydrolysed solvent extracts (ACN + ACN/H<sub>2</sub>O).

\*\*\* acidic phase remained after back extraction with diethyl ether

**Table 7.6.1-4 Wheat straw: Distribution of radioactivity in samples grown in aged soil treated with <sup>14</sup>C-ethofumesate; characterization before and after acidic hydrolysis (6 M HCl) (distribution of residue (%))**

(distribution of Residue (%))				
Compound (ethofumesate)	% TRR	mg/kg	% TRR	mg/kg
	1x, maturity, 5 months			
TRR [mg a.s. equiv./kg] =	0.70			
Conventional extraction				
Combined extracts (ACN + ACN/H2O)	72.0	0.50	72.0	0.50
C18 clean-up	before acidic hydrolysis		after acidic hydrolysis	
C18 eluent	71.8	0.50	71.8	0.50
Ethofumesate	1.1	0.01	<0.1	<0.01
NC 20645	5.7	0.04	10.8	0.08
NC 8493	2.0	0.01	6.5	0.05
NC 9607	1.9	0.01	26.6	0.19
Unknowns (polar)	58.9	0.41	19.6	0.14
Remainder	2.2	0.02	8.3	0.06
Ethyl acetate extract*	0.2	<0.01	0.2	<0.01
Exhaustive extraction(2 M HCl)				
HCl extract	11.1	0.08	11.1	0.08
Solids	17.0	0.12	17.0	0.12

Compound (ethofumesate)	% TRR	mg/kg	% TRR	mg/kg
Total identified	10.7	0.07	43.9	0.31
Total characterised	72.4	0.51	39.2	0.27
Total analysed	83.1	0.58	83.1	0.58
Solids	17.0	0.12	17.0	0.12
Accountability	100.0	0.70	100.0	0.70
	1x, maturity, 9 months			
TRR [mg a.s. equiv./kg] =	0.16			
Conventional extraction				
Combined extracts (ACN + ACN/H2O)	61.5	0.10	61.5	0.10
C18 clean-up	before acidic hydrolysis		after acidic hydrolysis	
C18 eluent	61.5	0.10	61.5	0.10
Ethofumesate	2.7	<0.01	0.0	<0.01
NC 20645	6.5	0.01	12.3	0.02
NC 8493	<0.1	<0.01	3.5	0.01
NC 9607	2.9	<0.01	16.8	0.03
Unknowns (polar)	44.4	0.07	23.5	0.04
Remainder	5.0	0.01	5.4	0.01
Ethyl acetate extract*	<0.1	<0.01	<0.1	<0.01
Exhaustive extraction(2 M HCl)				
HCl extract	23.1	0.04	23.1	0.04
Solids	15.4	0.02	15.4	0.02
Total identified	12.1	0.02	32.6	0.05
Total characterised	72.5	0.12	52.0	0.08
Total analysed	84.6	0.14	84.6	0.14
Solids	15.4	0.02	15.4	0.02
Accountability	100.0	0.16	100.0	0.16
	1x, maturity, 12 months			
TRR [mg a.s. equiv./kg] =	1.27			
Conventional extraction				
Combined extracts (ACN + ACN/H2O)	55.9	0.71	55.9	0.71
C18 clean-up	before acidic hydrolysis		after acidic hydrolysis	
C18 eluent	54.1	0.69	54.1	0.69
Ethofumesate	0.7	0.01	0.0	0.00
NC 20645	13.0	0.17	7.2	0.09
NC 8493	1.3	0.02	4.8	0.06
NC 9607	2.8	0.04	28.5	0.36
Unknowns (polar)	33.4	0.42	7.2	0.09
Remainder	2.9	0.04	6.4	0.08
Ethyl acetate extract*	1.8	0.02	1.8	0.02
Exhaustive extraction(4 M HCl)				
HCl extract	22.0	0.28	22.0	0.28
Ethofumesate	<0.1	<0.01	<0.1	<0.01
NC 20645	3.1	0.04	3.1	0.04
NC 8493	1.7	0.02	1.7	0.02
NC 9607	2.0	0.03	2.0	0.03
Unknown (polar)	12.3	0.16	12.3	0.16
Unknown (remainder)	2.9	0.04	2.9	0.04
Solids	22.1	0.28	22.1	0.28
Total identified	24.6	0.31	47.3	0.60
Total characterised	53.3	0.68	30.6	0.39
Total analysed	77.9	0.99	77.9	0.99
Solids	22.1	0.28	22.1	0.28
Accountability	100.0	1.27	100.0	1.27

The results given in this table are summarised from Tables 13, 19 and 20 of the original report.

\* ethyl acetate fraction generated during the clean-up of solvent extracts (not subjected to acidic hydrolysis and not analysed)

**Table 7.6.1-5 Wheat chaff: Distribution of radioactivity in samples grown in aged soil treated with  $^{14}\text{C}$ -ethofumesate; characterization before and after acidic hydrolysis (6 M HCl) (distribution of residue (%))**

Residue (%)	Compound (ethofumesate)	% TRR	mg/kg	% TRR	mg/kg
		1x, maturity, 12 months			
	TRR [mg a.s. equiv./kg] =	0.16			
	Conventional extraction				
	Combined extracts (ACN + ACN/H2O)	59.7	0.10	59.7	0.10
	C18 clean-up	before acidic hydrolysis		after acidic hydrolysis	
	C18 eluent	58.9	0.09		
	Ethofumesate	1.6	<0.01	-	-
	NC 20645	5.8	0.01	-	-
	NC 8493	0	0.00	-	-
	NC 9607	3.6	0.01	-	-
	Unknowns (polar)	42.9	0.07	-	-
	Remainder	5.0	0.01	-	-
	Ethyl acetate extract*	0.8	<0.01	0.8	<0.01
	Hydrolysis of C18 eluent (6 M HCl)			58.9	0.09
	Diethyl ether extract**	-	-	34.7	0.06
	Ethofumesate	-	-	1.4	<0.01
	NC 20645	-	-	3.5	0.01
	NC 8493	-	-	2.9	<0.01
	NC 9607	-	-	22.0	0.04
	Unknowns (polar)	-	-	3.2	0.01
	Remainder	-	-	1.7	<0.01
	HCl extract***	-	-	24.2	0.04
	Exhaustive extraction(4 M HCl)				
	HCl extract	17.1	0.03	17.1	0.03
	Solids	23.3	0.04	23.3	0.04
	Total identified	11.0	0.02	29.8	0.05
	Total characterised	65.8	0.11	47.0	0.08
	Total analysed	76.8	0.12	76.8	0.12
	Solids	23.3	0.04	23.3	0.04
	Accountability	100.0	0.16	100.0	0.16

The results given in this table are summarised from tables 13, 23 and 24 of the original report.

\* ethyl acetate fraction generated during the clean-up of solvent extracts (not subjected to acidic hydrolysis and not analysed)

\*\* diethyl ether extract produced by back extraction of the acidic (6 M HCl) hydrolysed solvent extracts (ACN + ACN/H<sub>2</sub>O).

\*\*\* acidic phase remained after back extraction with diethyl ether

The unknown polar fraction was characterized by subjecting the conventional extracts to an acidic hydrolysis step with 3 M or 6 M HCl in a microwave digester. After the hydrolysis, the concentration of the metabolites NC 9607 and NC 8493 had increased significantly, while the concentration of the polar metabolite had decreased in the same proportion. The formation of NC 9607 and NC 8493 indicates the presence of conjugates, either of the metabolite itself or in the case of NC 9607 of its carboxy analogue NC 20645. Parent ethofumesate was found to be acid labile. Therefore small amounts of NC 8493 (detected in the hydrolysate) can result from the acidic decomposition of parent ethofumesate.

Ethofumesate was moderately metabolised in confined rotational crops. The following metabolic routes were observed:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can either undergo conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to the carboxy analogue NC 20645 which can also undergo conjugation to give polar metabolites.

On the basis of these results, the same metabolic pathway of [ $^{14}$ C-benzene]-ethofumesate in confined rotational crops can be proposed as in primary crops.

### B.7.6.1.2. Confined rotational crop study (2)

#### New data for AIR:

A plant-back interval of crop failure (plant-back interval of 30 days) was not covered by the study, which has already been evaluated in the Monograph prepared in the framework of Directive 91/414/EEC. Therefore a new study on the metabolism in rotational crops was submitted which covers the plant-back interval of 30 days.

This study is summarised and discussed below.

Report:	KCA 6.6.1/01, Chapleo, S. (2003)
Title:	THE UPTAKE OF [ $^{14}$ C]-ETHOFUMESATE RESIDUES IN SOIL BY ROTATIONAL CROPS UNDER CONFINED CONDITIONS
Document No:	22558
Guidelines:	7524/VI/95 rev. 2
GLP:	Yes

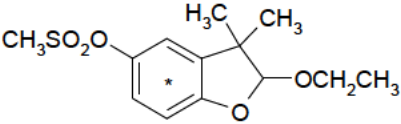
## I. Materials and Methods

### Test site information

Testing environment: glasshouse using an open wooden box constructed of 20 mm thick plywood. This was divided by wooden partitions into separate compartments for each species

Soil characteristics: soil as a sand or loamy sand; sand/ silt / clay of 89.8:4.8:5.5; pH 5.4; organic carbon content of 3.9%.

### Test material

Chemical structure	 <p>*position of the radiolabel</p>
Radiolabelled test material	[ $^{14}$ C]-ethofumesate (+)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulphonate
Specific radioactivity	185 MBq/mmol = 28 mCi/mmol 288.4 g/mol (at this specific activity)
Radiochemical purity	≥ 95.9% (TLC)
Formulation:	EC formulation, 200 g ethofumesate /L

### Application data

Application rate: 5 L/ha formulated product; corresponding to 1 kg a.s./ha

Date of application: 22.11.2002

Application method: hand-held sprayer system; no ploughing of the soil was carried out before sowing.

### Crop information

Representative rotational crops, i.e., annual ryegrass, carrots, French beans and spinach, were sown into the treated soil at a plant-back interval of 30 days. Plant samples were taken at an immature and a mature stage.

Crops / crop groups: ryegrass (cereals), carrots (root vegetables), French beans (pulses) and spinach (leafy vegetables), were sown into the treated soil at a plant-back interval of 30 days.

For plant sampling of immature stage, spinach and French beans were sampled 77 days after application, annual ryegrass was sampled 84 days after application and carrots were sampled 95 days after application. For plant sampling at mature stage, spinach was sampled 98 days after application, French beans were sampled 109 days after application, ryegrass was sampled 132 days after application and carrots were sampled 133 days after application.

Three soil cores were taken to a 20 cm depth shortly after application, 30 days after application (prior to sowing) and on the day of each plant sampling. Plant and soil samples were stored deep frozen (-20°C) until analysis.

### **Analytical methodology**

Plant and soil samples were homogenised and analysed by oxidative combustion to determine the total radioactive residue (TRR).

Homogenised plant samples (foliage, stems and foliage, root and pod tissues) were extracted twice with acetonitrile. The remaining residues were re-extracted twice with acetonitrile/water (1:1, v/v). The extracts were combined, dried and re-dissolved in acetonitrile/water (1:1, v/v) prior to analysis. The unextracted radioactive residues of annual ryegrass (mature stage), French beans foliage (immature stage) and spinach foliage (immature stage) were extracted using acetonitrile/water (3:1, v/v; 17 h, soxhlet), 1 M HCl (17 h, reflux) and 5 M HCl (17 h, reflux). Acid hydrolysis was carried out with the concentrated combined acetonitrile and aqueous acetonitrile extracts from annual ryegrass, French bean foliage, French bean pods and spinach (mature stage) to identify/quantify conjugated Ethofumesate metabolites. A subsample of each extract was combined with an equal volume of concentrated HCl (11.7 M) and incubated in a boiling water bath (approximately 100°C) for 75 min. After cooling, each hydrolysate was extracted twice with diethyl ether. The extracts were dried and re-dissolved in acetonitrile/water (1:1, v/v) for chromatographic analysis. In addition, the combined acetonitrile and aqueous acetonitrile extracts of French bean foliage and spinach foliage samples were partitioned with hexane. The aqueous phase was then hydrolysed as described before.

Extracts were analysed for Ethofumesate and its metabolites using two reversed-phase HPLC systems with either water and methanol or water and acetonitrile as mobile phases and three TLC systems. For TLC, two normal phase TLCs with either dichloromethane or toluene/methanol (8:2, v/v) as mobile phase and one reversed phase TLC with methanol/water (9:1, v/v) as mobile phase were carried out. Ethofumesate and its metabolites were identified using the following reference substances: Ethofumesate, NC 9607, NC 8493, NC 20645 and NC 10458.

## **II. Results and discussion**

A consistent gradual reduction in soil TRR between the days of application and the days of maturity harvest was observed. The TRRs in soil ranged between 0.701 and 1.507 mg a.s. equiv./kg on the day of application and decreased to 0.207-0.542 mg a.s. equiv./kg on the days of maturity harvest. The majority of the losses occurred during the initial 30 day period post-application. Between 14% and 52% of the TRR measured in soil on the day of application remained in the upper 20 cm layer of soil at maturity. The TRR in annual ryegrass at immature and mature stages were 0.460 mg a.s. equiv./kg and 1.229 mg a.s. equiv./kg, respectively, showing an uptake of soil residues throughout plant development. The TRR in carrot foliage at immature stage was 1.021 mg a.s.

equiv./kg and 0.535 mg a.s. equiv./kg at mature stage. The TRR in carrot roots at mature stage was 0.330 mg a.s. equiv./kg. The TRR in French bean at immature stage was 0.338 mg a.s. equiv./kg. The TRR in French bean foliage, pods and grain at maturity were 0.509 mg a.s. equiv./kg, 0.050 mg a.s. equiv./kg and 0.054 mg a.s. equiv./kg, respectively, showing that translocation into the pods and grain was very low. The TRRs in spinach at immature and mature stage were 0.270 mg a.s. equiv./kg and 0.148 mg a.s. equiv./kg.

In **ryegrass**, the majority of the radioactivity was readily extractable using acetonitrile and aqueous acetonitrile. Unextracted residues accounted only for 1.8-8.9% TRR. Low levels of ethofumesate and NC 9607 were detected at immature stage (6.8% TRR and 0.7% TRR) together with a negligible concentration of radioactivity which eluted with the void volume of HPLC system (N1, 1.2% TRR, 0.006 mg p.e./kg). The main component was a polar fraction (N2, 80.7-85.6% TRR) of greater polarity than the reference standards during the HPLC measurement.

Analysis of the extracts by TLC confirmed the presence of ethofumesate and NC 9607 and separated the main fraction detected by HPLC (N2, not identified, RT = 8-10 min) into 3 unidentified but well resolved components with greater polarity than NC 9607 and a component which was assigned to NC 20645. The latter was assumed to be built out of NC 9607 during TLC analysis. The main radioactivity remained associated with the origin of the TLC plate. Acidic hydrolysis showed that the 3 unidentified but well resolved components on TLC plates with greater polarity than NC 9607 comprise several conjugates which degraded to NC 9607, NC 8493 and NC 20645, whereas NC 9607 was the major metabolite.

In **carrots**, the majority of the radioactivity was readily extractable using acetonitrile and aqueous acetonitrile. Unextracted residues accounted only for 2.6-4.3% TRR. The main radioactive component was ethofumesate which ranged between 56.4% TRR and 77.2% TRR in carrot foliage. In carrot roots at harvest, ethofumesate accounted for 98.0% TRR. An unresolved mixture of at least 2 polar fractions, of greater polarity than the reference standards, was 10.6% TRR in foliage at immature stage. This was resolved in foliage at mature stage into two separate fractions at 14.8% and 1.9% TRR. Low levels of one of these fractions were detected in roots (1.9% TRR) as well. Low levels of NC 9607 and NC 8493 were detected in foliage (0.9-1.4% TRR) together with radioactivity which eluted with the void volume (0.3-5.0% TRR).

In **French beans**, the majority of the radioactivity was readily extractable using acetonitrile and aqueous acetonitrile. Unextracted residues accounted only for 5.7-17.9% TRR. In French beans foliage, the principle radioactive fraction at immature stage was an unresolved mixture of at least two polar components (67.8% TRR) which possessed greater polarity than the reference standards (using HPLC systems). This peak separated in foliage at maturity into two separated fractions (84.3% and 20.5% TRR) in HPLC systems. These peaks were shown to be conjugates that degraded after acidic hydrolysis into NC 8493, NC 9607, NC 20645 and traces of NC 10458 and several unidentified components. Radioactivity with similar chromatographic properties was also detected in pods as unresolved peaks (46.6% TRR) which was characterised as acid-labile releasing highly polar radioactivity on hydrolysis. Low levels of radioactivity which eluted with the void volume in HPLC systems were detected in foliage at both harvests (3.0-4.3% TRR) but much higher levels were found in pods (24.8% TRR) and grain (21.9% TRR). Ethofumesate was found at low levels in foliage at both harvests (0.2-2.0% TRR). The major metabolite in French bean grains was NC 9607 which accounted for 0.014 mg p.e./kg corresponding to 26.7% TRR.



In **spinach**, the majority of the radioactivity was readily extractable using acetonitrile and aqueous acetonitrile. Unextracted residues accounted only for 2.1-5.4% TRR. In spinach at immature stage, the principle radioactive fractions were two polar fractions of greater polarity than the reference standards which accounted for 61.2% and 20.0% TRR: The principle radioactive fraction at mature stage was radioactivity which eluted with the void volume of the HPLC system (69.5% TRR) and one fraction found as well at earlier harvest (21.3% TRR). These fractions were shown to be conjugates which degraded to NC 8493, NC 9607 and traces of NC 10458 and several unidentified components by acidic hydrolysis. Low levels of ethofumesate were detected at both harvests which accounted for 1.4-6.3% TRR.

**Table 7.6.1-6 Characterisation/identification of radioactive residues in samples**

	Ryegrass		carrots		French beans		spinach	
	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg
Time interval between application of the test material to the soil and sowing or planting	84		95		77		77	
TRR – Total radioactive Residues	100	0.460	100	1,021	100	0,338	100	0,270
ERR - Extracted Radioactive Residues (as measured)	102	0.468	95.3	0.973	71.9	0.243	90.4	0.244
AN-Extract	90.2	0.415	85.1	0.869	70.7	0.239	94.1	0,254
AN/water extract	10.4	0.048	8.1	0.083	11.8	0.040	5.6	0,015
AN/water Soxhlet extraction	---	---	---	---	3.5	0.012	0.7	0,002
1M HCl reflux extraction	---	---	---	---	5.5	0.019	2.2	0,006
5M HCl reflux extraction	---	---	---	---	0.9	0.003	0.3	0,001
Identification / characterization								
Ethofumesate	6.8	0.031	77.2	0.789	2.0	0.007	1.4	0,004
NC 9607	0.7	0.003	1.3	0.013	ND	ND	ND	ND
NC 20645	ND	ND	ND	ND	ND	ND	ND	ND
NC 8493	ND	ND	ND	ND	ND	ND	ND	ND
Unknown 1 (RT: 4-7)	1.2	0.006	0.3	0.003	3.0	0.010	ND	ND
Unknown 2 (RT: 8-10)	85.6	0.394	10.6*	0.109	67.8*	0.230	61.2	0,165
Unknown 3 (RT: 10-13)	ND	ND	ND	ND	ND	ND	20.0	0,054
<i>Total identified</i>	7.5	0.034	78.5	0.802	2.0	0.007	1.4	0,004
<i>Total characterized</i>	86.8	0.400	10.3	0.911	70.8	0.240	81.2	0,219
URR – Unextracted Radioactive Residues	8.9	0.041	2.6	0.027	5.7	0.019	2.1	0.006
Accountability (sum ERR and URR)	109.6	0.504	92	0.941	98.2	0.332	105.2	0.284
Time interval between application of the test material to the soil and sowing or planting	maturity		Foliage - maturity		Foliage - maturity		maturity	
TRR – Total radioactive Residues	100	1.229	100	0.535	100	0.509	100	0.148
ERR - Extracted Radioactive Residues	86.4	1.062	95.3	0.973	103.9	0.529	95.3	0.141
AN-Extract	74.4	0.914	84.5**	0.452	103.9**	0.529	87.8	0,130
AN/water extract	14.6	0.179	---	---	---	---	9.5	0,014
AN/water Soxhlet extraction	1.4	0.017	N/A	N/A	N/A	N/A	N/A	N/A
1M HCl reflux extraction	2.0	0.024	N/A	N/A	N/A	N/A	N/A	N/A
5M HCl reflux extraction	0.6	0.007	N/A	N/A	N/A	N/A	N/A	N/A
Identification / characterization								
Ethofumesate	ND	ND	56.4	0.302	0.2	0.001	6.3	0,009

	Ryegrass		carrots		French beans		spinach	
	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg
NC 9607	ND	ND	1.4	0.007	ND	ND	ND	ND
NC 20645	ND	ND	ND	ND	ND	ND	ND	ND
NC 8493	ND	ND	0.9	0.005	ND	ND	ND	ND
Unknown 1 (RT: 4-7)	ND	ND	5.0	0.027	4.3	0.022	69.5	0.103
Unknown 2 (RT: 8-10)	100	1.229	14.8	0.080	84.3	0.429	ND	ND
Unknown 3 (RT: 10-13)	ND	ND	1.9	0.010	20.5	0.104	21.3	0.032
Unknown 4 (RT: 28-29)	ND	ND	ND	ND	0.2	0.001	ND	ND
<i>Total identified</i>	--	--	57.8	0.309	0.2	0.001	6.3	0.009
<i>Total characterized</i>	100	1.229	21.7	0.207	109.3	0.556	90.8	0.135
URR – Unextracted Radioactive Residues	1.8	0.022	4.3	0.023	9.9	0.050	5.4	0.008
Accountability (sum ERR and URR)	86.4	1.059	84.7	0.454	119.4	0.607	102.7	0.152
Time interval between application of the test material to the soil and sowing or planting			Roots - maturity		Pods - maturity			
TRR – Total radioactive Residues			100	0.330	100	0.050		
ERR - Extracted Radioactive Residues			100	0.330	96	0.048		
AN-Extract			100**	0.330	80.0	0.040		
AN/water extract			---	---	16.0	0.008		
AN/water Soxhlet extraction			N/A	N/A	N/A	N/A		
1M HCl reflux extraction			N/A	N/A	N/A	N/A		
5M HCl reflux extraction			N/A	N/A	N/A	N/A		
	Identification / characterization							
Ethofumesate			98.0	0.323	ND	ND		
NC 9607			ND	ND	ND	ND		
NC 20645			ND	ND	ND	ND		
NC 8493			ND	ND	ND	ND		
Unknown 1 (RT: 4-7)			ND	ND	24.8	0.013		
Unknown 2 (RT: 8-10)			ND	ND	46.6*	0.024		
Unknown 3 (RT: 10-13)			1.9	0.006	---	---		
<i>Total identified</i>			98.0	0.323	ND	ND		
<i>Total characterized</i>			1.9	0.006	87.8	0.037		
URR – Unextracted Radioactive Residues			2.6	0.009	17.9	0.009		
Accountability (sum ERR and URR)			102.5	0.338	105.7	0.054		
Time interval between application of the test material to the soil and sowing or planting					Grain – maturity			
TRR – Total radioactive Residues					100	0.054		
ERR - Extracted Radioactive Residues					109.3	0.059		
AN-Extract					109.3**	0.059		
AN/water extract					---	---		
	Identification / characterization							
Ethofumesate					ND	ND		
NC 9607					26.7	0.014		
NC 20645					ND	ND		
NC 8493					ND	ND		
Unknown 1 (RT: 4-7)					21.9	0.012		

	Ryegrass		carrots		French beans		spinach	
	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg	TRR (%)	mg a.s. eq /kg
Unknown 3 (RT: 10-13)					5.1	0.003		
<i>Total identified</i>					26.7	0.014		
<i>Total characterized</i>					27.0	0.015		
URR – Unextracted Radioactive Residues					14.6	0.008		
Accountability (sum ERR and URR)					124.1	0.067		

ND not detected

N/A not analysed

\* Radioactive components were not resolved

\*\* The TRR measured in the initial acetonitrile extract was subsequently shown to be invalid, since the TRR measured in the combined extracts (see below) gave a more acceptable total recovery.

### III. Conclusion

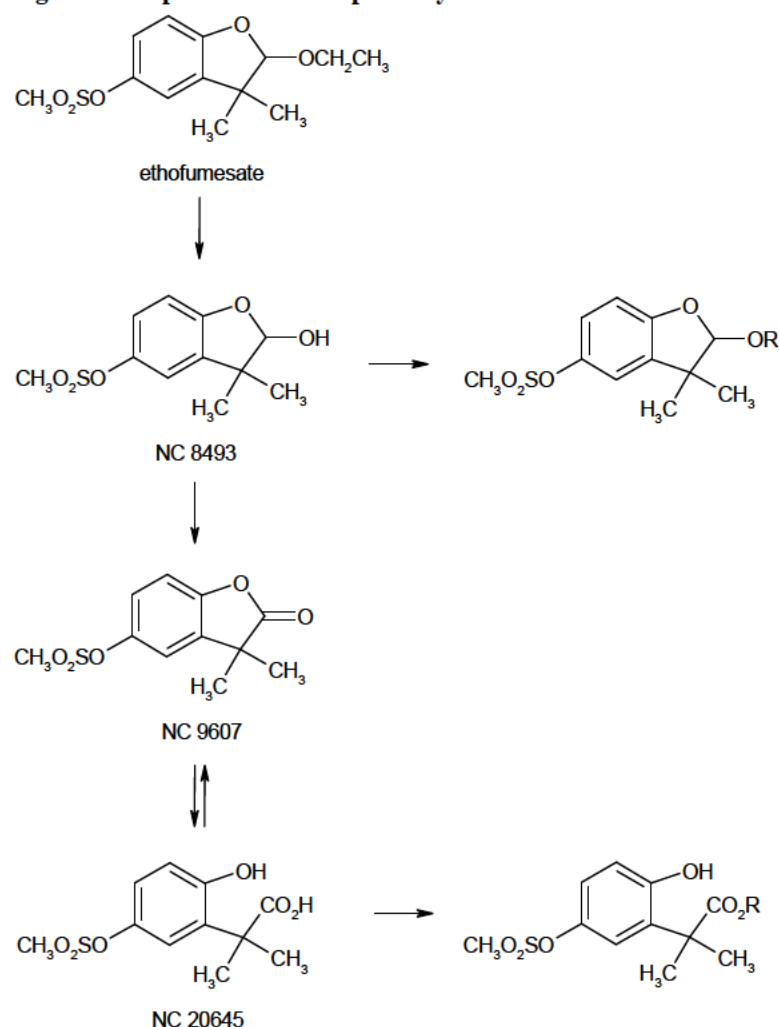
This report corroborates the results of the confined rotational crop study which have already been reviewed on EU level. Based on the results of this study it can be concluded that the nature of residues in rotational crops is similar to that in primary crops.

Based on the metabolites identified in following crops, the following metabolic routes were deduced:

- Cleavage of the ethoxy side chain, with hydroxylation at the 2 position, to give NC 8493.
- NC 8493 can either undergo conjugation to give polar metabolites, or oxidation to the lactone NC 9607.
- The lactone ring of NC 9607 opens to the carboxy analogue NC 20645 which can also undergo conjugation to give polar metabolites.

The metabolic routes detected are in line with those observed in primary crops. On the basis of these results it can be concluded that the metabolism of [<sup>14</sup>C]-ethofumesate in confined rotational crops follows the same metabolic path as primary crops:

The proposed residue definition for plants for monitoring and risk assessment is therefore still: the sum of Ethofumesate, 2-keto-ethofumesate (NC 9607), open-ring-2-keto-ethofumesate (NC 20645) and its conjugate.

**Figure 4 Proposed metabolic pathway of ethofumesate in rotational (succeeding) crops**

(R = unknown conjugate)

**B.7.6.2. Magnitude of residues in rotational crops**

Several field rotational crop studies – either as “multi-crop” study (containing data for two rotations with three crop groups: leafy, root, and cereal crops) or as “limited” study (containing data for one rotation with one crop) were conducted. The studies were run with exaggerated field rates, either in Europe or the US. The studies were evaluated during the Annex I inclusion and were considered acceptable. However, since none of the studies were conducted with the current application rate of 1.0 kg as./ha, additional field rotational crop studies (a “multi-crop” study containing data for three rotations with three crop groups) were conducted in addition.

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

Report:	KCA 6.6.2 /01;Castro, L. E.;1994;M-155392-01
Title:	ETHOFUMESATE EMULSIFIABLE CONCENTRATE 200 g/l CR 13768: AT-HARVEST RESIDUES OF ETHOFUMESATE AND METABOLITES IN ROTATIONAL CROPS AND SOIL FOLLOWING APPLICATIONS OF NORTON EC TO SUGARBEETS, USA, 1990
Report No:	A83117
Document No:	M-155392-01-1
Guidelines:	Deviation not specified
GLP/GEP:	Yes

Report:	KCA 6.6.2 /02;Crofts, M.; Whiteoak, R. J.;1974;M-155272-01
Title:	RESIDUE ANALYSIS OF WHEAT GROWN IN THE UK AS A FOLLOWING CROP AFTER SUGAR BEET TREATED WITH NORTRON (1973)
Report No:	A82995
Document No:	M-155272-01-1
Guidelines:	Deviation not specified
GLP/GEP:	No
Report:	KCA 6.6.2 /03;Crofts, M.; Whiteoak, R. J.;1974;M-155271-01
Title:	RESIDUE ANALYSIS OF WHEAT AND CORN (MAIZE) GROWN AS FOLLOWING CROPS AFTER SUGAR BEET TREATED WITH NORTRON (1973)
Report No:	A82994
Document No:	M-155271-01-1
Guidelines:	Deviation not specified
GLP/GEP:	No
Report:	KCA 6.6.2 /04;Peatman, M. H.; Snowdon, P. J.;1991;M-155644-01
Title:	RESIDUES OF SOIL AND EMERGENCY CROPS FOLLOWING APPLICATION OF ETHOFUMESATE AS A 50 SC FORMULATION IN THE UK 1990/91
Report No:	A83376
Document No(s):	Report includes Trial Nos.: 041/04/057 M-155644-01-1
Guidelines:	Deviation not specified
GLP/GEP:	Yes

### B.7.6.2.1. Magnitude of residues in rotational crops (1)

#### New data for AIR:

Report:	KCA 6.6.2 /05;Schulte, G.; Diehl, P.;2013;M-463906-02; Amended: 2013-09-13
Title:	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
Report No:	10-2501
Document No(s):	Report includes Trial Nos.: 10-2501-02 10-2501-03 10-2501-04 10-2501-05 M-463906-02-1
Guidelines:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD Guidelines for the Testing of Chemicals. Residues in Rotational Crops (Limited Field Studies). 504. 2007-01-10 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 US EPA OCSPP Guideline No. 860.1900;not specified
GLP/GEP:	yes

## I. Materials and Methods

Altogether 4 field rotational crop residue trials were conducted in Europe, two in the northern and two in the southern residue region, as follows:

In 2010/2011, 4 trials (one each in Germany, the Netherlands, Italy, and Spain) were conducted to support the use of Ethofumesate SC 500 in field-grown, non-perennial crops. A single application was made at a nominal rate of 2 L/ha, corresponding to 1.0 kg/ha ethofumesate a.s., which reflected the projected rate for foliar

treatment with ethofumesate in arable, non-perennial crops, such as sugar beet. Water rates were 100-300 L/ha. Applications were either made to bare soil or to the target crop sugar beet (2nd and 3rd rotation); followed by sowing or planting of the rotational crops (carrot, lettuce barley or wheat). All treatments were made at the scheduled rates.

At various intervals, crops were planted back onto the test area in order to simulate a crop failure ("rotation 1", plant-back interval [PBI] 25-33 days), a second use of the plot in the same year ("rotation 2", PBI 54-259 days), or use of the same plot in the succeeding year ("rotation 3", PBI 284-354 days). In each rotation, 3 different crops representing different botanical groups were planted: a root crop (carrots), a leafy crop (lettuce), or a small grain cereal (barley or wheat).

Samples of the rotational crops were taken at their respective harvest times, as well as at one earlier interval (immature RACs for lettuce and carrots, or fodder ["green material"] for barley and wheat). The samples were analysed for the parent compound and the common moiety NC 9607 using method 00955/M002 (cf. KCA 4.1.2/26). The respective LOQs for the two analytes were 0.01 mg/kg.

## II. Results and discussion

Concurrent recoveries of ethofumesate and the common moiety NC 9607 were obtained from samples of carrots, lettuce, barley and wheat. (Validation recoveries of method 00955/M002 were conducted separately. Details of the validation recoveries are presented in chapter CA 4.1.2 of the dossier.)

To calculate the total residue of ethofumesate the residues of parent and the common moiety NC 9607 were summed. Residues below the LOQ of 0.01 mg/kg were calculated as 0.01 mg/kg.

### Root crops:

Concurrent recovery samples for ethofumesate and the common moiety NC 9607 were spiked at levels of 0.01 mg/kg as well as at 0.10 mg/kg. Mean recoveries in carrot (roots or leaves) were 64-86%, with RSDs of 4.8-24.0%; n=3-6. Low recoveries and high variability were often seen at spiking levels of 0.1 mg/kg (10x LOQ); at spiking levels of 0.01 mg/kg, recoveries were in an acceptable range of 70 – 110% and RSD values were at or below 20%.

Details of recovery data are shown in Table 7.6.2-5. All trial data are summarised below in Table 7.6.2-2 and in greater detail in the Annex II.

The total residues of ethofumesate (parent compound plus common moiety NC 9607) in the harvested roots of carrots were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-33 days, when they ranged at an "intermediate" growth stage (including "early harvest", BBCH 47-49) from <0.02-0.05 mg/kg, and at typical harvest ripeness (BBCH 49) from <0.02-0.04 mg/kg. By the second and third rotation, residues in harvestable roots were always below the LOQ of 0.01 mg/kg for each analyte. Thus the total residue was always <0.02 mg/kg.

In carrot leaves, highest total residue levels were seen in the first rotation (0.04 mg/kg). By the second and third rotation, total residues in carrot leaves were always below the combined LOQ of 0.02 mg/kg.

To calculate the total residue of ethofumesate the residues of parent and the common moiety NC 9607 were summed. The residues of parent ethofumesate ranged between <0.01 mg/kg and 0.04 mg/kg in roots and leaves, whereas the residues in the common moiety NC 9607 were always below the LOQ of 0.01 mg/kg.

**Leafy crops:**

Concurrent recovery samples for ethofumesate and the common moiety NC 9607 were spiked at levels of 0.01 mg/kg as well as at 0.10 mg/kg. Mean recoveries in lettuce (head) were 69-85%, with RSDs of 7.3-14.8%; n=3-5. Low recoveries and high variability were often seen at spiking levels of 0.1 mg/kg (10x LOQ); at spiking levels of 0.01 mg/kg, recoveries were in an acceptable range of 70 – 110% and RSD values were below 20%.

Details of recovery data are shown in Table 7.6.2-5. All trial data are summarised below in Table 7.6.2-3 and in greater detail in the Annex II.

The total residues of ethofumesate in the harvested heads of lettuce were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-33 days, when they ranged at an "intermediate" growth stage (including "early harvest", BBCH 41-48) from <0.02-0.03 mg/kg, and were below the LOQ of 0.02 mg/kg at typical harvest ripeness (BBCH 49).

By the second and third rotation, residues in harvestable lettuce were always below the LOQ of 0.02 mg/kg.

Residues of the common moiety NC 9607 were in all rotations below the LOQ of 0.01 mg/kg, except for one sample which was collected at an "intermediate" growth stage (BBCH 41) and showed residues of 0.01 mg/kg.

**Cereal crops:**

Concurrent recovery samples for ethofumesate and the common moiety NC 9607 were spiked at levels of 0.01 mg/kg and at 0.10 mg/kg. Mean recoveries in cereal crop samples (green material, grain, and straw) were 75-95%, with RSDs values of the larger validations sets (n>2, at the LOQ) of 3.0-19.5%. All values were within acceptable ranges.

Details of recovery data are shown in Table 7.6.2-5. All trial data are summarised below in Table 7.6.2-4 and in greater detail in the Annex II.

The total residues of ethofumesate in barley and wheat green material were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-33 days, when they ranged from <0.02-0.03 mg/kg (BBCH 29-30).

By the second and third rotation, residues in green material of cereals were always below the LOQ of 0.02 mg/kg. The residues resulted from the common moiety NC 9607, which showed residues between <0.01 and 0.02 mg/kg; ethofumesate residues were always <0.01 mg/kg.

The total residues of ethofumesate in barley and wheat grain and straw were in all rotations below the LOQ of 0.02 mg/kg.

**Table 7.6.2-1 Application scenario in field rotational crop trials (study 10-2501): Spray treatment with Ethofumesate SC 500 to soil or a target crop**

Trial No. Plot No Country Location Year	Target Crop, Variety	FL	Application			GS
			No.	kg/ha (a.s.)	kg/hl (a.s.)	
N-EU						
10-2501-02 10-2501-02-T-1A Netherlands 1681 ND Zwaagdijk 2010	soil	500 SC	1	1.0	0.333	

Trial No. Plot No Country Location Year	Target Crop, Variety	FL	Application			GS
			No.	kg/ha (a.s.)	kg/hl (a.s.)	
10-2501-02 10-2501-02-T-1B Netherlands 1681 ND Zwaagdijk 2010	soil	500 SC	1	1.0	0.333	
10-2501-02 10-2501-02-T-1C Netherlands 1681 ND Zwaagdijk 2010	soil	500 SC	1	1.0	0.333	
10-2501-02 10-2501-02-T-2A Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-02 10-2501-02-T-2B Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-02 10-2501-02-T-2C Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-02 10-2501-02-T-3A Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-02 10-2501-02-T-3B Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-02 10-2501-02-T-3C Netherlands 1681 ND Zwaagdijk 2010	beet, sugar Heron	500 SC	1	1.0	0.333	16
10-2501-05 10-2501-05-T-1A Germany 51399 Burscheid 2010	soil	500 SC	1	1.0	0.333	
10-2501-05 10-2501-05-T-1B Germany 51399 Burscheid 2010	soil	500 SC	1	1.0	0.333	
10-2501-05 10-2501-05-T-1C Germany 51399 Burscheid 2010	soil	500 SC	1	1.0	0.333	
10-2501-05 10-2501-05-T-2A Germany 51399 Burscheid 2010	beet, sugar Williams	500 SC	1	1.0	0.333	16



Trial No. Plot No Country Location Year	Target Crop, Variety	FL	Application			GS
			No.	kg/ha (a.s.)	kg/hl (a.s.)	
10-2501-05 10-2501-05-T-2B Germany 51399 Burscheid 2010	beet, sugar  Wiiliams	500 SC	1	1.0	0.333	16
10-2501-05 10-2501-05-T-2C Germany 51399 Burscheid 2010	beet, sugar  Wiiliams	500 SC	1	1.0	0.333	16
10-2501-05 10-2501-05-T-3A Germany 51399 Burscheid 2010	beet, sugar  Wiiliams	500 SC	1	1.0	0.333	16
10-2501-05 10-2501-05-T-3B Germany 51399 Burscheid 2010	beet, sugar  Wiiliams	500 SC	1	1.0	0.333	16
10-2501-05 10-2501-05-T-3C Germany 51399 Burscheid 2010	beet, sugar  Wiiliams	500 SC	1	1.0	0.333	16
<b>S-EU</b>						
10-2501-03 10-2501-03-T-1A Italy 40128 Bologna 2010	soil	500 SC	1	1.0	0.333	
10-2501-03 10-2501-03-T-1B Italy 40128 Bologna 2010	soil	500 SC	1	1.0	0.333	
10-2501-03 10-2501-03-T-1C Italy 40128 Bologna 2010	soil	500 SC	1	1.0	0.333	
10-2501-03 10-2501-03-T-2A Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18
10-2501-03 10-2501-03-T-2B Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18
10-2501-03 10-2501-03-T-2C Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18

Trial No. Plot No Country Location Year	Target Crop, Variety	FL	Application			GS
			No.	kg/ha (a.s.)	kg/hl (a.s.)	
10-2501-03 10-2501-03-T-3A Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18
10-2501-03 10-2501-03-T-3B Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18
10-2501-03 10-2501-03-T-3C Italy 40128 Bologna 2010	beet, sugar  Houston	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-1A Spain 41310 Brenes Sevilla 2010	soil	500 SC	1	1.0	0.333	
10-2501-04 10-2501-04-T-1B Spain 41310 Brenes Sevilla 2010	soil	500 SC	1	1.0	0.333	
10-2501-04 10-2501-04-T-1C Spain 41310 Brenes Sevilla 2010	soil	500 SC	1	1.0	0.333	
10-2501-04 0-2503-03-T-2A Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-2B Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-2C Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-3A Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-3B Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18
10-2501-04 10-2501-04-T-3C Spain 41310 Brenes Sevilla 2010	beet, sugar  Barbate	500 SC	1	1.0	0.333	18

FL = formulation GS = growth stage (BBCH-code) at last treatment  
EU-S = southern European residue region

The results of the rotational crop field trials are summarised in the following tables. Those trials, where the application was made on bare soil, the information is written in italics.

**Table 7.6.2-2 Results of field rotational crop trials following spray treatment with Ethofumesate SC 500 to soil or a target crop and then planting back and sampling of various rotational crops;  
Here: root crops**

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		
					ethofumesate	NC 9607	Total residue
<b>N-EU</b>							
10-2501-02 10-2501-02-T-1A Netherlands 1681 ND Zwaagdijk 2010	Carrot, Nerja  (Rotation 1) PBI 25 days	leaf	48 49	92 106	0.03 0.03	<0.01 <0.01	0.04 0.04
		root	48 49	92 106	0.04 <u>0.02</u>	<0.01 <u>&lt;0.01</u>	0.05 <u>0.03</u>
10-2501-02 10-2501-02-T-2A Netherlands 1681 ND Zwaagdijk 2010	Carrot, Nerja  (Rotation 2) PBI 54 days	leaf	48 49	144 158	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	48 49	144 158	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-02 10-2501-02-T-3A Netherlands 1681 ND Zwaagdijk 2010	Carrot, Nerja  (Rotation 3) PBI 321 days	leaf	47 49	399 413	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	47 49	399 413	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-05 10-2501-05-T-1A Germany 51399 Burscheid 2010	Carrot Cesta  (Rotation 1) PBI 26 days	leaf	46 49	100 114	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	46 49	100 114	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-05 10-2501-05-T-2A Germany 51399 Burscheid 2010	Carrot Cesta  (Rotation 2) PBI 70days	leaf	48 49	150 164	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	48 49	150 164	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-05 10-2501-05-T-3A Germany 51399 Burscheid 2010	Carrot Cesta  (Rotation 3) PBI 320 days	leaf	48 49	396 410	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	48 49	396 410	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
<b>S-EU</b>							
10-2501-03 10-2501-03-T-1A Italy 40128 Bologna 2010	Carrot Nantes-Clodia  (Rotation 1) PBI 29 days	leaf	49 49	140 154	0.03 0.03	<0.01 <0.01	0.04 0.04
		root	49 49	140 154	0.02 <u>0.03</u>	<0.01 <u>&lt;0.01</u>	0.03 <u>0.04</u>
10-2501-03 10-2501-03-T-2A Italy 40128 Bologna 2010	Carrot Nantes-Clodia  (Rotation 2) PBI 121 days	leaf	48 49	339 353	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	48 49	339 353	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-03 10-2501-03-T-3A Italy 40128 Bologna 2010	Carrot Nantes-Clodia  (Rotation 3) PBI 354 days	leaf	48 49	472 486	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
		root	48 49	472 486	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-04 10-2501-04-T-1A	Carrot Coral	leaf	47 49	201 216	0.02 0.02	<0.01 <0.01	<0.03 0.03

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		
					ethofumesate	NC 9607	Total residue
<i>Spain</i> <i>41310 Brenes Sevilla</i> <i>2010</i>	<i>(Rotation 1)</i> <i>PBI 26 days</i>	<i>root</i>	47 49	201 216	0.03 <u>0.02</u>	<0.01 <u>&lt;0.01</u>	0.04 <u>0.03</u>
10-2501-04 10-2501-04-T-2A Spain	Carrot Coral	leaf	45 49	434 448	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
41310 Brenes Sevilla 2010	(Rotation 2) PBI 259 days	root	43 49	434 448	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-04 10-2501-04-T-3A Spain	Carrot Coral	leaf	45 49	463 477	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02
41310 Brenes Sevilla 2010	(Rotation 3) PBI 330 days	root	41 49	463 477	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>

**Table 7.6.2-3 Results of field rotational crop trials following spray treatment with Ethofumesate SC 500 to soil or a target crop and then planting back and sampling of various rotational crops Here: leafy crops**

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		Total residue
					ethofumesate	NC9607	
<b>N-EU</b>							
10-2501-02 10-2501-02-T-1B Netherlands 1681 ND Zwaagdijk	Lettuce Lucan Butterhead variety	head	41 49	46 60	0.02 <u>&lt;0.01</u>	0.01 <u>&lt;0.01</u>	0.03 <u>&lt;0.02</u>
EU-N 2010	(Rotation 1) PBI 25 days						
10-2501-02 10-2501-02-T-2B Netherlands 1681 ND Zwaagdijk	Lettuce Lucan Butterhead variety	head	46 49	88 102	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
EU-N 2010	(Rotation 2) PBI 57 days						
10-2501-02 10-2501-02-T-3B Netherlands 1681 ND Zwaagdijk	Lettuce Lucan Butterhead variety	head	45 49	351 365	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
EU-N 2010	(Rotation 3) PBI 322 days						
10-2501-05 10-2501-05-T-1B Germany 51399 Burscheid	Lettuce Argentinos Loose leaf variety	head	46 49	82 96	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
EU-N 2010	(Rotation 1) PBI 26 days						

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		Total residue
					ethofumesate	NC9607	
10-2501-05 10-2501-05-T-2B Germany 51399 Burscheid  EU-N 2010	Lettuce Aleppo Loose leaf variety  (Rotation 2) PBI 77 days	head	46 49	124 138	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-05 10-2501-05-T-3B Germany 51399 Burscheid  EU-N 2010	Lettuce Aleppo Loose leaf variety  (Rotation 3) PBI 320 days	head	46 49	354 368	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
<b>S-EU</b>							
10-2501-03 10-2501-03-T-1B Italy 40128 Bologna  EU-S 2010	Lettuce Genti Lina Loose leaf variety  (Rotation 1) PBI 29 days	head	48 49	62 76	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-03 10-2501-03-T-2B Italy 40128 Bologna  EU-S 2010	Lettuce Palomis Loose leaf variety  (Rotation 2) PBI 122days	head	48 49	157 171	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-03 10-2501-03-T-3B Italy 40128 Bologna  EU-S 2010	Lettuce Gentile Funride Loose leaf variety  (Rotation 3) PBI 354 days	head	47 49	387 401	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-04 10-2501-04-T-1B Spain 41310 Brenes Sevilla  EU-S 2010	Lettuce Filipo Loose leaf variety  (Rotation 1) PBI 26 days	head	45 49	131 145	0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	0.02 <u>&lt;0.02</u>
10-2501-04 10-2501-04-T-2B Spain 41310 Brenes Sevilla  EU-S 2010	Lettuce Carolus Loose leaf variety  (Rotation 2) PBI 259 days	head	45 49	378 392	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>
10-2501-04 10-2501-04-T-3B Spain 41310 Brenes Sevilla  EU-S 2010	Lettuce Carolus Loose leaf variety  (Rotation 3) PBI 330 days	head	45 49	419 434	<0.01 <u>&lt;0.01</u>	<0.01 <u>&lt;0.01</u>	<0.02 <u>&lt;0.02</u>

**Table 7.6.2-4 Results of field rotational crop trials following spray treatment with Ethofumesate SC 500 to soil or a target crop and then planting back and sampling of various rotational crops**  
**Here: cereal crops**

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		Total residue
					ethofumesate	NC 9607	
<b>N-EU</b>							
10-2501-02 10-2501-02-T-1C Netherlands 1681 ND Zwaagdijk 2010	Barley Cervoise  (Rotation 1) PBI 33 days	green material	30	226	<0.01	<0.01	<0.02
		straw	89	329	<0.01	<0.01	<0.02
10-2501-02 10-2501-02-T-2C Netherlands 1681 ND Zwaagdijk 2010	Wheat Tatarus  (Rotation 2) PBI 176 days	green material	30	327	<0.01	<0.01	<0.02
		grain	89	428	<0.01	<0.01	<0.02
		straw	89	428	<0.01	<0.01	<0.02
10-2501-02 10-2501-02-T-3C Netherlands 1681 ND Zwaagdijk 2010	Barley Tripple  (Rotation 3) PBI 288 days	green material	30	343	<0.01	<0.01	<0.02
		grain	89	452	<0.01	<0.01	<0.02
		straw	89	452	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-1C Germany 51399 Burscheid 2010	Barley Simba Summer variety  (Rotation 1) PBI 26 days	green material	29	64	<0.01	0.02	0.03
		grain	89	135	<0.01	<0.01	<0.02
		straw	89	135	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-2C Germany 51399 Burscheid 2010	Wheat Hermann Winter variety  (Rotation 2) PBI 131 days	green material	30	311	<0.01	<0.01	<0.02
		grain	89	417	<0.01	<0.01	<0.02
		straw	89	417	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-3C Germany 51399 Burscheid 2010	Barley Simba Summer variety  (Rotation 3) PBI 284 days	green material	29	333	<0.01	<0.01	<0.02
		grain	89	420	<0.01	<0.01	<0.02
		straw	89	420	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-1C Germany 51399 Burscheid 2010	Barley Simba Summer variety  (Rotation 1) PBI 26 days	green material	29	64	<0.01	0.02	0.03
		grain	89	135	<0.01	<0.01	<0.02
		straw	89	135	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-2C Germany 51399 Burscheid 2010	Wheat Hermann Winter variety  (Rotation 2) PBI 131 days	green material	30	311	<0.01	<0.01	<0.02
		grain	89	417	<0.01	<0.01	<0.02
		straw	89	417	<0.01	<0.01	<0.02
10-2501-05 10-2501-05-T-3C Germany 51399 Burscheid 2010	Barley Simba Summer variety  (Rotation 3) PBI 284 days	green material	29	333	<0.01	<0.01	<0.02
		grain	89	420	<0.01	<0.01	<0.02
		straw	89	420	<0.01	<0.01	<0.02

Study No. (Trial No.) Plot No Country	Rotational Crop, Variety  (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg)		Total residue
					ethofumesate	NC 9607	
<b>S-EU</b>							
10-2501-03 10-2501-03-T-1C Italy 40128 Bologna 2010	Barley Otis  (Rotation 1) PBI 26 days	green material	30	64	<0.01	0.02	0.03
		grain	89	117	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	117	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
10-2501-03 10-2501-03-T-2C Italy 40128 Bologna 2010	Wheat Mieti  (Rotation 2) PBI 151 days	green material	30	311	<0.01	<0.01	<0.02
		grain	89	401	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	401	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
10-2501-03 10-2501-03-T-3C Italy 40128 Bologna 2010	Barley Otis  (Rotation 3) PBI 284 days	green material	30	354	<0.01	<0.01	<0.02
		grain	89	417	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	417	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
10-2501-04 10-2501-04-T-1C Spain 41310 Brenes Sevilla 2010	Barley Garbo  (Rotation 1) PBI 26 days	green material	30	134	<0.01	0.02	0.03
		grain	89	242	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	242	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
10-2501-04 10-2501-04-T-2C Spain 41310 Brenes Sevilla 2010	Wheat Semolero  (Rotation 2) PBI 259 days	green material	30	322	<0.01	<0.01	<0.02
		grain	89	463	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	463	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
10-2501-04 10-2501-04-T-3C Spain 41310 Brenes Sevilla 2010	Barley Garbo  (Rotation 3) PBI 330 days	green material	30	414	<0.01	<0.01	<0.02
		grain	89	477	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>
		straw	89	477	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.02</u>

DALT = days after last treatment

GS = growth stage (BBCH-code) at sampling

PBI = plant-back interval

**Table 7.6.2-5 Recovery data for ethofumesate in rotational crop matrices (root, leafy, and cereal crops)**

Study Trial No. Plot No.	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2501 (10-2501-02), (10-2501-03), (10-2501-04), (10-2501-05)  Plots T-1A to T-3A  GLP: yes 2010	Carrot	leaf	ethofumesate	3	0.01	66;95;97	66	97	86	20.2
				6	0.10	70;76;79;81;82;95	70	95	81	10.3
				9	overall		66	97	82	13.6
		root	NC 9607	3	0.01	63;77;87	63	87	76	15.9
				6	0.10	54;58;60;62;71;98	54	98	67	24.0
				9	overall		54	98	70	21.0
	Lettuce	head	ethofumesate	4	0.01	71;82;85;86	71	86	81	8.5
				5	0.10	69;73;76;81;85	69	85	77	8.3
				9	overall		69	86	79	8.3
		head	NC 9607	4	0.01	73;79;81;81	73	81	79	4.8
				5	0.10	45;57;61;76;81	45	81	64	22.8
				9	overall		45	81	70	18.5
10-2501 (10-2501-02), (10-2501-03), (10-2501-04),	Lettuce	head	ethofumesate	3	0.01	76;86;93	76	93	85	10.1
				5	0.10	71;75;83;87;93	71	93	82	10.9
				8	overall		71	93	83	10.0
			NC 9607	3	0.01	70;77;81	70	81	76	7.3

(10-2501-05)  Plots T-1A to T-3A  GLP: yes 2010				5 8	0.10 overall	56;65;68;71;84	56 56	84 84	69 72	14.8 12.7
10-2501 (10-2501-02), (10-2501-03), (10-2501-04), (10-2501-05)  Plots T-1A to T-3A  GLP: yes 2010	Barley/ Wheat	green material	ethofumesate	4	0.01	65;90;91;93	65	93	85	15.6
				3	0.10	81;86;84	81	86	84	3.0
				7	overall		65	93	84	11.2
			NC 9607	4	0.01	60;83;92;96	60	96	83	19.5
				3	0.10	76;78;85	76	85	80	5.9
				7	overall		60	96	81	14.5
		grain	ethofumesate	3	0.01	81;89;93	81	93	88	7.0
				4	0.10	81;84;86;90	81	90	85	4.4
				7	overall		81	93	86	5.3
			NC 9607	3	0.01	86;90;110	86	110	95	13.5
				3	0.10	77;80;94	77	94	84	10.8
				6	overall		77	110	90	13.2
		straw	ethofumesate	4	0.01	73;76;80;85	73	85	79	6.6
				3	0.10	70;87;84	70	87	80	11.3
				7	overall		70	87	79	8.2
			NC 9607	3	0.01	65;81;79	65	81	75	11.6
				2	0.10	78;80	78	80	79	
				5	overall		65	81	77	8.6

### III. Conclusions

Four rotational crop field trials were conducted in Europe (2 each in the northern and southern residue regions) in the years 2010-2011. Ethofumesate was applied once as an SC 500 formulation either to bare soil or to a target crop (sugar beet) at an active substance rate of 1.0 kg/ha, the target crop was then harvested, and crops representing 3 different botanical groups (roots, leafy veg., cereals) were planted on the plots at 3 intervals thereafter.

To evaluate the potential residues in following crops, samples of the rotated crops were taken at an intermediate stage and at usual full harvest ripeness. Samples were analysed for ethofumesate and the common moiety NC9607.

Residues of ethofumesate in all rotational crops were only detected in the first rotation, i.e. grown after a plant-back interval (PBI) of 25-33 days.

The highest total residues of ethofumesate in rotational **root crops** (here: immature carrot roots sampled approx. 14 days prior to the mature crop) ranged from <0.02-0.05 mg/kg (median 0.04 mg/kg; n=4). Residues in the mature crop ranged from <0.02-0.04 mg/kg (median 0.03 mg/kg; n= 4). Residues were also determined in the leaves and ranged from <0.02-0.04 mg/kg (median 0.04 mg/kg; n=4), independent if harvested from the immature or the mature crop. These results are confirmed by the confined rotational crop studies.

Detectable residues were only found as ethofumesate; residues of the common moiety NC 9607 were always below the LOQ of 0.01 mg/kg in carrot roots and leaves.

In **lettuce, cereals grain and straw** no residues of ethofumesate and NC 9607 above the LOQ of 0.01 mg/kg were detected.

Only 3 trials could be evaluated for cereal grains as geese ate the grain in the 4th trial. Samples were also taken of the **fodder-relevant commodities** green material and straw. In green material taken earlier in the rotation,



ethofumesate residues were below the LOQ of 0.01 mg/kg and the residues of the common moiety NC 9607 ranged from <0.01-0.02 mg/kg. Thus the total residue ranged from <0.02-0.03 mg/kg (median 0.03 mg/kg, n= 4) in green material of the first rotation.

### B.7.6.2.2. Magnitude of residues in rotational crops (2)

#### New data for AIR:

Report:	KCA 6.6.2/01, Spence, Ch. (2014)
Title:	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)
Document No:	Test Facility Study No. 697614, Report No. 34890
Guidelines:	OECD 504 and OECD 509
GLP:	Yes

### I. Material and methods

Two field rotation trials, one trial in Northern EU (UK) and one trial in Southern EU (Italy), were carried out during the growing season 2012-2013. Each of these trials contains one control and one treated plot. At both trials, ethofumesate formulated as ethofumesate 500 g/L was applied once at a rate ranging from 960.00 g as/ha to 1125.08 g as/ha to sugar beets. The application was carried out at a BBCH 14-16 except for the plot with a plant-back interval of 30-31 days and the rotation with spring barley. In the other trial, the application was carried out pre-emergence. Spinach, carrots or radish and spring barley were sown/planted 30-41 days after application; spinach, radish and winter wheat or winter barley were sown/planted 90-180 days after application; spinach, radish and spring barley were sown/planted 335 days after application. Please note that prior to sowing/planting either the soil was inverted to a depth of 26-30 cm with a fork or spat followed by raking to prepare a seed bed or the soil was ploughed to a depth of 50 cm using a single furrow plough followed by power harrowing or the soil was ploughed to a depth of 20 cm. Samples have been taken from immature leafy vegetables, mature leafy vegetables, roots and tops of root vegetables, cereal forage, cereal hay, cereal grain and cereal straw. Due to crop failure the 90 day plant back interval root crop sample could not be taken from trial 1. The samples were stored deep frozen at -18°C until analysis. The maximum storage period between sampling and extraction was 12 months. The storage stability of ethofumesate and its relevant metabolites in the crop commodities of the rotational crops is covered by available storage stability data.

The samples were analysed according to the analytical method of Hamberger (study No. 11A04042-01-VMSB). The percent recoveries for ethofumesate and NC 9607 and NC 20645 from control crop specimens freshly fortified (Ethofumesate: 0.01 and 0.1 mg/kg for root and leafy vegetables and 0.05 and 0.5 mg/kg for cereal matrices; NC 9607 and NC 20645: 0.005 and 0.05 mg/kg for root and leafy vegetables and 0.025 and 0.25 for cereal matrices) are between 70 and 110%.

No Ethofumesate residues were detected in unfortified control specimens from any of the tests. Residues were extracted with acetone or acetone/water. The extract was made alkaline (conversion of NC 9607 into NC 20645) and partitioned with hexane. The remaining plant material from acetone extraction and the alkaline aqueous phase were combined and neutralised. After adding 10% acetone and cooking under reflux for one hour, acidic hydrolysis was carried out (cracking conjugates, conversion of NC 20645 into NC 9607). The clean-up was done by solid phase extraction. All final extracts were analysed with GC/MS. The metabolite NC 20645 was

converted into NC 9607 within the analytical method and was finally detected as NC 9607. Since an acidic hydrolysis step was included in this method, also conjugated residues were extracted.

## II. Results and discussion

No residues of Ethofumesate and the sum of its metabolites NC9607 + NC20645 above the LOQ (0.01 mg/kg for each analyte for root and leafy vegetable matrices and 0.05 mg/kg for each analyte for cereal matrices) were found in any of the control and treated specimens. Detailed results are given in the tables below.

**Table 7.6.2-6 Results of Ethofumesate and its metabolites included in the proposed residue definition in rotational crops for trial 1 located in Cambridge, UK**

Commodity	Plantback Interval (days)	Days After Last Application	Days Between Sowing and Sampling	Ethofumesate Residues (mg/kg)	Sum of NC9607 + NC20645 (mg/kg)
Immature leafy vegetable	41	79	38	<0.01	<<0.01
	103	343	240	<0.01	<<0.01
	335	383	48	<0.01	<<0.01
Mature leafy vegetable	41	98	57	<0.01	<<0.01
	103	347	244	<0.01	<<0.01
	335	389	54	<0.01	<<0.01
Root vegetable - roots	40	179	139	<0.01	<<0.01
	103	No sample could be taken as unusually cold weather caused crop failure			
	335	390	55	<0.01	<0.01
Root vegetable - tops	40	179	139	<0.01	<0.01
	103	No sample could be taken as unusually cold weather caused crop failure			
	335	390	55	<0.01	<0.01
Cereal - forage	31	82	51	<0.05	<0.05
	176	347	171	<0.05	<0.05
	335	376	41	<0.05	<0.05
Cereal - hay	31	127	96	<0.05	<0.05
	176	396	220	<0.05	<0.05
	335	417	82	<0.05	<0.05
Cereal - grain	31	157	126	<0.05	<0.05
	176	431	255	<0.05	<0.05
	335	453	118	<0.05	<0.05
Cereal - straw	31	157	126	<0.05	<0.05
	176	431	255	<0.05	<0.05
	335	453	118	<0.05	<0.05

**Table 7.6.2-7 Results of Ethofumesate and its metabolites included in the proposed residue definition in rotational crops for trial 2 located in Lombardia, Italy**

Commodity	Plantback Interval (days)	Days After Last Application	Days Between Sowing and Sampling	Ethofumesate Residues (mg/kg)	Sum of NC9607 + NC20645 (mg/kg)
Immature leafy vegetable	30	83	53	<0.01	<0.01
	90	135	45	<0.01	<0.01
	335	365	30	<0.01	<0.01
Mature leafy vegetable	30	99	69	<0.01	<0.01
	90	147	57	<0.01	<0.01
	335	376	41	<0.01	<0.01
Root vegetable - roots	30	118	88	<0.01	<0.01
	90	147	57	<0.01	<0.01
	335	365	30	<0.01	<0.01
Root vegetable - tops	30	118	88	<0.01	<0.01
	90	147	57	<0.01	<0.01
	335	365	30	<0.01	<0.01
Cereal - forage	30	79	49	<0.05	<0.05
	180	335	155	<0.05	<0.05
	335	365	30	<0.05	<0.05

Commodity	Plantback Interval (days)	Days After Last Application	Days Between Sowing and Sampling	Ethofumesate Residues (mg/kg)	Sum of NC9607 + NC20645 (mg/kg)
Cereal - hay	30	100	70	<0.05	<0.05
	180	350	170	<0.05	<0.05
	335	386	51	<0.05	<0.05
Cereal - grain	30	142	112	<0.05	<0.05
	180	410	230	<0.05	<0.05
	335	428	93	<0.05	<0.05
Cereal - straw	30	142	112	<0.05	<0.05
	180	410	230	<0.05	<0.05
	335	428	93	<0.05	<0.05

### III. Conclusion

This study confirms the results of the field rotation studies already reviewed on EU level. No residues of Ethofumesate and its metabolites included in the proposed residue definition need to be expected in rotational crops after application of Ethofumesate according to the intended GAP.

## B.7.7. OTHER STUDIES

### B.7.7.1. Supplementary information

The search of open literature revealed a publication about stereoselective degradation of ethofumesate in turfgrass and soil (Wang, P. et al.; 2005; M-458577-01-1; published). The article is summarized in the following for the sake of completeness. This supplementary information has no impact on the data presented in this section since degradation of ethofumesate in plants was found to be fast and resulted in non-chiral metabolites. Moreover, ethofumesate related residues (comprising parent compound and the non-chiral metabolites NC 20645, in free and conjugated form, and NC 9607) were generally low in mature crops at harvest.

Livestock studies have also shown that a transfer of ethofumesate related residues (taken up by feedstuffs) in edible matrices of the animals is negligible and will not lead to any residues above 0.01 mg/kg in edible matrices of livestock.

Thus, food items of plant and animal origin will show no ethofumesate related residues and therefore no dietary exposure is expected, neither to the enantiomers of ethofumesate nor to any metabolite and therefore no enantioselective analysis is needed.

Report:	KCA 6.10 /01;Wang, P.; Wang, Q.; Jiang, S.; Qiu, J.; Wang, P.; Zhou, Z.;2005; M-458577-01
Title:	Stereoselective degradation of ethofumesate in turfgrass and soil.
Source:	Pesticide Biochemistry and Physiology, Volume 82, Issue 3, Page 197-204, Publication Year 2005
Document No:	M-458577-01-1
Guidelines:	not applicable
GLP/GEP:	no

## I. Summary

The stereoselective degradation of ethofumesate in turfgrasses and several agricultural soils was investigated to provide details of the fate of this chiral herbicide. Racemic ethofumesate was either foliar applied to two species of turfgrass or fortified into four types of agricultural soils. (+)- and (-)-enantiomers were extracted and analyzed

by a validated chiral HPLC method which involved extraction of samples with organic solvent followed by separation on a cellulose-tris-(3,5-dimethylphenyl-carbamate)-based chiral column and quantification by UV absorbance at 230 nm. Mean recoveries of each enantiomer fortified at 0.5, 5, and 10 µg/g ranged from  $82.3 \pm 5.84$  to  $92.5 \pm 2.87\%$  in turfgrasses and from  $86.0 \pm 5.09$  to  $98.1 \pm 2.51\%$  in soil. As a measure of this composition, the enantiomeric ratio (ER) was used, defined as the concentration ratio of (+)/(-)-enantiomer. Similarly, preferential degradation of the (-)-enantiomer was observed in both grass species with the largest ER of about 3, and in one of the test soil with ER = 1.65, resulting in residues enriched with (+)-enantiomer. The stereoselective degradation in this soil led to a significant difference on half-lives between the two enantiomers. No stereoselective degradation was observed in other soils.

## II. Materials and methods

### A. Material

#### 1. Test material

Test item:	Racemic ethofumesate standard
Active substance(s):	Ethofumesate
Chemical state and description:	Technical grade substance
Source of test item:	Institute for the Control of Agrochemicals, Ministry of Agriculture (Beijing, China)
Batch number:	None
Purity:	96%
Storage conditions:	Stock solution in 2-propanol at -20 °C
Water solubility:	Not stated

#### 2. Site description (for field study in turfgrass):

Location/country:	Gardening department of China Agricultural University
Test turfgrass species:	Kentucky bluegrass ( <i>Poa pratensis</i> L.) and tall fescue ( <i>Festuca arundinacea</i> Schreb.)
Plot size:	3 x 2 m
Pesticides used on fields:	No other pesticides than ethofumesate and irrigation were applied during the study.
History of pesticides on field plot:	Not stated
Weather conditions:	Natural rainfalls at 2 and 4 days after treatment; no further weather conditions reported.

#### 3. Soil (for laboratory study):

Soil sample and texture:	#1	#2	#3	#4
	Silt sand loam	Sand loam	Silt sand loam	Sand loam
Source/origin of soils:	Heilongjiang	Fujian,	Sichuan	Beijing
	NE China	SE China	West China	Central China
Particle size (%): Sand	50	72	40	67
Silt	47	26	57	17
Clay	3	2	3	16
pH:	5.0	4.3	5.3	7.9
Organic matter:	6.85	3.51	1.98	1.31

### B. Study design and methods

#### 1. Study design

Study type:	Field study in turfgrass And Laboratory study in soil
Study duration:	10 days
Treatments:	Turfgrass field study: 1 foliar application treatment, no untreated control plot instead 1 pre-treatment control sample 2 days before spraying. Soil laboratory study: each of 4 soils were treated by racemic ethofumesate and not treated

Preparation prior to treatment:	Turfgrass field study: turf species were mown to height of 5 cm 2 days before treatment.
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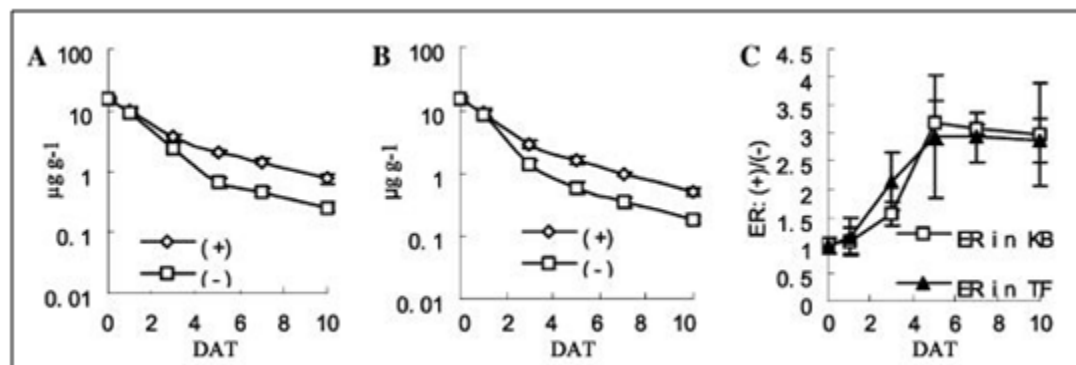
Application method:	Soil laboratory study: soils were air-dried and passed through 2 mm sieve; adjusted to moisture content of 30% for experiment. Turfgrass field study: foliar application, dissolved in acetone/Milli-Q water (1:5, v/v)
Application date:	Soil laboratory study: mixed into soil 2 July 2004
Application rate per treatment:	Turfgrass field study: 1.5 kg ethofumesate/ha at 250 L/ha (water volume) Soil laboratory study: 100 µL test item/100 g dry weight soil (corresponding to 10 µg/g for each of two enantiomers)
2. Sampling	
Sampling technique:	Turfgrass field study: collected from plots and prepared for storage Soil laboratory study: 5 g dry weight soil (corr. 6.5 g wet soil) for immediate analysis.
Sampling frequency:	Immediately after treatment and at 1, 3, 5, 7, and 10 days after treatment (DAT).
Number of samples per site/soil type: storage of samples:	1 sample per sampling event per soil type or turfgrass species Turfgrass samples were rinsed with distilled water for 10 min., sopped up with filter paper, homogenized in a mechanical Waring blender and stored at -20°C until analysis.
3. Measurements	
Parameters:	Concentrations of each of the two enantiomers of ethofumesate
Calibration and method validation:	Extract of ethofumesate-free control grass and soil samples was fortified with racemic ethofumesate to give final concentrations of each enantiomer ranging from 0.5 to 150 µg/g. The limit of detection (LOD) for each enantiomer was considered to be the concentration that produced a signal-to-noise (S/N) ratio of 3, and the limit of determination of the method was accordingly obtained by dividing the LOD by concentrated volume times of 10.
4. Chemical analysis	
Guideline/protocol:	None
Method:	High performance liquid chromatography-chiral stationary phase (HPLC-CSP)
Preparation for analysis:	Samples were extracted repeatedly with acetic acid and drying over anhydrous Na <sub>2</sub> SO <sub>4</sub> . Residue was finally reconstituted in 1ml of 2-propanol and filtered through a filter (0.45 µm in pore size).
Conduction:	The enantiomers were separated on cellulose-Tris-(3,5-dimethylphenyl-carbamate) (CDMPC)-based CSP at room temperature. Mobile phase made up of n-hexane and 2-propanol and monitored at 230 nm.
Reference item:	Ethofumesate racemic standard solution
Recovery:	Recoveries of each enantiomer fortified at 0.5, 5, and 10 µg/g ranged from 82.3 ± 5.84 to 98.1 ± 2.51%
Limit of detection:	0.03 µg/g sample for each of the 2 enantiomers
Limit of quantification:	0.3 µg/g sample for each of the 2 enantiomers

### III. Results and discussion

#### 1. Analytical findings:

The enantiomeric ratio (ER) in Kentucky bluegrass (KB) reached the highest value of 3.2 at 5 days after treatment (Figure 6.10-1, A and C). Similar stereoselective tendency was observed in tall fescue (TF), and the highest ER was 2.9 at 5 and 7 days after treatment (Figure 6.10-1, B and C). The increasing ERs confirmed that the (-)-ethofumesate disappeared faster than its antipode in the test plants.

**Figure 5: Stereoselective dissipation of two enantiomers in (A) KB, (B) in TF, and (C) variation of ERs in test plants. Each point represents the mean value  $\pm$  SE of three replicates**



The degradation of ethofumesate in soils was significantly slower than that in the grass. The enantiomers degraded much faster in soils 2 and 3 with half-lives of less than 3.6 weeks than in soil 1 with half-lives more than 6 weeks. Curves of the concentration in soils (Y,  $\mu\text{g/g}$ ) versus incubation times (x, weeks) were regressed by the first-order kinetics model with the R2 value ranging from 0.9038 to 0.9857 (Table 6.10-1).

**Table 7.7.1-1 Regressive metabolizing functions of two enantiomers in test soils**

Soils	Enantiomer	Regressive functions	R2	Half-life (weeks)
1	(+)	$Y = 8.6344 e^{-0.1105x}$	0.9038	6.27
	(-)	$Y = 8.7668 e^{-0.1118x}$	0.9266	6.20
2	(+)	$Y = 8.6254 e^{-0.1941x}$	0.9554	3.57
	(-)	$Y = 8.8143 e^{-0.2109x}$	0.9758	3.29
3	(+)	$Y = 8.9841 e^{-0.1973x}$	0.9614	3.51b
	(-)	$Y = 9.2979 e^{-0.2707x}$	0.9857	2.56b
4	(+)	$Y = 10.73 e^{-0.1671x}$	0.9278	4.15
	(-)	$Y = 10.854 e^{-0.1705x}$	0.9346	4.07

a The regressive functions were obtained based on the mean value of three replicates

b Significantly different from each other,  $P = 0.05$  (Student's paired t test) n.d.: not detectable

#### IV. Conclusion

Preferential degradation of the (-)-enantiomer was observed in both grass species and in one of the tested soils. In grass the enantiomeric ratio (ER) amounted to about 3 and in the soil to a value of  $ER = 1.65$ , resulting in residues enriched with (+)-enantiomer. The stereoselective degradation in one of the tested soils led to a significant difference in the half-lives of the two enantiomers. No stereoselective degradation was observed in the other two soils under investigation.

However, the preferential degradation of the (-)-enantiomer, as observed in both grass species and in one soil, has no influence on the dietary exposure of man. Plant metabolism studies have shown a fast degradation of ethofumesate to non-chiral metabolites – and moreover - mature edible matrices show generally low ethofumesate related residues at or slightly above the LOQ after post- and as well pre-emergence treatment. Therefore no dietary exposure is expected, neither to the enantiomers of ethofumesate nor to any metabolite and thus a different degradation of enantiomers is not of concern.

### B.7.7.2. Effect on the residue level in pollen and bee products

Sugar beets, fodder beets and beetroots are harvested before flowering and are therefore no feeding crops for bees. In addition, no internationally agreed guidelines are available for conducting a study addressing this data requirement.

Nevertheless, UPL submitted a statement is provided within this dossier which outlines the situation for honeybees in detail. This statement is given below.

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Report:	KCA 6.10.1/01, Lückmann, J. (2013)
Title:	Ethofumesate - exposure of honeybees to residues in nectar, pollen and guttation fluid in sugar and fodder beets
Document No:	---
Guidelines:	---
GLP/GEP:	---

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The risk for honeybees to get in contact with contaminated nectar and pollen is negligible as sugar and fodder beets do not build flowers within the first year. Sugar and fodder beets are harvested by the end of the first year. In the rare case that shoots with flowers are produced in the first year or beets are flowering in the second year (if beets are grown for seed production) no risk for honeybees is expected as beet flowers are wind pollinated. Sugar and fodder beet flowers are not mentioned in any standard or handbook on honey bee foraging plants (e.g. Maurizio & Schaper, 1994; Pritsch, 2007).


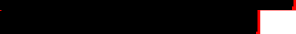

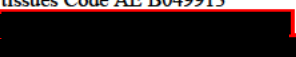



The beet structure does not allow formation of water reservoirs in leaf axils. The risk for honeybees to get in contact with Ethofumesate residues in guttation fluid being present at the leaf edges as guttation droplets at sugar and fodder beets after Ethofumesate treatment is very low as well, since beets display guttation in a very low frequency and intensity (Joachimsmeier et al., 2012; Pistorius et al., 2012). Hence, beets are very unattractive water sources for honeybees.

## B.7.8. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny 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 6.1	Whiteoak, R. J.	1975	STABILITY OF RESIDUES DURING STORAGE OF CROP AND SOIL SAMPLES FROM TRIALS WITH NORTON Fisons plc, United Kingdom Bayer CropScience, Report No.: A83296, Edition Number: <u>M-155565-01-1</u> Date: 1975-08-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	DAR (1998)
KCA 6.1	Bright, J. H. M.	1991	STABILITY OF ETHOFUMESATE AND NC 9607 RESIDUES IN SUGARBEET ROOTS AND TOPS DURING DEEP FREEZE STORAGE Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83111, Report includes Trial Nos.: 041/02/001 Edition Number: <u>M-155386-01-1</u> Date: 1991-03-08 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	DAR (1998)
KCA 6.1	Cole, M. G.	1995	ETHOFUMESATE: STABILITY OF ETHOFUMESATE, NC 9607 AND NC 8493 IN GRASS DURING FROZEN STORAGE, USA, 1993 Hoechst NOR-AM AgrEvo Inc., USA Bayer CropScience, Report No.: A54281, Edition Number: <u>M-134863-01-1</u> EPA MRID No.: 43765701 Date: 1995-06-14 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	DAR (1998)
KCA 6.1	Schulte, G.	2013	Storage stability of open-ring-2-keto ethofumesate (AE C520645) in plant matrices for 24 months - Phase report after 6 months Bayer CropScience, Report No.: MR-13/086, Edition Number: <u>M-459806-01-1</u> Date: 2013-07-11 GLP/GEP: yes, unpublished	N	Y	Data requirement: Storage stability of metabolite was not yet demonstrated.	Task Force Ethofumesate	Submitted for the purpose of renewal (2014)
KCA 6.1	Hamberger, R.	2013	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 AgriChem B.V., 12A04042-01-SSSB CIP Chemisches Institut Pforzheim GmbH GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	
KCA 6.1	Schlewitz, P.	2014	FROZEN storage stability of residues of ethofumesate metabolite NC 20645 in sugar beet (roots and tops with leaves) United Phosphorus Ltd., R B1312 ANADIAG GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.1	Perez, R.; Schmitt, J. L.; Patel, D.	2014	Freezer storage stability of ethofumesate in animal matrix samples - interim report ADPEN Laboratories, Inc., Jacksonville, FL, USA Bayer CropScience, Report No.: RAADP031, Edition Number: <u>M-467206-02-1</u>	Y	Y	Data requirement: Storage stability of metabolite was not yet demonstrated	Task Force Ethofumesate	Submitted for the purpose of renewal (2014)



Data Point	Author(s)	Year	Title Compagny 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
			Date: 2013-09-26 GLP/GEP: yes, unpublished			d.		
KCA 6.2.1 /01	Miller, C.	1999	Summary of the metabolism of ethofumesate in plants Ethofumesate AE B049913 AgrEvo UK Crop Protection Ltd., Chesterford Park, United Kingdom Bayer CropScience, Report No.: C003349, Edition Number: <u>M-185979-01-1</u> GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /01	Adcock, J. W.; Warner, P. A., Challis, I. R.	1976	THE METABOLISM OF 14C-ETHOFUMESATE IN THE ONION Fisons plc, United Kingdom Bayer CropScience, Report No.: A82959, Edition Number: <u>M-155236-01-1</u> Date: 1976-10-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /02	Warner, P. A.; Adcock, J. W.	1977	METABOLISM OF ETHOFUMESATE IN TOBACCO Fisons plc, United Kingdom Bayer CropScience, Report No.: A82963, Edition Number: <u>M-155240-01-1</u> Date: 1977-12-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /03	Lines, D. S.; Adcock, J. W.	1978	THE METABOLISM OF ETHOFUMESATE BY SUGAR BEET UNDER GREENHOUSE CONDITIONS Fisons plc, United Kingdom Bayer CropScience, Report No.: A82964, Edition Number: <u>M-155241-01-1</u> Date: 1978-12-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /04	Lines, D. S.; Adcock, J. W.	1979	THE METABOLISM OF ETHOFUMESATE (98% PURE 14C-ETHOFUMESATE) BY SUGAR BEET UNDER FIELD CONDITIONS Fisons plc, United Kingdom Bayer CropScience, Report No.: A82965, Edition Number: <u>M-155242-01-1</u> Date: 1979-01-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /05	Chapleo, S.	1992	THE METABOLISM OF [14C]-ETHOFUMESATE IN SUGAR BEET - A GLASSHOUSE STUDY Inveresk Research Int. Ltd., Tranent, Scotland Bayer CropScience, Report No.: A82970, Report includes Trial Nos.: 381174 ENVIR 84B Edition Number: <u>M-155247-01-1</u> Date: 1992-09-22 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /06	Caley, C. Y.; Chapleo, S.; Haswell, A.	1994	THE METABOLISM OF 14C-ETHOFUMESATE IN SUGAR BEET Inveresk Research Int. Ltd., Tranent, Scotland Bayer CropScience, Report No.: A87553, Report includes Trial Nos.: 382445 Edition Number: <u>M-161455-01-1</u> Date: 1994-06-01	N	N	-	Bayer CropScience	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			GLP/GEP: yes, unpublished					
KCA 6.2.1 /07	Chapleo, S.	1992	THE METABOLISM OF [14C]-ETHOFUMESATE IN ANNUAL RYEGRASS - A GLASSHOUSE STUDY Inveresk Research Int. Ltd., Tranent, Scotland Bayer CropScience, Report No.: A82971, Report includes Trial Nos.: 381169 ENVIR 85B Edition Number: <u>M-155248-01-1</u> Date: 1992-09-17 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.1 /08	Mellet, M.	1993	Determination of the residues of ethofumesate, ethofumesate-2- keto and the conjugates in sugar beets after application of Ethosat 500 SC in France, 1992 ANADIAG S.A., Haguenau, France Feinchemie Schwebda, Report No.: <u>M-468491-01-1</u> , Report includes Trial Nos.: 92HBEBI01 92HBEBI06 Edition Number: <u>M-468491-01-1</u> Date: 1993-06-07 GLP/GEP: yes, unpublished	N	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 6.2.1/01	Hennecke, D.	2003	Metabolism of Ethofumesate in sugar beets United Phosphorus Ltd., GAB-002/7-08 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	
KCA 6.2.2 /01		1992	THE METABOLISM OF 14C ETHOFUMESATE IN LAYING HENS  Bayer CropScience, Report No.: A82969, Report includes Trial Nos.: SMS 297/920431 TOX 90542 Edition Number: <u>M-155246-01-1</u> Date: 1992-06-09 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.2 /02		1999	Poultry - Metabolism, Distribution and nature of the residues in eggs and edible tissues Code AE B049913  Bayer CropScience, Report No.: C002998, Report includes Trial Nos.: Tox97227 Edition Number: <u>M-185380-01-1</u> Date: 1999-06-01 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.3 /01		1976	THE METABOLISM OF 14C-ETHOFUMESATE IN THE SHEEP  Bayer CropScience, Report No.: A82958, Edition Number: <u>M-155235-01-1</u> Date: 1976-09-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.2.3 /02		1992	THE METABOLISM OF 14C-ETHOFUMESATE IN THE COW	Y	N	-	Bayer CropScience	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			Huntingdon Research Centre Ltd., Bayer CropScience, Report No.: A82968, Report includes Trial Nos.: SMS 296/920441 TOX 90541 Edition Number: <u>M-155245-01-1</u> Date: 1992-06-04 GLP/GEP: yes, unpublished				nce	
KCA 6.2.3 /03		1999	Metabolism, distribution and nature of the residues in milk and edible tissues Ethofumesate ruminant Code: AE B049913 Bayer CropScience, Report No.: C003362, Report includes Trial Nos.: TOX97226 Edition Number: <u>M-185993-01-1</u> Date: 1999-04-07 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /01	Crofts, M.	1975	RESIDUES IN FODDER BEET AND RED BEET FROM 1974 APPLICATIONS OF NORTON IN THE UK Fisons plc, United Kingdom Bayer CropScience, Report No.: A83007, Edition Number: <u>M-155284-01-1</u> EPA MRID No.: 41214220 Date: 1975-05-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /02	Crofts, M.; Whiteoak, R. J.	1976	RESIDUES IN MANGOLDS, FODDER BEET AND RED BEET FROM 1975 AND 1976 APPLICATIONS OF NORTON IN THE UK (AND 1 RED BEET TRIAL IN SWEDEN) Fisons plc, United Kingdom Bayer CropScience, Report No.: A83020, Edition Number: <u>M-155297-01-1</u> EPA MRID No.: 41214219 Date: 1976-09-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /03	Crofts, M.; Whiteoak, R. J.	1977	RESIDUES IN RED BEET ROOTS FROM 1976 TRIALS WITH NORTON IN AUSTRALIA Fisons plc, United Kingdom Bayer CropScience, Report No.: A83022, Edition Number: <u>M-155299-01-1</u> EPA MRID No.: 41214220 Date: 1977-02-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /04	Crofts, M.	1978	HARVEST RESIDUES IN RED BEET FROM NORTON TRIALS IN THE USA (NEW YORK, TEXAS AND WISCONSIN) IN 1976/77 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83036, Edition Number: <u>M-155313-01-1</u> EPA MRID No.: 41214219 Date: 1978-01-31 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /05	Crofts, M.	1978	HARVEST RESIDUES IN RED BEET FROM A NORTON TRIAL IN CANADA IN 1977 Fisons plc, United Kingdom	N	N	-	Bayer CropScie nce	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			Bayer CropScience, Report No.: A83039, Edition Number: <u>M-155316-01-1</u> EPA MRID No.: 41214233 Date: 1978-11-02 GLP/GEP: no, unpublished					
KCA 6.3.5 /06	Wrede, A.	1995	Residues in red beet after application of Betanal progress in France 1993 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A83118, Report includes Trial Nos.: PF-R 93 098 Edition Number: <u>M-155393-01-1</u> Date: 1995-04-04 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /07	Crofts, M.; Whiteoak, R. J.	1973	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM 1972 TRIALS WITH NORTON IN THE UK Fisons plc, United Kingdom Bayer CropScience, Report No.: A82975, Edition Number: <u>M-155252-01-1</u> EPA MRID No.: acc.36374 Date: 1973-07-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /08	Crofts, M.; Whiteoak, R. J.	1973	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM FRENCH TRIALS WITH NORTON IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82976, Edition Number: <u>M-155253-01-1</u> EPA MRID No.: 41214219 Date: 1973-07-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /09	Crofts, M.; Whiteoak, R. J.	1973	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM DANISH TRIALS WITH NORTON IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82977, Edition Number: <u>M-155254-01-1</u> EPA MRID No.: 41214219 Date: 1973-08-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /10	Crofts, M.; Whiteoak, R. J.	1973	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM AUSTRIAN TRIALS WITH NORTON IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82978, Edition Number: <u>M-155255-01-1</u> Date: 1973-08-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /11	Crofts, M.; Whiteoak, R. J.	1973	HARVEST RESIDUES IN SUGAR BEET (ROOTS AND LEAVES) FROM 1972 TRIALS WITH NORTON IN YUGOSLAVIA Fisons plc, United Kingdom Bayer CropScience, Report No.: A82979, Edition Number: <u>M-155256-01-1</u> EPA MRID No.: 41414219 Date: 1973-08-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5	Whiteoak, R.	1973	RESIDUES IN SUGAR BEET (ROOTS	N	N	-	Bayer	In DAR

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/12	J.; Crofts, M.; Harris, R. J.		AND LEAVES) FROM 1972 TRIALS WITH NORTON IN W. GERMANY (UPDATED) Fisons plc, United Kingdom Bayer CropScience, Report No.: A82980, Edition Number: <u>M-155257-01-1</u> EPA MRID No.: 41214220 Date: 1973-10-01 GLP/GEP: no, unpublished				CropScie nce	(1998)
KCA 6.3.5 /13	Whiteoak, R. J.	1973	RESIDUE DECLINE STUDIES IN COLORADO (USA) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTON IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82982, Edition Number: <u>M-155259-01-1</u> EPA MRID No.: acc.36365 Date: 1973-12-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /14	Whiteoak, R. J.; Crofts, M.	1974	RESIDUE DECLINE STUDIES IN MICHIGAN (USA) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTON IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82983, Edition Number: <u>M-155260-01-1</u> EPA MRID No.: acc.37839 Date: 1974-02-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /15	Crofts, M.; Whiteoak, R. J.	1974	NORTON RESIDUE IN HARVEST SUGAR BEET FROM NINE REGIONS OF THE USA IN 1972 Fisons plc, United Kingdom Bayer CropScience, Report No.: A82986, Edition Number: <u>M-155263-01-1</u> EPA MRID No.: acc.36366 Date: 1974-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /16	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN SUGAR BEET FROM 1973 PRE-EMERGENCE APPLICATIONS OF NORTON (TRAMAT) IN ITALY Fisons plc, United Kingdom Bayer CropScience, Report No.: A82990, Edition Number: <u>M-155267-01-1</u> Date: 1974-06-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /17	Crofts, M.; Whiteoak, R. J.	1974	RESIDUE DECLINE STUDY IN THE UK (1973) WITH SUGAR BEET TREATED PRE-EMERGENCE WITH NORTON Fisons plc, United Kingdom Bayer CropScience, Report No.: A82992, Edition Number: <u>M-155269-01-1</u> Date: 1974-07-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /18	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN FODDER BEET FROM 1973 PRE-EMERGENCE APPLICATION OF NORTON (TRAMAT) IN W. GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A82993, Edition Number: <u>M-155270-01-1</u>	N	N	-	Bayer CropScie nce	In DAR (1998)



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			Date: 1974-11-01 GLP/GEP: no, unpublished					
KCA 6.3.5 /19	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN FODDER BEET FROM 1972 AND 1973 POST- EMERGENCE APPLICATIONS OF NORTRON (TRAMAT) IN W. GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A82996, Edition Number: <u>M-155273-01-1</u> Date: 1974-11-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /20	Crofts, M.; Whiteoak, R. J.	1974	RESIDUE DECLINE STUDY IN THE UK (1973) WITH SUGAR BEET TREATED POST-EMERGENCE WITH NORTRON Fisons plc, United Kingdom Bayer CropScience, Report No.: A82997, Edition Number: <u>M-155274-01-1</u> Date: 1974-11-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /21	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN SUGAR BEET FROM 1973 PRE-EMERGENCE APPLICATIONS OF NORTRON (TRAMAT) IN W. GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A82998, Edition Number: <u>M-155275-01-1</u> Date: 1974-12-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /22	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN SUGAR BEET AND SOIL FROM 1973 POST- EMERGENCE APPLICATIONS OF NORTRON (TRAMAT) IN ITALY Fisons plc, United Kingdom Bayer CropScience, Report No.: A83290, Edition Number: <u>M-155559-01-1</u> Date: 1974-12-01 GLP/GEP: no, unpublished ...also filed: KCA 7.1.2.2.1 /10	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /23	Crofts, M.; Whiteoak, R. J.	1974	HARVEST RESIDUES IN SUGAR BEET FROM 1973 POST-EMERGENCE APPLICATIONS OF NORTRON IN THE UK Fisons plc, United Kingdom Bayer CropScience, Report No.: A82999, Edition Number: <u>M-155276-01-1</u> Date: 1974-12-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /24	Crofts, M.	1975	HARVEST RESIDUES IN SUGAR BEET FROM 1974 PRE-EMERGENCE APPLICATIONS OF NORTRON IN CANADA Fisons plc, United Kingdom Bayer CropScience, Report No.: A83004, Edition Number: <u>M-155281-01-1</u> EPA MRID No.: 41214220 Date: 1975-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /25	Crofts, M.	1975	DECLINE IN RESIDUES IN SUGAR BEET TREATED PRE-EMERGENCE WITH NORTRON (TRAMAT) IN ITALY (1974) Fisons plc, United Kingdom	N	N	-	Bayer CropScie nce	In DAR (1998)

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			Bayer CropScience, Report No.: A83005, Edition Number: <u>M-155282-01-1</u> Date: 1975-04-01 GLP/GEP: no, unpublished					
KCA 6.3.5 /26	Crofts, M.	1975	DECLINE OF RESIDUES IN SUGAR BEET TREATED POST-EMERGENCE WITH NORTRON (TRAMAT) IN ITALY (1974) Fisons plc, United Kingdom Bayer CropScience, Report No.: A83006, Edition Number: <u>M-155283-01-1</u> Date: 1975-04-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /27	Crofts, M.; Whiteoak, R. J.	1976	NORTON RESIDUES IN MATURE SUGAR BEET FOLLOWING POST- EMERGENCE APPLICATIONS AS A TANK MIX WITH DESMEDIPHAM IN THE USA Fisons plc, United Kingdom Bayer CropScience, Report No.: A83012, Edition Number: <u>M-155289-01-1</u> Date: 1976-02-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /28	Crofts, M.; Harris, R. J.; Wilkie, P. M.	1976	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED PRE- EMERGENCE WITH NORTRON 20 EC OR TCA OR A TANK MIX OF BOTH COMPONENTS IN THE USA Fisons plc, United Kingdom Bayer CropScience, Report No.: A83013, Edition Number: <u>M-155290-01-1</u> Date: 1976-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /29	Crofts, M.; Harris, R. J.; Wilkie, P. M.	1976	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED PRE- EMERGENCE WITH NORTRON OR PYRAMIN OR A TANK MIX OR BOTH COMPONENTS IN THE USA IN 1975 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83016, Edition Number: <u>M-155293-01-1</u> Date: 1976-05-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /30	Crofts, M.	1976	NORTON AND RO-NEET RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE-EMERGENCE APPLICATION AND TANK MIX IN THE USA IN 1974 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83017, Edition Number: <u>M-155294-01-1</u> Date: 1976-05-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /31	Crofts, M.	1976	COMPARISON OF RESIDUES IN MATURE SUGAR BEET TREATED WITH AN SC OR AN EC FORMULATION OF NORTON IN UK, 1975 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83019, Edition Number: <u>M-155296-01-1</u> Date: 1976-06-01	N	N	-	Bayer CropScie nce	In DAR (1998)

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			GLP/GEP: no, unpublished					
KCA 6.3.5 /32	Crofts, M.	1978	HARVEST RESIDUES IN SUGAR BEET FROM PRE- EMERGENCE APPLICATIONS OF TRAMAT (NORTON) SC FORMULATION IN W. GERMANY IN 1976. Fisons plc, United Kingdom Bayer CropScience, Report No.: A83034, Edition Number: <u>M-155311-01-1</u> Date: 1978-01-23 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /33	Crofts, M.	1978	HARVEST RESIDUES IN SUGAR BEET FROM 1977 TRIALS WITH TRAMAT (NORTON) SC AND EC FORMULATIONS IN W. GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A83035, Edition Number: <u>M-155312-01-1</u> Date: 1978-01-23 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /34	Harris, R. J.; Reary, J. B.	1979	RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE-EMERGENCE APPLICATIONS OF SEPARATE OR TANK-MIX FORMULATIONS OF ETHOFUMESATE AND CHLORIDAZON IN MICHIGAN AND OHIO 1978 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83045, Edition Number: <u>M-155322-01-1</u> Date: 1979-09-13 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /35	Browne, P. M.; Reary, J. B.	1979	ETHOFUMESATE RESIDUES IN MATURE SUGAR BEET TREATED POST EMERGENCE IN MIXTURES WITH PHENMEDIPHAM AND/OR DESMEDIPHAM IN USA 1977 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83046, Edition Number: <u>M-155323-01-1</u> EPA MRID No.: 41214220 Date: 1979-08-30 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /36	Reary, J. B.	1980	RESIDUES IN MATURE SUGAR BEET TREATED POST-EMERGENCE WITH MIXTURES OF ETHOFUMESATE AND/OR PHENMEDIPHAM AND DESMEDIPHAM (COMMERCIAL EC FORMULATIONS) IN USA 1979 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83049, Edition Number: <u>M-155326-01-1</u> EPA MRID No.: 41214219 Date: 1980-05-23 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /37	Reary, J. B.	1980	RESIDUES IN MATURE SUGAR BEET FOLLOWING PRE AND POST- EMERGENCE APPLICATION OF ETHOFUMESATE (20 EC) IN CALIFORNIA 1977 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83050, Edition Number: <u>M-155327-01-1</u> EPA MRID No.: 41214220	N	N	-	Bayer CropScie nce	In DAR (1998)



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			Date: 1980-06-20 GLP/GEP: no, unpublished					
KCA 6.3.5 /38	Browne, P. M.; Reary, J. B.	1980	RESIDUES IN SUGAR BEET TREATED PRE- EMERGENCE WITH A SUSPENSION CONCENTRATE FORMULATION (50 SC) OF ETHOFUMESATE IN WEST GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A83053, Edition Number: <u>M-155330-01-1</u> Date: 1980-09-12 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /39	Reary, J. B.	1980	RESIDUES IN MATURE SUGAR BEET TREATED PRE-EMERGENCE WITH MIXTURES OF ETHOFUMESATE AND/OR PEBULATE OR CYCLOATE (COMMERCIAL EC FORMULATIONS) IN CALIFORNIA 1979 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83051, Edition Number: <u>M-155328-01-1</u> EPA MRID No.: 41214220 Date: 1980-08-14 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /40	Housden, M. C.; Reary, J. B.	1981	Residues of Ethofumestae and metabolites in sugar beet treated pre-emergence with a one-pack mixture of Ethofumestae and Lenacil in West Germany 1980 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83057, Edition Number: <u>M-155334-01-1</u> Date: 1981-01-14 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /41	Reary, J. B.	1981	Residues of Ethofumesate and metabolites in sugar beet treated pre-emergence with a one-pack mixture of Ethofumesate and Chloridazon in West Germany 1980 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83058, Edition Number: <u>M-155335-01-1</u> Date: 1981-01-15 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /42	Reary, J. B.	1981	Residues in sugar beet treated post- emergence with a suspension concentrate formulation (50 SC) of Ethofumesate in West Germany 1980 Fisons plc, United Kingdom Bayer CropScience, Report No.: A83059, Edition Number: <u>M-155336-01-1</u> Date: 1981-01-15 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /43	Haldeman, J. K.; Ford, J. J.	1982	ANTOR AND NORTON HERBICIDE RESIDUES IN SUGAR BEETS FROM TREATED PLOTS FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A89134, Edition Number: <u>M-164269-01-1</u> Date: 1982-02-22 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.3.5 /44	Cron, J. H.	1982	Residues of Ethofumesate and metabolites in sugar beet treated pre-emergence with a one-pack mixture of Ethofumesate and	N	N	-	Bayer CropScie nce	In DAR (1998)

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			Chloridazon in West Germany 1981 FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83064, Edition Number: <u>M-155341-01-1</u> Date: 1982-02-11 GLP/GEP: no, unpublished					
KCA 6.3.5 /45	Haldeman, J. K.	1982	NORTON HERBICIDE RESIDUES IN SUGAR BEETS TREATED PRE- AND POST-PLANTING Hercules Inc.; Bayer CropScience, Report No.: A83066, Edition Number: <u>M-155343-01-1</u> Date: 1982-04-29 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /46	Cron, J. H.	1982	Residues of Ethofumesate and metabolites in sugar/fodder beet treated post-emergence with Ethofumesate (50 SC) in West Germany 1981 FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83065, Edition Number: <u>M-155342-01-1</u> Date: 1982-04-02 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /47	Haldeman, J. K.	1982	ETHOFUMESATE RESIDUES IN SUGAR BEETS FROM TWO CALIFORNIA LOCATIONS FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83067, Edition Number: <u>M-155344-01-1</u> Date: 1982-05-07 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /48	Ford, J. J.	1983	DIETHATYL ETHYL (ANTOR HERBICIDE) AND ETHOFUMESATE (NORTON HERBICIDE) RESIDUES IN 6-MONTH SUGAR BEETS FROM CALIFORNIA FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A89135, Report includes Trial Nos.: H41/3/81 H79/3/3 Edition Number: <u>M-164271-01-1</u> EPA MRID No.: 41214220 Date: 1983-07-13 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /49	Lee, G. E.; Weishedel, B. C.	1984	ETHOFUMESATE (NORTON HERBICIDE) RESIDUES IN SUGAR BEETS FROM QUEBEC AND MANITOBA FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83069, Edition Number: <u>M-155346-01-1</u> Date: 1984-04-16 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /50	Manley, J. D.; Snowdon, P. J.	1984	Residues of Ethofumesate and major metabolites in sugarbeet treated in West Germany 1982 and 1983, with a Co-formulation of Ethofumesate and	N	N	-	Bayer CropScience	In DAR (1998)

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			Phenmedipham FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83072, Report includes Trial Nos.: 041/03/080 Edition Number: <u>M-155349-01-1</u> Date: 1984-09-20 GLP/GEP: no, unpublished					
KCA 6.3.5 /51	Snowdon, P. J.	1985	Residues of Ethofumesate and major metabolites in sugarbeet treated in France 1984 with Ethofumesate and Phenmedipham as either a Co-formulation of a Tank-mix FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83071, Report includes Trial Nos.: 041/03/082 Edition Number: <u>M-155348-01-1</u> Date: 1985-04-18 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /52	Manley, J. D.; Snowdon, P. J.	1986	Residues of Ethofumesate and major metabolites in sugar beet treated in the Federal Republic of Germany, 1983 with a Co-formulation of Ethofumesate and Phenmedipham FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83077, Report includes Trial Nos.: 041/03/080 Edition Number: <u>M-155353-01-1</u> Date: 1986-08-04 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /53	Manley, J. D.; Snowdon, P. J.	1986	Residues of Ethofumesate and major metabolites in sugarbeet treated in the Federal Republic of Germany 1985 with a Co-formulation of Ethofumesate and Phenmedipham FBC Limited, Chesterford Park, United Kingdom Bayer CropScience, Report No.: A83078, Report includes Trial Nos.: 041/03/085 Edition Number: <u>M-155354-01-1</u> EPA MRID No.: 41214220 Date: 1986-09-16 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /54	Banwell, M.; Bright, J. H. M.	1990	Residues of Ethofumesate and its major metabolites in sugar beet following multiple post-emergence application of an EC Co-formulation with Penmedipham and Desmedipham in Denmark 1989 (2nd Edition) Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83095, Report includes Trial Nos.: 041/03/116 Edition Number: <u>M-155764-02-1</u> Date: 1990-07-11 ...Amended: 1990-11-01 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5	Straszewski,	1993	Ethofumesate: SC (CQ 1273/01): Residues	N	N	-	Bayer	In DAR

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/55	A.		of Ethofumesate and its major metabolite in sugar beets France 1992 Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83115, Edition Number: <u>M-155390-01-1</u> Date: 1993-09-23 GLP/GEP: yes, unpublished				CropScience	(1998)
KCA 6.3.5 /56	Wrede, A.	1995	Residues in sugar beet after application of Betanal progress of in France 1993 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A62042, Edition Number: <u>M-145562-01-1</u> Date: 1995-04-04 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.3.5 /57	Helgers, A.	1997	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 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A89772, Edition Number: <u>M-165366-02-1</u> Date: 1997-01-27 ...Amended: 1997-02-27 GLP/GEP: yes, unpublished	N	Y	8 residue trials in S-EU are required according to SANCO 7525/VI/95 rev. 9 (data gap in EFSA MRL review)	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 6.3.5 /58	Schulte, G.	2013	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 Bayer CropScience, Report No.: 10-2109, Report includes Trial Nos.: 10-2109-01 10-2109-02 10-2109-03 10-2109-04 Edition Number: <u>M-444836-02-1</u> Date: 2013-01-15 ...Amended: 2013-07-09 GLP/GEP: yes, unpublished	N	Y	8 residue trials in S-EU are required according to SANCO 7525/VI/95 rev. 9 (data gap in EFSA MRL review)	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 6.3.1/01	Tandy, R.	2012a	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 United Phosphorus Ltd., S09-01656 Eurofins Agrosience Services LTD, UK GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.3.1/02	Perny, A.	2002	Residue study in sugar beets following treatments with a formulated product containing Ethofumesate 128 g/l, Phenmedipham 62 g/l and Desmedipham 16 g/l on sugar beet fields under field conditions in France and in the Netherlands in 2000 AgriChem B.V., R A0015 Anadiag S.A., Haguenau, France GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)

Data Point	Author(s)	Year	Title Compagny 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 6.3.1/03	Perny, A.	2003	Residue study in sugar beets following treatments with a formulated product containing Ethofumesate 128 g/l, Phenmedipham 62 g/l and Desmedipham 16 g/l on sugar beet fields under field conditions in France and in The Netherlands in 2001 AgriChem B.V., R A1114 Anadiag S.A., Haguenau, France GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/04	Huaultmé, J.-M.	2013a	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 AgriChem B.V., BPL12/436/GC BIOTEK Agriculture GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/05	Chevallier, E.	2012	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 AgriChem B.V., BPL11/380/GC BIOTEK Agriculture GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/06	Waalkens, W.M., Hamberger, R.	2005a	Determination of the decline of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l EC, Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2003 AgriChem B.V., R03-16-NF-08 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/07	Waalkens, W.M., Hamberger, R.	2005b	Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l EC, Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2003 AgriChem B.V., R03-16-NF-09 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/08	Waalkens, W.M., Hamberger, R.	2005c	Determination of the decline of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on	N	Y	New data for active ingredient, not	ACM*	Submitted for the purpose of renewal



Data Point	Author(s)	Year	Title Compagny 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
			sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2004 AgriChem B.V., R04-16-NF-08 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no			previously submitted nor evaluated		(2014)
KCA 6.3.1/09	Waalkens, W.M., Hamberger, R.	2005d	Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in / on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in the Netherlands and northern France, 2004 AgriChem B.V., R04-16-NF-09 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/10	Anspach, T.	2001	Magnitude of the residue of Phenmedipham, Desmedipham, Ethofumesate and its metabolite 2-oxo-Ethofumesate in sugar beets (roots and leaves/tops) after the application of Betasana Trio under filed conditions in Germany, 2000 United Phosphorus Ltd., ADN-0004 Dr. Specht Partner, Chemische Laboratorien GmbH, Germany GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.3.1/11	Tandy, R.	2013	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 United Phosphorus Ltd., S10-00258 Eurofins Agrosience Services LTD, UK GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.3.1/12	Waalkens, W.M., Hamberger, R.	2005e	Determination of the magnitude of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, aniline, Ethofumesate, 2-Keto-Ethofumesate in/on sugar beet plants and roots after foliar applications of Phenmedipham 157 g/l SE and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to sugar beets in northern Spain, 2003 AgriChem B.V., R03-16-SP-06 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/13	Waalkens, W.M., Hamberger, R.	2005f	Determination of the decline of the residues of Phenmedipham, MHPC, Methylaniline, Desmedipham, EHPC, Aniline, Ethofumesate, 2-Keto-Ethofumesate in/on fodder beet plants and roots after foliar applications of Phenmedipham 157 g/l SE	N	Y	New data for active ingredient, not previously submitted	ACM*	Submitted for the purpose of renewal (2014)

Data Point	Author(s)	Year	Title Compagny 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
			and Ethofumesate / Phenmedipham / Desmedipham 128/62/21 g/l EC to fodder beets in southern France, 2003 AgriChem B.V., R03-16-FR-07 Res.Comp. for Plant Protec. "De Bredelaar" B.V., Elst, NL GLP: yes Published: no			nor evaluated		
KCA 6.3.1/14	Huauilmé, J.- M.	2013b	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 AgriChem B.V., BPL12/435/GC BIOTEK Agriculture GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.3.1/15	Tandy, R.	2012b	Validation of the analytical method A0019 to confirm the conversion of NC 20645 to NC 9607 in sugar beet roots and tops and wheat grain and straw United Phosphorus Ltd., S11-03715 Eurofins Agroscience Service GmbH GLP/GEP: no Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.3.1/16	Weir, A.	2014	METHOD MODIFICATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ETHOFUMESATE AND ITS METABOLITES NC 20645 AND NC 9607 IN SUGARBEET ROOTS AND TOPS United Phosphorus Ltd., S13-03837 Eurofins Agroscience Services LTD, UK GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	UPL	Submitted for the purpose of renewal (2014)
KCA 6.4.1 /01		1975	INVESTIGATION OF TISSUE AND EGG RESIDUES FROM HENS FOLLOWING DIETARY INTAKE OF NC 8438 FOR 21 DAYS  Bayer CropScience, Report No.: A83011, Edition Number: <u>M-155288-01-1</u> Date: 1975-09-01 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /27	Y	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.4.1 /02		1999	Review of animal metabolism data; maximum estimated dietary concentration for poultry and cattle; rebuttal for further animal feeding studies Ethofumesate Code: AE B049913  Bayer CropScience, Report No.: C003329, Edition Number: <u>M-185950-01-1</u> GLP/GEP: no, unpublished ...also filed: KCA 6.4.2 /04	Y	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.4.2 /01		1977	RESIDUES IN MILK AND TISSUES FOLLOWING A 28-DAY FEEDING STUDY WITH ETHOFUMESATE IN DAIRY COWS - PART 1  Bayer CropScience, Report No.: A83024, Edition Number: <u>M-155301-01-1</u> EPA MRID No.: 41214208	Y	N	-	Bayer CropScie nce	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			Date: 1977-06-01 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /25					
KCA 6.4.2 /02		1977	RESIDUES IN MILK AND TISSUES FOLLOWING A 28-DAY FEEDING STUDY WITH ETHOFUMESATE IN DAIRY COWS - PART 2 Bayer CropScience, Report No.: A89223, Edition Number: <u>M-164398-01-1</u> Date: 1977-06-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.4.2 /03		1994	Ethofumesate-derived residues in the meat and milk of dairy cows: resulting from oral ingestion of ethofumesate Bayer CropScience, Report No.: B002201, Report includes Trial Nos.: B93R04/05 Edition Number: <u>M-237976-01-1</u> EPA MRID No.: 43458701 Date: 1994-10-12 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /29	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.4.2 /04		1999	Review of animal metabolism data; maximum estimated dietary concentration for poultry and cattle; rebuttal for further animal feeding studies Ethofumesate Code: AE B049913 Bayer CropScience, Report No.: C003329, Edition Number: <u>M-185950-01-1</u> GLP/GEP: no, unpublished ...also filed: KCA 6.4.1 /02	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 6.5.1 /01	Miebach, D.; Bongartz, R.	2010	Nature of the residues of ethofumesate in processed commodities - High temperature hydrolysis Bayer CropScience, Report No.: MEF-10/803, Edition Number: <u>M-397800-01-1</u> Date: 2010-12-09 GLP/GEP: yes, unpublished	N	Y	Required according to SANCO/703 5/VI/95 rev 5 and SANCO/118 02/2010	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 6.5.3 /01	Whiteoak, R. J.; Crofts, M.	1973	CONJUGATED RESIDUES IN FRACTIONS PROCESSED FROM SUGAR BEET TREATED WITH NORTON Fisons plc, United Kingdom Bayer CropScience, Report No.: A82973, Edition Number: <u>M-155250-01-1</u> EPA MRID No.: acc.36368 Date: 1973-05-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 6.5.3 /02	Crofts, M.; Whiteoak, R. J.	1974	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET Fisons plc, United Kingdom Bayer CropScience, Report No.: A82985, Edition Number: <u>M-155262-01-1</u> EPA MRID No.: acc.36369 Date: 1974-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScience	In DAR (1998)



Data Point	Author(s)	Year	Title Compagny 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
			...also filed: KCA 4.1.2 /10					
KCA 6.5.3 /03	Crofts, M.; Whiteoak, R. J.	1975	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET - ARTIFICIALLY HIGH RESIDUES IN BEET GROWN AND PROCESSED IN THE UNITED KINGDOM Fisons plc, United Kingdom Bayer CropScience, Report No.: A83002, Edition Number: <u>M-155279-01-1</u> Date: 1975-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.5.3 /04	Crofts, M.; Whiteoak, R. J.	1975	FATE OF THE METABOLITE CONJUGATED NC 9607 DURING PRODUCTION OF SUGAR FROM NORTON TREATED SUGAR BEET - ARTIFICIALLY HIGH RESIDUE IN BEET GROWN AND PROCESSED IN W. GERMANY Fisons plc, United Kingdom Bayer CropScience, Report No.: A83003, Edition Number: <u>M-155280-01-1</u> Date: 1975-03-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.6.1 /01	Carlton, R.; Cordell, P.	1993	THE UPTAKE AND METABOLISM OF ETHOFUMESATE AND ITS SOIL METABOLITES IN A CONFINED ROTATIONAL CROP STUDY Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83396, Report includes Trial Nos.: 90B Edition Number: <u>M-155664-01-1</u> Date: 1993-06-18 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.6.1 /02	Schneider, E.	1994	PR94/025 - Ethofumesate - Determination of ethofumesate residues in soil of a long time field study after the application of Ethosat (FSG031894) to sugar beet plants Dr. G. Krebs Analytik, Köln, Germany Feinchemie Schwebda, Report No.: <u>M-468487-01-1</u> , Edition Number: <u>M-468487-01-1</u> Date: 1994-08-12 GLP/GEP: yes, unpublished	N	N	-	Adama (former Feinchem ie Schwebd a)	In DAR (1998)
KCA 6.6.1/01	Chapleo, S.	2003	The uptake of [ <sup>14</sup> C]-Ethofumesate residues in soil by rotational crops under confined conditions AgriChem B.V., 22558 Inveresk Research International, Tranent, Scotland GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.6.2 /01	Castro, L. E.	1994	ETHOFUMESATE EMULSIFIABLE CONCENTRATE 200 g/l CR 13768: AT- HARVEST RESIDUES OF ETHOFUMESATE AND METABOLITES IN ROTATIONAL CROPS AND SOIL FOLLOWING APPLICATIONS OF NORTON EC TO SUGARBEETS, USA, 1990 Nor-Am Chemical Company, Pikeville, NC, USA Bayer CropScience, Report No.: A83117,	N	N	-	Bayer CropScie nce	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			Edition Number: <u>M-155392-01-1</u> EPA MRID No.: 43298104 Date: 1994-05-05 GLP/GEP: yes, unpublished					
KCA 6.6.2 /02	Crofts, M.; Whiteoak, R. J.	1974	RESIDUE ANALYSIS OF WHEAT GROWN IN THE UK AS A FOLLOWING CROP AFTER SUGAR BEET TREATED WITH NORTON (1973) Fisons plc, United Kingdom Bayer CropScience, Report No.: A82995, Edition Number: <u>M-155272-01-1</u> Date: 1974-11-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.6.2 /03	Crofts, M.; Whiteoak, R. J.	1974	RESIDUE ANALYSIS OF WHEAT AND CORN (MAIZE) GROWN AS FOLLOWING CROPS AFTER SUGAR BEET TREATED WITH NORTON (1973) Fisons plc, United Kingdom Bayer CropScience, Report No.: A82994, Edition Number: <u>M-155271-01-1</u> Date: 1974-09-01 GLP/GEP: no, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.6.2 /04	Peatman, M. H.; Snowdon, P. J.	1991	RESIDUES OF SOIL AND EMERGENCY CROPS FOLLOWING APPLICATION OF ETHOFUMESATE AS A 50 SC FORMULATION IN THE UK 1990/91 Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83376, Report includes Trial Nos.: 041/04/057 Edition Number: <u>M-155644-01-1</u> Date: 1991-12-20 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScie nce	In DAR (1998)
KCA 6.6.2 /05	Schulte, G.; Diehl, P.	2013	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 Bayer CropScience, Report No.: 10-2501, Report includes Trial Nos.: 10-2501-02 10-2501-03 10-2501-04 10-2501-05 Edition Number: <u>M-463906-02-1</u> Date: 2013-08-22 ...Amended: 2013-09-13 GLP/GEP: yes, unpublished	N	Y	data gap in EFSA MRL review	Task Force Ethofume sate	Submitted for the purpose of renewal (2014)
KCA 6.6.2/01	Spence, C.	2014	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) AgriChem B.V., 697614, 34890 Charles River Laboratories, Edinburgh, UK GLP: yes Published: no	N	Y	New data for active ingredient, not previously submitted nor evaluated	ACM*	Submitted for the purpose of renewal (2014)
KCA 6.7.2 /01	Peatman, M. H.	1999	MRL proposal for peas and beans following a review of available residues data from the EU Ethofumesate AE B049913 AgrEvo UK Crop Protection Ltd.,	N	N	-	Bayer CropScie nce	In DAR (1998)

Data Point	Author(s)	Year	Title Compagny 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
			Chesterford Park, United Kingdom Bayer CropScience, Report No.: C003327, Edition Number: <u>M-185947-01-1</u> GLP/GEP: no, unpublished					
KCA 6.10 /01	Wang, P.; Wang, Q.; Jiang, S.; Qiu, J.; Wang, P.; Zhou, Z.	2005	Stereoselective degradation of ethofumesate in turfgrass and soil. Journal: Pestic. Biochem. Physiol., Volume: 82, Issue: 3, Pages: 197-204, Year: 2005, Report No.: <u>M-458577-01-1</u> , Edition Number: <u>M-458577-01-1</u> Date: 2005-12-31 GLP/GEP: no, published ...also filed: KCA 7.1.2.1.1 /14	N	N	-	LIT	Submitted for the purpose of renewal (2014)
KCA 6.10.1/01	Lückmann, J.	2013	Ethofumesate - exposure of honeybees to residues in nectar, pollen and guttation fluid in sugar and fodder beets United Phosphorus Ltd., P13096 RIFCon GmbH, Hirschberg, Germany GLP/GEP: no Published: no	N	N	-	UPL	Submitted 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

LIT: public literature



## ANNEX I - RESIDUE TRIALS (PRIMARY CROPS)

## Sugar beets (southern European) – Bayer CropSciences AG

Study Trial No.; Plot Location incl. postal code	Commodity Variety	Date of 1) sowing or 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues (mg/kg)		DALA [days]	Remark
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
A89772 ER95ECS446 ER95ECS446ITA0002 -P3 40052 Baricella Emilia Romagna IT	Beet, sugar Adrienne	1) 16.03.1995 3) 05.09.1995	1.050	300	0.350	24.03.1995/0	Seed imbibition complete - Radicle emerged	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	165 165	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 ER95ECS446 ER95ECS446ITA0001 -P3 40128 Bologna Emilia Romagna Italy	Beet, sugar Monodoro	1) 15.03.1995 3) 07.09.1995	1.050	300	0.350	21.03.1995/0	Beginning of imbibition - Seed imbibition complete	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	170 170	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 R95ECS446 ER95ECS446ITA0003 ER95ECS446ITA0003 -P3 40012 Caldera di Reno Emilia Romagna Italy	Beet, sugar Break	1) 22.03.1995 3) 06.09.1995	1.050	300	0.350	27.03.1995/0	Beginning of imbibition - Seed imbibition complete	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	163 163	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 ER95ECS446 ER95ECS446ITA0004 ER95ECS446ITA0004 -P3 44020 Gallo Emilia Romagna Italy	Beet, sugar Adige	1) 23.03.1995 3) 11.08.1995	1.050	300	0.350	31.03.1995/0	Seed imbibition complete - Radicle emerged	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	133 133	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1

Study Trial No.; Plot Location incl. postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues (mg/kg)		DALA [days]	Remark
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
A89772 ER95ECS446 ER95ECS446ITA0004 ER95ECS446ITA0004 -P2 44020 Gallo Emilia Romagna Italy	Beet, sugar Adige	1) 23.03.1995 3) 11.08.1995	1.050	300	0.350	31.03.1995/0	Seed imbibition complete - Radicle emerged	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	133 133	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 ER95ECS446 ER95ECS446ITA0002 ER95ECS446ITA0002 -P2 40052 Baricella Emilia Romagna Italy	Beet, sugar Adrienne	1) 16.03.1995 3) 05.09.1995	1.050	300	0.350	24.03.1995/0	Seed imbibition complete - Radicle emerged	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	165 165	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 ER95ECS446 ER95ECS446ITA0001 ER95ECS446ITA0001 -P2 40128 Bologna Emilia Romagna Italy	Beet, sugar Monodoro	1) 15.03.1995 3) 07.09.1995	1.050	300	0.350	21.03.1995/0	Beginning of imbibition - Seed imbibition complete	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	170 170	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1
A89772 ER95ECS446 ER95ECS446ITA0003 ER95ECS446ITA0003 -P2 40012 Caldera di Reno Emilia Romagna Italy	Beet, sugar Break	1) 22.03.1995 3) 06.09.1995	1.050	300	0.350	27.03.1995/0	Beginning of imbibition - Seed imbibition complete	root leaf with root collar	<0.05 <0.05	<0.05 <0.05	163 163	LOQ: 0.05 mg/kg formulation: AE B049913 02 WP42 A1

Study Trial No.; Plot Location incl. postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues (mg/kg)		DALA [days]	Remark
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2109 10-2109-01 10-2109-01-T E-41730 Las Cabezas Spain	Beet, sugar Sanlucar	1) 22.11.2010 3) 10.06.2011 - 30.08.2011	1.0	300	0.333	03.02.2011/0	Seven leaves unfolded	whole plant with root leaf with root collar body	25 <0.01 <0.01	0.17 <0.01 <0.01	0 155 155	LOQ: 0.01 mg/kg formulation: Ethofumesate SC 500
10-2109 10-2109-02 10-2109-02-T I-44012 Settepolesini Italy	Beet, sugar Leila	1) 25.03.2010	1.0	300	0.333	01.05.2010/0	Eight leaves unfolded	whole plant with root leaf with root collar body	25 <0.01 <0.01	0.14 <0.01 <0.01	0 116 116	LOQ: 0.01 mg/kg formulation: Ethofumesate SC 500
10-2109 10-2109-03 10-2109-03-T I-40128 Bologna Italy	Beet, sugar Houston	3) 01.08.2010 - 31.08.2010	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	whole plant with root body leaf with root collar	12 <0.01 <0.01	0.05 <0.01 0.02	0 84 84	LOQ: 0.01 mg/kg formulation: Ethofumesate SC 500
10-2109 10-2109-04 10-2109-04-T GR-50100 Drepano Greece	Beet, sugar Greta	1) 28.03.2010 3) 09.09.2010 - 10.09.2010	1.0	300	0.333	14.05.2010/0	Seven leaves unfolded	whole plant with root body leaf with root collar	34 <0.01 <0.01	0.93 <0.01 <0.01	0 116 116	LOQ: 0.01 mg/kg formulation: Ethofumesate SC 500

## Sugar beets (northern and southern European) – United Phosphorus Ltd

### Northern Europe

Residue data summary from supervised trials of Ethofumesate on sugar beets in Northern Europe; analysis of residues of Ethofumesate; Ethofumesate "free"-2-keto (NC 9607) and Ethofumesate "conjugated"-2-keto (= conjugated NC 20645). Free NC 20645 has been included in the results for its conjugate

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC 20645- conj		
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-01 NG32 1PN Harston, Notts, UK	Sugar beet, BEAVA, Opta	1) 19.03.09 2) n/a 3) 02.09.2009- 23.09.2009	1031	206	n/a	30.04.2009	BBCH 10- 12	Leaves with top	n/d	n/d	<0.05	125	LOQ=0.05 mg/kg
								Roots	n/d	n/d	<0.05	125	
								Leaves with top	n/d	n/d	n/d	146	
								Roots	n/d	n/d	<0.05	146	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-01 NG32 1PN Harston, Notts, UK	Sugar beet, BEAVA, Opta	1) 19.03.09 2) n/a 3) 02.09.2009- 23.09.2009	1078	108	n/a	04.06.2009	BBCH 18	Leaves with top	n/d	n/d	0.06	90	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	90	
								Leaves with top	n/d	n/d	0.06	111	
								Roots	n/d	n/d	<0.05	111	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-01 NG32 1PN Harston, Notts, UK	Sugar beet, BEAVA, Opta	1) 19.03.09 2) n/a 3) 02.09.2009- 23.09.2009	224 245 225	122 107 98	n/a n/a n/a	21.05.2009 28.05.2009 04.06.2009	BBCH 14 BBCH 17 BBCH 18	Leaves with top	n/d	n/d	<0.05	90	LOQ=0.05 mg/kg
								Roots	n/d	n/d	<0.05	90	
								Leaves with top	n/d	n/d	<0.05	111	
								Roots	n/d	n/d	n/d	111	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-02 C07 6PE Colchester, Essex, UK	Sugar beet, BEAVA, Bullfinch	1) 24.03.09 2) n/a 3) 08.09.2009- 19.11.2009	1050	210	n/a	15.05.2009	BBCH 12- 13	Leaves with top	n/d	n/d	0.06	116	LOQ=0.05 mg/kg
								Roots	n/d	n/d	<0.05	116	
								Leaves with top	n/d	n/d	n/d	188	
								Roots	n/d	n/d	<0.05	188	
KCA 6.3.1/01,	Sugar beet,	1) 24.03.09	1022	102	n/a	10.06.2009	BBCH 18	Leaves with top	n/d	n/d	0.25	90	LOQ=0.05



Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC 20645- conj		
Tandy, R. (2012a) S09-01656 S09-01656-02 C07 6PE Colchester, Essex, UK	BEAVA, Bullfinch	2) n/a 3) 08.09.2009- 19.11.2009						Roots	n/d	n/d	<0.05	90	mg/kg
								Leaves with top	n/d	n/d	<0.05	162	
								Roots	<b>n/d</b>	<b>n/d</b>	<b>&lt;0.05</b>	<b>162</b>	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-02 C07 6PE Colchester, Essex, UK	Sugar beet, BEAVA, Bullfinch	1) 24.03.09 2) n/a 3) 08.09.2009- 19.11.2009	184 232 229	100 101 100	n/a n/a n/a	28.06.2009 03.06.2009 10.06.2009	BBCH 14 BBCH 16 BBCH 18	Leaves with top	n/d	n/d	0.07	90	mg/kg
								Roots	n/d	n/d	n/d	90	
								Leaves with top	n/d	n/d	n/d	162	
								Roots	n/d	n/d	n/d	162	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-03 YO42 4RU North Yorkshire, UK	Sugar beet, BEAVA, Bobcat	1) 03.05.09 2) n/a 3) 08.09.2009- 06.10.2009	1000	200	n/a	10.05.2009	BBCH 07	Leaves with top	n/d	n/d	<0.05	121	mg/kg
								Roots	n/d	n/d	n/d	121	
								Leaves with top	n/d	n/d	<0.05	149	
								Roots	n/d	n/d	n/d	149	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-03 YO42 4RU North Yorkshire, UK	Sugar beet, BEAVA, Bobcat	1) 03.05.09 2) n/a 3) 08.09.2009- 06.10.2009	1067	107	n/a	01.07.2009	BBCH 18	Leaves with top	n/d	n/d	<0.05	121	mg/kg
								Roots	n/d	n/d	n/d	121	
								Leaves with top	<b>n/d</b>	<b>n/d</b>	<b>&lt;0.05</b>	<b>149</b>	
								Roots	n/d	n/d	n/d	149	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-03 YO42 4RU North Yorkshire, UK	Sugar beet, BEAVA, Bobcat	1) 03.05.09 2) n/a 3) 08.09.2009- 06.10.2009	190 238 245	103 103 107	n/a n/a n/a	15.06.2009 23.06.2009 01.07.2009	BBCH 15 BBCH 16 BBCH 18	Leaves with top	n/d	n/d	<0.05	69	mg/kg
								Roots	n/d	n/d	n/d	69	
KCA 6.3.1/01, Tandy, R. (2012a)	Sugar beet, BEAVA,	1) 08.05.09 2) n/a	983	197	n/a	14.05.2009	BBCH 07	Whole plant	<0.05	n/d	0.14	43	mg/kg
								Whole plant	<0.05	n/d	0.12	51	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC 20645- conj		
S09-01656 S09-01656-04 YO8 5LF Osgodby North Yorkshire, UK	Ace	3) 26.06.2009 04.07.2009 17.07.2009 23.09.2009 06.10.2009						Whole plant	n/d	n/d	<0.05	64	"Conjugated" 2-keto Ethofumesate residues were found in untreated plant specimen
								Leaves with top	n/d	n/d	n/d	132	
								Roots	<0.05	n/d	n/d	132	
								Leaves with top	n/d	n/d	n/d	145	
								Roots	n/d	n/d	n/d	145	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-04 YO8 5LF Osgodby North Yorkshire, UK	Sugar beet, BEAVA, Ace	1) 08.05.09 2) n/a 3) 26.06.2009 04.07.2009 17.07.2009 23.09.2009 06.10.2009	967	97	n/a	26.06.2009	BBCH 18	Whole plant	19.18	<0.05	0.61	0	LOQ=0.05 mg/kg "Conjugated" 2-keto Ethofumesate residues were found in untreated plant specimen
								Whole plant	0.82	n/d	3.40	8	
								Whole plant	<0.05	n/d	0.87	21	
								Leaves with top	n/d	n/d	0.15	89	
								Roots	n/d	n/d	n/d	89	
								Leaves with top	n/d	n/d	n/d	102	
								<b>Roots</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>102</b>	
KCA 6.3.1/01, Tandy, R. (2012a) S09-01656 S09-01656-04 YO8 5LF Osgodby North Yorkshire, UK	Sugar beet, BEAVA, Ace	1) 08.05.09 2) n/a 3) 26.06.2009 04.07.2009 17.07.2009 23.09.2009 06.10.2009	196 215 215	107 93 93	n/a n/a n/a	10.06.2009 18.06.2009 26.06.2009	BBCH 15 BBCH 16 BBCH 18	Whole plant	3.18	<0.05	1.92	0	LOQ=0.05 mg/kg "Conjugated" 2-keto Ethofumesate residues were found in untreated plant specimen
								Whole plant	<0.05	n/d	2.68	8	
								Whole plant	n/d	n/d	0.66	21	
								Leaves with top	n/d	n/d	0.12	89	
								Roots	n/d	n/d	n/d	89	
								Leaves with top	n/d	n/d	0.14	102	
								Roots	n/d	n/d	n/d	102	

**Residue data summary from supervised trials of Ethofumesate on sugar beets in Northern Europe, analysis of residues of Ethofumesate as sum of Ethofumesate, 2-keto-Ethofumesate in free (NC 9607) and conjugated form (= conjugated NC 20645) expressed as Ethofumesate. Free NC 20645 has been included in the results for 2-keto ethofumesate.**

Results for 2-Keto-chloroformate:											
Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]	DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				Sum of a.s., and relevant metabolites		
KCA 6.3.1/02, Perny, A. (2002) R A0015 A0015 ND1 60400 Appilly, N-F	Sugar beet, Somate	1) 20.03.2000	372.3	291	128	22.05.2000 31.05.2000	BBCH 16 BBCH 33-39	Leaves and collar	<0.05	175	LOQ=0.05 mg/kg LOD=0.02 mg/kg application rate per season: 768.4 g/ha
			369.1	288	128			Roots	<0.05	175	
KCA 6.3.1/02, Perny, A. (2002) R A0015 A0015 AN1 67160 Seebach, N-F	Sugar beet, Rifle	1) 29.03.2000	387.0 383.3	302 299	128 128	16.05.2000 23.05.2000	BBCH 16 BBCH 16-18	Plant	21.0	0	LOQ=0.05 mg/kg LOD=0.02 mg/kg application rate per season: 770.3 g/ha
								Plant	0.19	29	
								Plant	<0.05	58	
								Plant	0.06	100	
								Leaves and collar	<0.05	134	
								Roots	<0.05	134	
KCA 6.3.1/02, Perny, A. (2002) R A0015 A0015 HL1 6085NR Horn, NL	Sugar beets, Lonera	1) 07.04.2000	394.6 365.3	308 285	128 128	26.05.2000 06.06.2000	BBCH 16-18 BBCH 19	Leaves and collar	<0.05	164	LOQ=0.05 mg/kg LOD=0.02 mg/kg application rate per season: 759.9 g/ha
								Roots	<0.05	164	
KCA 6.3.1/02, Perny, A. (2002) R A0015 A0015 HL2 6201AB Mechelen NL	Sugar beets, Aresto	1) 11.04.2000	399.4 394.0	312 308	128 128	19.05.2000 26.05.2000	BBCH 14 BBCH 16-17	Plant	14.6	0	LOQ=0.05 mg/kg LOD=0.02 mg/kg application rate per season: 793.4 g/ha
								Plant	0.18	32	
								Plant	<0.05	61	
								Plant	<0.05	105	
								Leaves and collar	<0.05	127	
								Roots	<0.02	127	
KCA 6.3.1/03, Perny, A. (2003) R A1114 R A1114 BP1 45170 Attray N-F	Sugar beets, Sterna	1) 17.04.2001	381 400	297 313	128 128	29.05.2001 10.06.2001	BBCH 16 BBCH 31	Leaves and collar	<0.05	103	LOQ=0.05 mg/kg LOD=0.003 mg/kg application rate per season: 781 g/ha
								Roots	<0.05	103	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]	DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				Sum of a.s., and relevant metabolites		
KCA 6.3.1/03, Perny, A. (2003) R A1114 67160 Seebach, N-F	Sugar beets, Rifle	1) 05.05.2001	376	293	128	19.06.2001 29.06.2001	BBCH 16 BBCH 16-18	Plant	24.8	0	LOQ=0.05 mg/kg LOD=0.003 mg/kg application rate per season: 773 g/ha
			397	310	128			Plant	<0.05	31	
								Plant	<0.05	60	
								Plant	<0.05	103	
								Leaves and collar	<0.05	110	
								Roots	<0.05	110	
KCA 6.3.1/03, Perny, A. (2003) R A1114 A1114 HL1 6281AB Mechelen NL	Sugar beets, Toledo	1) 10.05.2001	402	314	128	14.06.2001 23.06.2001	BBCH 16 BBCH 18	Leaves and collar	<0.05	134	LOQ=0.05 mg/kg LOD=0.003 mg/kg application rate per season: 766 g/ha
			364	284	128			Roots	<0.05	134	
KCA 6.3.1/03, Perny, A. (2003) R A1114 A1114 HL2 6085NR Horn NL	Sugar beets, Hiteatia	1) 12.05.2001	386	302	128	19.06.2001 29.06.2001	BBCH 16 BBCH 18-19	Plant	14.8	0	LOQ=0.05 mg/kg LOD=0.003 mg/kg application rate per season: 783 g/ha
			394	308	128			Plant	0.08	29	
								Plant	<0.05	63	
								Plant	<0.05	99	
								Leaves and collar	<0.05	119	
								Roots	<b>&lt;0.05</b>	<b>119</b>	

**Residue data summary from supervised trials of Ethofumesate on sugar beets in Northern Europe, analysis of residues of Ethofumesate, sum of NC20645 and NC9607 (expressed as NC9607), and NC8493 (the analysis includes the free and conjugated forms of NC20645 and NC8493)**

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607	NC8493		
KCA 6.3.1/04, Huauhmé, J.-M. (2013a) BPL 12/436/GC BPL12/436/GC- 01-NL 5973 AC Lottum Limburg – NL	Sugar Beet, Shakira	1) 28.03.2012 2) Not applicable 3) 12.09.2012	1021	311	329	22.05.2012	BBCH 18	Leaves with top	<0.01	<0.01	<0.01	91	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Roots	<0.01	<0.01	<0.01	91	
								Leaves with top	<0.01	<0.01	<0.01	113	
								Roots	<0.01	<0.01	<0.01	113	
KCA 6.3.1/04, Huauhmé, J.-M. (2013a) BPL 12/436/GC BPL12/436/GC- 02-NL 6599 CJ Ven- Zelderheide Limburg – NL	Sugar beet, Coyote	1) 09.04.2012 2) Not applicable 3) 17.09.2012- 18.09.2012	975	297	329	24.05.2012	BBCH 18	Whole plant	16.8	0.12	0.86	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Whole plant	0.31	0.25	0.04	8	
								Whole plant	<0.01	0.03	<0.01	29	
								Leaves with top	<0.01	<0.01	<0.01	90	
								Roots	<0.01	<0.01	<0.01	90	
								Leaves with top	<0.01	<0.01	<0.01	116	
								Roots	<0.01	<0.01	<0.01	116	
KCA 6.3.1/04, Huauhmé, J.-M. (2013a) BPL 12/436/GC BPL12/436/GC- 03-BE 3470 Kortenaken Brabant – BE	Sugar beet, Rubens	1) 10.04.2012 2) Not applicable 3) 12.09.2012 to 14.09.2012	1046	319	327	22.05.2012	BBCH 18	Leaves with top	<0.01	<0.01	<0.01	92	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Roots	<0.01	<0.01	<0.01	92	
								Leaves with top	<0.01	<0.01	<0.01	113	
								Roots	<0.01	<0.01	<0.01	113	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607	NC8493		
KCA 6.3.1/04, Huauhmé, J.-M. (2013a) BPL 12/436/GC BPL12/436/GC- 04-BE 3890 Kortij Limburg – BE	Sugar beet, Candama	1) 22.03.2012 2) Not applicable 3) 12.09.2012 to 13.09.2012	1002	306	327	22.05.2012	BBCH 18	Whole plant	19.9	0.72	0.86	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Whole plant	1.12	0.44	0.15	7	
								Whole plant	<0.01	0.03	<0.01	28	
								Leaves with top	<0.01	<0.01	<0.01	92	
								Roots	<0.01	<0.01	<0.01	92	
								Leaves with top	<0.01	<0.01	<0.01	113	
								Roots	<0.01	<0.01	<0.01	113	
KCA 6.3.1/05, Chevallier, E. (2012) BPL 11/380/GC BPL11/380/GC- 01-NL, Plot T3 5973 RC Lottum Limburg– NL	Sugar beet, Arrival	1) 12.04.2011 2) Not applicable 3) 07.09.2011	1032	314	329	06.05.2011	BBCH 14	Leaves with top	<0.01	<0.01	<0.01	124	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Root	<0.01	<0.01	<0.01	124	
KCA 6.3.1/05, Chevallier, E. (2012) BPL 11/380/GC BPL11/380/GC- 02-NL, Plot T3 6595 CJ Ottersum Limburg– NL	Sugar beet, Coyote	1) 14.04.2011 2) Not applicable 3) 13.09.2011	1014	309	329	11.05.2011	BBCH 14	Whole plant	67.8	0.36	0.69	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Whole plant	1.43	0.29	0.09	7	
								Whole plant	0.02	0.02	<0.01	28	
								Leaves with top	<0.01	<0.01	<0.01	96	
								Root	<0.01	<0.01	<0.01	96	
								Leaves with top	<0.01	<0.01	<0.01	125	
								Root	<0.01	<0.01	<0.01	125	
KCA 6.3.1/05, Chevallier, E. (2012) BPL 11/380/GC BPL11/380/GC- 03-BE, Plot T3 3473 Waanrode Brabant – BE	Sugar beet, Rubens	1) 25.03.2011 2) Not applicable 3) 13.09.2011	1024	313	327	30.04.2011	BBCH 14	Leaves with top	<0.01	<0.01	<0.01	136	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Root	<0.01	<0.01	<0.01	136	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607	NC8493		
KCA 6.3.1/05, Chevallier, E. (2012) BPL 11/380/GC BPL11/380/GC-04-BE, Plot T3 3870 Opheers Limburg– BE	Sugar beet, Bernadette	1) 23.03.2011 2) Not applicable 3) 13.09.2011	1004	307	327	30.04.2011	BBCH 14	Whole plant	49.0	1.92	0.74	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices; LOD=0.003 mg/kg for Ethofumesate and its metabolites in all matrices
								Whole plant	4.78	0.38	0.25	6	
								Whole plant	0.03	0.04	0.01	26	
								Leaves with top	<0.01	<0.01	<0.01	87	
								Root	<0.01	<0.01	<0.01	87	
								Leaves with top	<0.01	<0.01	<0.01	136	
								Root	<0.01	<0.01	<0.01	136	

Residue data summary from supervised trials of Ethofumesate on sugar beets in Northern Europe, analysis of residues of Ethofumesate and Ethofumesate-2-keto (NC 9607, includes free and conjugated NC 20645)

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]		DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607		
KCA 6.3.1/06, Waalkens, W.M. and Hamberger, R. (2005a) R03-16-NF-08 R03-168-01 Elst, NL	Sugar beets, Cyntia	1) 25.03.2003	261.23 531.81	248 252		25.04.2003 07.05.2003	BBCH 12 BBCH 14	Whole plants	35.1	0.26	0	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Whole plants	<0.02	<0.02	20	
								Whole plants	<0.006	<0.006	43	
								Whole plants	<0.006	<0.006	90	
								Leaf and top	<0.006	<0.006	173	
								Root	<0.006	<0.006	173	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]		DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607		
KCA 6.3.1/06, Waalkens, W.M. and Hamberger, R. (2005a) R03-16-NF-08 R03-168-02 Le Gault Saint Denis N-F	Sugar beets, Guépard	1) 18.03.2003	278.9 536.7	264 254		22.05.2003 27.05.2003	BBCH 12 BBCH 14	Whole plants	26.9	0.76	0	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Whole plants	0.02	<0.006	21	
								Whole plants	<0.006	<0.006	42	
								Whole plants	<0.006	<0.006	91	
								Leaf and top	<0.006	<0.006	120	
								Root	<0.006	<0.006	120	
KCA 6.3.1/07, Waalkens, W.M. and Hamberger, R. (2005b) R03-16-NF-09 R03-169-01 Angeren NL	Sugar beets, Santesse	1) 22.03.2003	275.99 527.33	262 250		24.04.2003 05.05.2003	BBCH 12 BBCH 14	Leaf and top	<0.006	<0.006	142	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Root	<0.02	<0.006	142	
KCA 6.3.1/07, Waalkens, W.M. and Hamberger, R. (2005b) R03-16-NF-09 R03-169-02 Esbarres N-F	Sugar beets, Baccara	1) 12.03.2003	265.4 548.1	252 260		17.04.2003 01.05.2003	BBCH 12 BBCH 16	Leaf and top	<0.006	<0.006	146	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Root	<0.006	<0.006	146	
KCA 6.3.1/08, Waalkens, W.M. and Hamberger, R. (2005c) R04-16-NF-08 R04-168-01 Valburg NL	Sugar beets, Shakira	1) 31.03.2004	258.85 522.40	248 250		04.05.2004 17.05.2004	BBCH 12 BBCH 14-15	Whole plants	30.0	0.08	0	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Whole plants	<0.006	<0.006	21	
								Whole plants	<0.006	<0.006	42	
								Whole plants	<0.006	<0.006	90	
								Leaf and top	<0.006	<0.006	134	
								Root	<0.006	<0.006	134	



Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]		DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607		
KCA 6.3.1/08, Waalkens, W.M. and Hamberger, R. (2005c) R04-16-NF-08 R04-168-02 Inchy en Artois N-F	Sugar beets, Monarch	1) 27.03.2004	280.79 596.84	269 261		29.04.2004 05.05.2004	BBCH 12 BBCH 14	Whole plants	35.0	0.12	0	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Whole plants	<0.006	<0.006	21	
								Whole plants	<0.006	<0.006	42	
								Whole plants	<0.006	<0.006	90	
								Leaf and top	<0.006	<0.006	166	
								Root	<0.006	<0.006	166	
KCA 6.3.1/09, Waalkens, W.M. and Hamberger, R. (2005d) R04-16-NF-09 R04-169-01 Angeren NL	Sugar beets, Pursan	1) 02.04.2004	264.60 536.34	253 257		03.05.2004 17.05.2004	BBCH 12 BBCH 14-15	Leaf and top	<0.006	<0.006	135	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Root	<0.006	<0.006	135	
KCA 6.3.1/09, Waalkens, W.M. and Hamberger, R. (2005d) R04-16-NF-09 R04-169-02 Houdilcourt N- F	Sugar beets, Crocodile	1) 30.03.2004	283.01 545.26	271 261		06.05.2004 17.05.2004	BBCH 12 BBCH 14	Leaf and top	<0.006	<0.006	155	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Root	<0.006	<0.006	155	
KCA 6.3.1/10, Anspach, T. (2001) AND-0004 FR 20/00/50 02692 Gnashwitz DE	Sugar beet, Fox	1) 15.05.2000 2) no flowering 3) 06.10.2000	229 292 289	298 305 302		09.06.2000 23.06.2000 07.07.2000	BBCH 12 BBCH 14 BBCH 18	Leaf and top	<0.004	<0.004	91	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
								Root	<0.004	<0.004	91	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]		DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607		
KCA 6.3.1/10, Anspach, T. (2001) AND-0004 FR 20/00/70 04668 Motterwitz DE	Sugar beet, Ascona	1) 17.04.2000 2) no flowering 3) 09.10.2000	230 294 297	300 307 310		06.05.2000 18.05.2000 24.05.2000	BBCH 12 BBCH 16 BBCH 18	Leaf and top Root	<0.004 <0.004	<0.004 <0.004	138 138	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
KCA 6.3.1/10, Anspach, T. (2001) AND-0004 AR 0003 Velen-Ramsdorf, Westphalia DE	Sugar beet, Helix	1) 14.04.2000 2) no flowering 3) 28.09.2000	230 286 286	300 307 310		10.05.2000 23.05.2000 30.05.2000	BBCH 12 BBCH 16 BBCH 19	Leaf and top Root	<0.004 <0.004	<0.004 <0.004	121 121	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;
KCA 6.3.1/10, Anspach, T. (2001) AND-0004 AC/00/59 16833 Lentzke DE	Sugar beet, Ascona	1) 04.04.2000 2) no flowering 3) 07.09.2000	231 294 292	301 307 304		01.05.2000 11.05.2000 23.05.2000	BBCH 12 BBCH 14-16 BBCH 16-18	Leaf and top Root	<0.004 <0.004	<0.004 <0.004	107 107	LOQ=0.02 mg/kg for ethofumesate and ethofumesate-2-keto in all matrices;

**Residue data summary from supervised trials of Ethofumesate on sugar beets in Northern Europe, analysis of residues of Ethofumesate. Ethofumesate "free"-2-keto (NC 9607) and Ethofumesate "conjugated"-2-keto (= conjugated NC 20645). Free NC 20645 has been included in the results for its conjugate.**

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258	Sugar Beet BEAVA Carissma	1) 16.03.2010 2) not applicable 3) 17.06.2010	1040	208		22.04.2010	0	Whole plant with roots Whole plant with roots	n/d n/d	n/d n/d	n/d n/d	56 62	LOQ=0.05 mg/kg

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
S10-00258-01 PE6 OSY, Thorney, Cambridgeshire, UK		23.06.2010 07.07.2010 15.09.2010 22.09.2010						Whole plant with roots	n/d	n/d	n/d	76	
								Leaves with tops	n/d	n/d	n/d	146	
								Roots	n/d	n/d	n/d	146	
								Leaves with tops	n/d	n/d	n/d	153	
								Roots	n/d	n/d	n/d	153	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-01 PE6 OSY, Thorney, Cambridgeshire, UK	Sugar Beet BEAVA Carissma	1) 16.03.2010 2) not applicable 3) 17.06.2010 23.06.2010 07.07.2010 15.09.2010 22.09.2010	1070	107		17.06.2010	14-18	Whole plant with roots	25.20	<0.05	0.73	0	LOQ=0.05 mg/kg
								Whole plant with roots	6.23	<0.05	3.90	6	
								Whole plant with roots	0.08	n/d	1.32	20	
								Leaves with tops	n/d	n/d	n/d	90	
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	97	
								<b>Roots</b>	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>97</b>	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-01 PE6 OSY, Thorney, Cambridgeshire, UK	Sugar Beet BEAVA Carissma	1) 16.03.2010 2) not applicable 3) 17.06.2010 23.06.2010 07.07.2010 15.09.2010 22.09.2010	200 388 400	100 97 100		02.06.2010 10.06.2010 17.06.2010	BBCH 14- 18	Whole plant with roots	7.71	<0.05	4.94	0	LOQ=0.05 mg/kg
								Whole plant with roots	1.34	<0.05	2.65	6	
								Whole plant with roots	<0.05	n/d	0.69	20	
								Leaves with tops	n/d	n/d	n/d	90	
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	97	
								Roots	n/d	n/d	n/d	97	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-02 PE9 4BE, Little Casterton, Cambridgeshire, UK	Sugar Beet BEAVA bobcat	1) 25.03.2010 2) not applicable 3) 17.06.2010 23.06.2010 07.07.2010 15.09.2010 28.10.2010	1015	203		22.04.2010	0	Whole plant with roots	n/d	n/d	n/d	56	LOQ=0.05 mg/kg
								Whole plant with roots	n/d	n/d	n/d	62	
								Whole plant with roots	n/d	n/d	n/d	76	
								Leaves with tops	n/d	n/d	n/d	146	
								Roots	n/d	n/d	n/d	146	
								Leaves with tops	n/d	n/d	n/d	189	
								Roots	n/d	n/d	n/d	189	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-02 PE9 4BE, Little Casterton, Cambridgeshire, UK	Sugar Beet BEAVA bobcat	1) 25.03.2010 2) not applicable 3) 17.06.2010 23.06.2010 07.07.2010 15.09.2010 28.10.2010	1100	110		16.06.2010	16	Whole plant with roots	15.48	<0.05	0.68	1	LOQ=0.05 mg/kg
								Whole plant with roots	3.56	<0.05	2.77	7	
								Whole plant with roots	0.29	<0.05	2.77	21	
								Leaves with tops	n/d	n/d	n/d	91	
								Roots	n/d	n/d	n/d	91	
								Leaves with tops	n/d	n/d	n/d	134	
								Roots	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>134</b>	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-02 PE9 4BE, Little Casterton, Cambridgeshire, UK	Sugar Beet BEAVA bobcat	1) 25.03.2010 2) not applicable 3) 17.06.2010 23.06.2010 07.07.2010 15.09.2010 28.10.2010	200 452 408	100 113 102		02.06.2010 10.06.2010 16.06.2010	18	Whole plant with roots	2.88	<0.05	3.93	1	LOQ=0.05 mg/kg
								Whole plant with roots	0.65	<0.05	4.05	7	
								Whole plant with roots	<0.05	n/d	1.96	21	
								Leaves with tops	n/d	n/d	n/d	91	
								Roots	n/d	n/d	n/d	91	
								Leaves with tops	n/d	n/d	n/d	134	
								Roots	n/d	n/d	n/d	134	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-03 C07 8SD, Little Bentley, Essex, UK	Sugar Beet BEAVA Saracen	1) 15.04.2010 2) not applicable 3) 08.9.2010 12.11.2010	1000	200		20.04.2010	BBCH 5	Leaves with tops	n/d	n/d	n/d	141	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	141	
								Leaves with tops	n/d	n/d	n/d	206	
								Roots	n/d	n/d	n/d	206	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-03 C07 8SD, Little Bentley, Essex, UK	Sugar Beet BEAVA Saracen	1) 15.04.2010 2) not applicable 3) 08.9.2010 12.11.2010	996	99.6		10.06.2010	BBCH 18	Leaves with tops	n/d	n/d	n/d	90	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	155	
								Roots	n/d	n/d	n/d	155	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-03 C07 8SD, Little Bentley, Essex, UK	Sugar Beet BEAVA Saracen	1) 15.04.2010 2) not applicable 3) 08.9.2010 12.11.2010	202 416 404	101 104 101		27.05.2010 03.06.2010 10.06.2010	BBCH 18	Leaves with tops	n/d	n/d	n/d	90	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	155	
								Roots	n/d	n/d	n/d	155	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-04 IP21 4BQ, Thrandeston, Suffolk, UK	Sugar Beet BEAVA Bullfinch	1) 07.04.2010 2) not applicable 3) 09.09.2010 10.11.2010	1015	203		20.04.2010	5	Leaves with tops	n/d	n/d	n/d	142	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	142	
								Leaves with tops	n/d	n/d	n/d	204	
								Roots	n/d	n/d	n/d	204	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-04 IP21 4BQ, Thrandeston, Suffolk, UK	Sugar Beet BEAVA Bullfinch	1) 15.04.2010 2) not applicable 3) 08.09.2010 12.11.2010	1010	101		10.06.2010	18	Leaves with tops	n/d	n/d	n/d	91	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	91	
								Leaves with tops	n/d	n/d	n/d	153	
								Roots	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>153</b>	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-04 IP21 4BQ, Thrandeston, Suffolk, UK	Sugar Beet BEAVA Bullfinch	1) 15.04.2010 2) not applicable 3) 08.09.2010 12.11.2010	196 396 404	98 99 101		27.05.2010 02.06.2010 10.06.2010	18	Leaves with tops	n/d	n/d	n/d	91	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	91	
								Leaves with tops	n/d	n/d	n/d	153	
								Roots	n/d	n/d	n/d	153	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-09 PE9 4BE, Little Casterton, Cambridgeshire, UK	Sugar Beet BEAVA Bobcat	1) 25.03.2010 2) not applicable 3) 15.07.2010 22.07.2010 05.08.2010 13.10.2010 28.10.2010	1020	102		15.07.2010	18	Whole plant with roots	6.78	n/d	0.33	0	LOQ=0.05 mg/kg
								Whole plant with roots	0.75	n/d	0.92	7	
								Whole plant with roots	0.11	n/d	1.33	21	
								Leaves with tops	n/d	n/d	n/d	90	
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	105	
								Roots	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>105</b>	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC9607	NC20645 conjugated		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-09 PE9 4BE, Little Casterton, Cambridgeshire, UK	Sugar Beet BEAVA Bobcat	1) 25.03.2010 2) not applicable 3) 15.07.2010 22.07.2010 05.08.2010 13.10.2010 28.10.2010	206	103		02.07.2010 09.07.2010 15.07.2010	18	Whole plant with roots	4.90	n/d	1.83	0	LOQ=0.05 mg/kg
			432	108				Whole plant with roots	0.13	n/d	2.15	7	
			412	103				Whole plant with roots	<0.05	n/d	0.87	21	
								Leaves with tops	n/d	n/d	n/d	90	
								Roots	n/d	n/d	n/d	90	
								Leaves with tops	n/d	n/d	n/d	105	
								Roots	<b>n/d</b>	<b>n/d</b>	<b>n/d</b>	<b>105</b>	

**Southern Europe**

**Residue data summary from supervised trials of Ethofumesate on sugar beets in Southern Europe, analysis of residues of Ethofumesate and Ethofumesate-2-keto (NC 9607, includes free and conjugated NC 20645)**

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]		DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607		
KCA 6.3.1/12, Waalkens, W.M., Hamberger, R. (2005e) R03-16-SP-06 R03-166-01 Rodezno ES	Sugar beets, Alama	1) 10.03.2003	274.3 562.4	260 267		24.04.2003 02.05.2003	BBCH 12 BBCH 14	Leaf and top	<0.006	<0.006	165	LOQ=0.02 mg/kg for Ethofumesate and Ethofumesate-2-keto in all matrices;
								Root	<0.006	<0.006	165	
KCA 6.3.1/13, Waalkens, W.M., Hamberger, R. (2005f) R03-16-FR-07 R03-167-01 Niort S-F	Fodder beets, Boléro	1) 15.04.2003	260.0 524.8	251 249		10.05.2003 21.05.2003	BBCH 12 BBCH 14	Whole plants	23.3	0.22	0	LOQ=0.02 mg/kg for Ethofumesate and Ethofumesate-2-keto in all matrices;
								Whole plants	<0.006	<0.006	21	
								Whole plants	<0.02	<0.006	42	
								Whole plants	<0.006	<0.006	90	
								Leaf and top	<0.006	<0.006	146	
								Root	<0.006	<0.006	146	



**Residue data summary from supervised trials of Ethofumesate on sugar beets in Southern Europe, analysis of residues of Ethofumesate, sum of NC20645 and NC9607 (expressed as NC9607), and NC8493 (the analysis includes the free and conjugated forms of NC20645 and NC8493)**

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607	NC8493		
KCA 6.3.1/14, Huauhmé, J.-M. (2013b) BPL12/435/GC BPL12/435/GC- 01-SP 01213-Lantarón Alava, ES	Sugar Beet, Isabella KWS	1) 22.03.2012 2) Not applicable 3) 30.09.2012	937	283	331	25.05.2012	BBCH 18	Leaves with top	<0.01	0.01	0.01	90	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices;
								Roots	<0.01	<0.01	<0.01	90	
								Leaves with top	<0.01	<0.01	<0.01	128	
								Roots	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>128</b>	
KCA 6.3.1/14, Huauhmé, J.-M. (2013b) BPL12/435/GC BPL12/435/GC- 02-SP 01428-Iruna de Oca Alava ES	Sugar Beet, Sonja	1) 17.03.2012 2) Not applicable 3) 02.10.2012	1009	305	331	26.05.2012	BBCH 18	Whole plants with root	22.4	0.40	1.52	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices;
								Whole plants with root	0.25	0.30	0.07	8	
								Whole plants with root	<0.01	0.04	<0.01	32	
								Leaves with top	<0.01	0.03	<0.01	91	
								Roots	<0.01	<0.01	0.01	91	
								Leaves with top	<0.01	<0.01	<0.01	128	
								Roots	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>128</b>	
KCA 6.3.1/14, Huauhmé, J.-M. (2013b) BPL12/435/GC BPL12/435/GC- 03-IT 35040-Merlara Veneto, IT	Sugar Beet, Nektarine	1) 03.03.2012 2) Not applicable 3) 25.08.2012	974	296	329	04.05.2012	BBCH 18	Leaves with top	<0.01	0.07	0.02	91	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices;
								Roots	<0.01	<0.01	<0.01	91	
								Leaves with top	<0.01	0.03	0.01	108	
								Roots	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>108</b>	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	Sum of NC20645 and NC9607	NC8493		
KCA 6.3.1/14, Hualmé, J.-M. (2013b) BPL12/435/GC BPL12/435/GC- 04-IT 37055-Ronco all'Adige Verona IT	Sugar Beet, Montana	1) 28.02.2012 2) Not applicable 3) 20.08.2012	979	298	329	04.05.2012	BBCH 18	Whole plants with root	15.7	0.22	1.10	0	LOQ=0.01 mg/kg for Ethofumesate and its metabolites in all matrices;
								Whole plants with root	0.66	0.31	0.07	7	
								Whole plants with root	<0.01	0.06	<0.01	32	
								Leaves with top	<0.01	0.04	<0.01	90	
								Roots	<0.01	<0.01	<0.01	90	
								Leaves with top	<0.01	0.12	<0.01	108	
								Roots	<0.01	<0.01	<0.01	108	

**Residue data summary from supervised trials of Ethofumesate on sugar beets in Southern Europe, analysis of residues of Ethofumesate, Ethofumesate "free"-2-keto (NC 9607) and Ethofumesate "conjugated"-2-keto (= conjugated NC 20645). Free NC 20645 has been included in the results for its conjugate**

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC20645 conjugate		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-05 40054, Budrio, Bologna IT	Sugar Beet BEAVA Rizor	1) 18.03.2010 2) not applicable 3) 13.05.2010 03.06.2010 12.08.2010 16.08.2010	1035	207		01.04.2010	00-07	Whole plant with roots	<0.05	n/d	0.31	42	LOQ=0.05 mg/kg
								Whole plant with roots	n/d	n/d	n/d	63	
								Leaves with tops	n/d	n/d	n/d	133	
								Roots	n/d	n/d	n/d	133	
								Leaves with tops	n/d	n/d	n/d	137	
								Roots	n/d	n/d	n/d	137	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC20645 conjugate		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-06 48017, Conselice, Ravenna, IT	Sugar Beet BEAVA, Pauletta	1) 26.06.2010 2) not applicable 3) 04.08.2010 12.08.2010	915	183		02.04.2010	0	Leaves with tops	n/d	n/d	n/d	124	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	124	
								Leaves with tops	n/d	n/d	n/d	132	
								Roots	n/d	n/d	n/d	132	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-07 42.212, Covarrubias, Soria ES	Sugar Beet BEAVA Noelia	1) 10.04.2010 2) not applicable 3) 15.06.2010 21.06.2010 05.07.2010 13.09.2010 15.10.2010	1000	200		13.04.2010	0	Whole plant with roots	n/d	n/d	0.09	63	LOQ=0.05 mg/kg
								Whole plant with roots	n/d	n/d	n/d	69	
								Whole plant with roots	n/d	n/d	n/d	83	
								Leaves with tops	n/d	n/d	n/d	153	
								Roots	n/d	n/d	n/d	153	
								Leaves with tops	n/d	n/d	n/d	185	
								Roots	n/d	n/d	n/d	185	
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-08 42392, Velamazán, Soria, ES	Sugar Beet BEAVA Emestina	1) 07.04.2010 2) not applicable 3) 30.08.2010 15.10.2010	1050	210		09.04.2010	0	Leaves with tops	n/d	n/d	n/d	143	LOQ=0.05 mg/kg
								Roots	n/d	n/d	n/d	143	
								Leaves with tops	n/d	n/d	n/d	189	
								Roots	n/d	n/d	n/d	189	

Reference no. Report no Location including Postal code	Commodity Variety	Date of 1) sowing or planting 2) flowering 3) harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment	Portion analysed	Residues [mg/kg]			DALA [days]	Remark
			a.s. [g/ha]	water [L/ha]	a.s. [g/hL]				a.s.	NC 9607	NC20645 conjugate		
KCA 6.3.1/11, Tandy, R. (2013) S10-00258 S10-00258-10 Lebrija, ES	Sugar Beet BEAVA Newton	1) 07.11.2010 2) n.a. 3) 25.01.2011 02.02.2011 15.02.2011 26.04.2011 23.06.2011	1040	208		15.11.2010	0	Whole plants with roots	0.12	n/d	<0.05	71	LOQ=0.05 mg/kg
								Whole plants with roots	n/d	n/d	n/d	79	
								Whole plants with roots	n/d	n/d	n/d	92	
								Leaves with tops	n/d	n/d	n/d	162	
								Roots	n/d	n/d	n/d	162	
								Leaves with tops	n/d	n/d	n/d	220	
								Roots	n/d	n/d	n/d	220	

**ANNEX II - RESIDUE TRIALS (SUCCEEDING CROPS)****Representative rotational crops (leafy crop, root crop and cereals) – Ethofumesate Task Force****Lettuce – 1<sup>st</sup> rotation**

Study Trial No.; Plot Location incl. postal code	application on target crop  Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-1B 1681 ND Zwaagdijk NL	Soil/  Lettuce Lucan Butterhead variety	1) 02.07.2010	1.0	300	0.333	07.06.2010/0		head	0.02 <0.01	0.01 <0.01	46 60	Soil characteristics: soil texture: Clay; pH: 6.9 % organic C: 5.6 LOQ: 0.01 mg/kg  Plant-back interval 25 days
10-2501 10-2501-03 10-2501-03-T-1B 40128 Bologna IT	Soil/  Lettuce Genti Lina Loose leaf variety	1) 14.05.2010	1.0	300	0.333	15.04.2010/0		head	<0.01 <0.01	<0.01 <0.01	62 76	Soil characteristics: soil texture: Sandy Loam; pH: 8.0 % organic C: 1.9 LOQ: 0.01 mg/kg  Plant-back interval 29 days
10-2501 10-2501-04 10-2501-04-T-1B 41310 Brenes Sevilla ES	Soil/  Lettuce Filipo Loose leaf variety	1) 16.11.2010	1.0	300	0.333	21.10.2010/0		head	0.01 <0.01	<0.01 <0.01	131 145	Soil characteristics: soil texture: Sandy clay pH: 8.4 % organic C: 0.9 LOQ: 0.01 mg/kg  Plant-back interval 26 days
10-2501 10-2501-05 10-2501-05-T-1B 51399 Burscheid DE	Soil/  Lettuce Argentinos Loose leaf variety	1) 19.04.2010	1.0	300	0.333	24.03.2010/0		head	<0.01 <0.01	<0.01 <0.01	82 96	Soil characteristics: soil texture: Sandy Loam pH: 6.4 % organic C: 1.6 LOQ: 0.01 mg/kg  Plant-back interval 26 days

Lettuce – 2<sup>nd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T- 2B 1681 ND Zwaagdijk NL	Beet, sugar Heron/  Lettuce Lucan Butterhead variety	1) 14.04.2010  1) 30.07.2010	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	88 102	LOQ: 0.01 mg/kg  Plant-back interval 57 days
10-2501 10-2501-03 10-2501-03-T- 2B 40128 Bologna IT	Beet, sugar Houston/  Lettuce Palomis Loose leaf variety	1) 01.04.2010  1) 16.09.2010	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	157 171	LOQ: 0.01 mg/kg  Plant-back interval 122 days
10-2501 10-2501-04 10-2501-04-T- 2B 41310 Brenes Sevilla ES	Beet, sugar Barbate/  Lettuce Carolus Loose leaf variety	1) 19.11.2010  1) 09.11.2011	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	378 392	LOQ: 0.01 mg/kg  Plant-back interval 259 days
10-2501 10-2501-05 0-2501-05-T- 2B 51399 Burscheid DE	Beet, sugar William/  Lettuce Aleppo Loose leaf variety	1) 13.04.2010  1) 13.08.2010	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	124 138	LOQ: 0.01 mg/kg  Plant-back interval 77 days

Lettuce – 3<sup>rd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T- 3B 1681 ND Zwaagdijk NL	Beet, sugar Heron/  Lettuce Lucan Butterhead variety	1) 14.04.2010  1) 21.04.2011	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	351 365	(LOQ: 0.01 mg/kg  Plant-back interval 322 days
10-2501 10-2501-03 10-2501-03-T- 3B 40128 Bologna IT	Beet, sugar Houston/  Lettuce Gentile Funride Loose leaf variety	1) 01.04.2010  1) 06.05.2011	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	387 401	LOQ: 0.01 mg/kg  Plant-back interval 354 days
10-2501 10-2501-04 10-2501-04-T- 3B 41310 Brenes Sevilla ES	Beet, sugar Barbate/  Lettuce Carolus Loose leaf variety	1) 19.11.2010  1) 19.01.2012	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	419 434	LOQ: 0.01 mg/kg  Plant-back interval 330 days
10-2501 10-2501-05 10-2501-05-T- 3B 51399 Burscheid DE	Beet, sugar Williams/  Lettuce Aleppo Loose leaf variety	1) 13.04.2010  1) 13.04.2011	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	head	<0.01 <0.01	<0.01 <0.01	354 368	LOQ: 0.01 mg/kg  Plant-back interval 320 days

Carrots – 1<sup>st</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-1A 1681 ND Zwaagdijk NL	Soil/ Carrot Nerja	1) 04.06.2010	1.0	300	0.333	10.05.2010/0		Leaf  root	0.03 0.03 0.04 0.02	<0.01 <0.01 <0.01 <0.01	92 106 92 106	Soil characteristics: soil texture: Clay pH: 6.9 % organic C: 5.6  LOQ 0.01 mg/kg Plant-back interval 25 days
10-2501 10-2501-03 10-2501-03-T-1A 40128 Bologna IT	Soil/ Carrot Nantes- Clodia	1) 14.05.2010	1.0	300	0.333	15.04.2010/0		Leaf  root	0.03 0.03 0.02 0.03	<0.01 <0.01 <0.01 <0.01	140 154 140 154	Soil characteristics: soil texture: Sandy Loam pH: 8.0 % organic C: 1.9  LOQ 0.01 mg/kg Plant-back interval 29 days
10-2501 10-2501-04 10-2501-04-T-1A 41310 Brenes Sevilla ES	Soil/ Carrot Coral	1) 16.11.2010		300	0.333	21.10.2010/0		Leaf  root	0.02 0.02 0.03 0.02	<0.01 <0.01 <0.01 <0.01	201 216 201 216	Soil characteristics: soil texture: Sandy clay pH: 8.4 % organic C: 0.9  LOQ 0.01 mg/kg Plant-back interval 26 d
10-2501 10-2501-05 10-2501-05-T-1A 51399 Burscheid DE	Soil/ Carrot Cesta	1) 19.04.2010		300	0.333	24.03.2010/0		Leaf  root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	100 114 100 114	Soil characteristics: soil texture: Sandy Loam pH: 6.4 % organic C: 1.6  LOQ 0.01 mg/kg Plant-back interval 26 d



Carrots – 2<sup>nd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-2A 1681 ND Zwaagdijk NL	Beet, sugar Heron/ Carrot Nerja	1) 14.04.2010 1) 27.07.2010	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	leaf root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	144 158 144 158	LOQ 0.01 mg/kg Plant-back interval 54 days
10-2501 10-2501-03 10-2501-03-T-2A 40128 Bologna IT	Beet, sugar Houston/ Carrot Nantes-Clodia	1) 01.04.2010 1) 15.09.2010	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	leaf root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	339 353 339 353	LOQ 0.01 mg/kg Plant-back interval 121 days
10-2501 10-2501-04 10-2501-04-T-2A 41310 Brenes Sevilla ES	Beet, sugar Barbate/ Carrot Coral	1) 19.11.2010 1) 09.11.2011	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	leaf root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	434 448 434 448	LOQ 0.01 mg/kg Plant-back interval 259 days
10-2501 10-2501-05 10-2501-05-T-2A 51399 Burscheid DE	Beet, sugar William/ Carrot Cestas	1) 13.04.2010 1) 06.08.2010	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	Leaf root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	150 164 150 164	LOQ 0.01 mg/kg Plant-back interval 70 days

Carrots – 3<sup>rd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-3A 1681 ND Zwaagdijk NL	Beet, sugar Heron Carrot Nerja	1) 14.04.2010 1) 20.04.2011	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	Leaf  root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	399 413 399 413	LOQ 0.01 mg/kg  Plant-back interval 321 days
10-2501 10-2501-03 10-2501-03-T-3A 40128 Bologna IT	Beet, sugar Houston Carrot Nantes Clodia	1) 01.04.2010 1) 06.05.2011	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	Leaf  root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	472 486 472 486	LOQ 0.01 mg/kg  Plant-back interval 354 days
10-2501 10-2501-04 10-2501-04-T-3A 41310 Brenes Sevilla ES	Beet, sugar Barbate Carrot Coral	1) 19.11.2010 1) 19.01.2012	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	Leaf  root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	463 477 463 477	LOQ 0.01 mg/kg  Plant-back interval 330 days
10-2501 10-2501-05 10-2501-05-T-3A 51399 Burscheid DE	Beet, sugar William Carrot Cestas	1) 13.04.2010 1) 13.04.2011	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	Leaf  root	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	396 410 396 410	LOQ 0.01 mg/kg  Plant-back interval 320 days

Cereals – 1<sup>st</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-1C 1681 ND Zwaagdijk NL0	Soil/ Barley Cervoise	1) 11.10.2010	1.0	300	0.333	08.09.2010/0		green material straw	<0.01 <0.01	<0.01 <0.01	226 329	Soil characteristics: soil texture: Clay pH: 6.9 % organic C: 5.6 LOQ: 0.01 mg/kg  Plant-back interval 33 days
10-2501 10-2501-03 10-2501-03-T-1C 40128 Bologna IT	Soil/ Barley Otis	1) 20.04.2010	1.0	300	0.333	25.03.2010/0		green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	64 117 117	Soil characteristics: soil texture: Sandy Loam pH: 8.0 % organic C: 1.9  LOQ: 0.01 mg/kg Plant-back interval 26 days
10-2501 10-2501-04 10-2501-04-T-1C 41310 Brenes Sevilla ES	Soil/ Barley Garbo	1) 16.11.2010	1.0	300	0.333	21.10.2010/0		green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	134 242 242	Soil characteristics: soil texture: Sandy clay pH: 8.4 % organic C: 0.9  LOQ: 0.01 mg/kg Plant-back interval 26 days
10-2501 10-2501-05 10-2501-05-T-1C 51399 Burscheid DE	Soil/ Barley Simba Summer variety	1) 19.04.2010	1.0	300	0.333	24.03.2010/0		green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	64 135 135	Soil characteristics: soil texture: Sandy Loam pH: 6.4 % organic C: 1.6  LOQ: 0.01 mg/kg Plant-back interval 26 days

Cereals – 2<sup>nd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-2C 1681 ND Zwaagdijk NL	Beet, sugar Heron/ Wheat Tatarus	1) 14.04.2010  1) 26.11.2010	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	327 428 428	LOQ: 0.01 mg/kg  Plant-back interval 176 days
10-2501 10-2501-03 10-2501-03-T-2C 40128 Bologna IT	Beet, sugar Houston/ Wheat Mieti	1) 01.04.2010  1) 15.10.2010	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	311 401 401	LOQ: 0.01 mg/kg  Plant-back interval 151 days
10-2501 10-2501-04 10-2501-04-T-2C 41310 Brenes Sevilla ES	Beet, sugar Barbate/ Wheat Semolero	1) 19.11.2010  1) 09.11.2011	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	322 463 463	(LOQ: 0.01 mg/kg  Plant-back interval 259 days
10-2501 10-2501-05 10-2501-05-T-2C 51399 Burscheid DE	Beet, sugar William/ Wheat Hermann Winter variety	1) 13.04.2010  1) 06.10.2010	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	311 417 417	LOQ: 0.01 mg/kg  Plant-back interval 131 days

Cereals – 3<sup>rd</sup> rotation

Study Trial No.; Plot Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
10-2501 10-2501-02 10-2501-02-T-3C 1681 ND Zwaagdijk Netherlands 2010	Beet, sugar Heron/ Barley Tripple	1) 14.04.2010 1) 18.03.2011	1.0	300	0.333	03.06.2010/0	Six leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	343 452 452	LOQ: 0.01 mg/kg  Plant-back interval 288 days
10-2501 10-2501-03 10-2501-03-T-3C 40128 Bologna Italy 2010	Beet, sugar Houston/ Barley Otis	1) 01.04.2010 1) 25.02.2011	1.0	300	0.333	17.05.2010/0	Eight leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	354 417 417	LOQ: 0.01 mg/kg  Plant-back interval 284 days
10-2501 10-2501-04 10-2501-04-T-3C 41310 Brenes Sevilla, ES	Beet, sugar Houston/ Barley Garbo	1) 19.11.2010 1) 19.01.2012	1.0	300	0.333	23.02.2011/0	Eight leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	414 477 477	LOQ: 0.01 mg/kg  Plant-back interval 330 days
10-2501 10-2501-05 10-2501-05-T-2C 51399 Burscheid DE	Beet, sugar William/ Wheat Hermann Winter variety	1) 13.04.2010 1) 06.10.2010	1.0	300	0.333	28.05.2010/0	Six leaves unfolded	green material grain straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	311 417 417	LOQ: 0.01 mg/kg  Plant-back interval 131 days

## Representative rotational crops (leafy crop, root crop and cereals) – UPL

### Root vegetables

Study Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
697614 Haddenham, CB63TC UK 2012	sugar beets  Carrots / Maestro	4) 17/06/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	tops roots	<0.01 <0.01	<0.01 <0.01	179 179	LOQ: 0.01 mg/kg  Plant-back interval 40 days
697614 Haddenham, CB63TC UK 2012	Beet, sugar  radish/ French Breakfast	4)08/05/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	tops roots	<0.01 <0.01	<0.01 <0.01	390 390	LOQ: 0.01 mg/kg  Plant-back interval 335 days
697614 26857 Salerno, IT 2012	Beet, sugar  radish/ Milano	4)29/06/2012	1.0	200	0.5	30/05/2012	BBCH 16	tops roots	<0.01 <0.01	<0.01 <0.01	118 118	LOQ: 0.01 mg/kg  Plant-back interval 30 days
697614 26857 Salerno, IT 2012	Beet, sugar  radish/ Milano	4)28/08/2012	1.0	200	0.5	30/05/2012	BBCH 16	tops roots	<0.01 <0.01	<0.01 <0.01	147 147	LOQ: 0.01 mg/kg  Plant-back interval 90 days
697614 26857 Salerno, IT 2012	Beet, sugar  radish/ Milano	4)30/04/2012	1.0	200	0.5	30/05/2012	BBCH 16	tops roots	<0.01 <0.01	<0.01 <0.01	365 365	LOQ: 0.01 mg/kg  Plant-back interval 335 days

## Leafy vegetables

Study Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
697614 Haddenham, CB63TC UK 2012	sugar beets  Spinach / F1 Mississippi	4) 18/07/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	79 98	LOQ: 0.01 mg/kg  Plant-back interval 41 days
697614 Haddenham, CB63TC UK 2012	sugar beets  Spinach / F1 Mississippi	4) 18/09/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	343 347	LOQ: 0.01 mg/kg  Plant-back interval 103 days
697614 Haddenham, CB63TC UK 2012	sugar beets  Spinach / F1 Mississippi	4)08/05/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	383 389	LOQ: 0.01 mg/kg  Plant-back interval 335 days
697614 26857 Salerno, IT 2012	Beet, sugar  Spinach / Liscia	4)29/06/2012	1.0	200	0.5	30/05/2012	BBCH 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	83 99	LOQ: 0.01 mg/kg  Plant-back interval 30 days
697614 26857 Salerno, IT 2012	Beet, sugar  Spinach / Liscia	4)28/08/2012	1.0	200	0.5	30/05/2012	BBCH 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	135 147	LOQ: 0.01 mg/kg  Plant-back interval 90 days
697614 26857 Salerno, IT 2012	Beet, sugar  Spinach / Liscia	4)30/04/2012	1.0	200	0.5	30/05/2012	BBCH 16	immature leaves mature leaves	<0.01 <0.01	<0.01 <0.01	365 376	LOQ: 0.01 mg/kg  Plant-back interval 335 days

## Cereals

Study Location incl. postal code Year of Trial	application on target crop  Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Application rate per treatment			Dates of treatment(s) or no. of treatments and last date	Growth stage at last treatment	Portion analysed succeeding crop	Residues (mg/kg)		DALT/ PHI (days)	Remarks
			kg a.s./ha	Water (L/ha)	kg a.s./hL				a.s.	NC 9607		
697614 Haddenham, CB63TC UK 2012	sugar beets  Spring barley/ KWS Aurelia	4) 04/04/2013	1.0	200	0.5	04/03/2013	pre emergence	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	82 127 157 157	LOQ: 0.05 mg/kg  Plant-back interval 31 days
697614 Haddenham, CB63TC UK 2012	sugar beets  Winter wheat/ Torch	4) 30/11/2012	1.0	200	0.5	07/06/2012	BBCH 14- 16	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	347 396 431 431	LOQ: 0.05 mg/kg  Plant-back interval 176 days
697614 Haddenham, CB63TC UK 2012	sugar beets  Spring barley/ KWS Aurelia	4)08/05/2013	1.0	200	0.5	07/06/2012	BBCH 14- 16	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	376 417 453 453	LOQ: 0.05 mg/kg  Plant-back interval 335 days
697614 26857 Salerno, IT 2012	sugar beets  Spring barley/ Arda	4)11/04/2013	1.0	200	0.5	12/03/2013	pre emergence	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	79 100 142 142	LOQ: 0.05 mg/kg  Plant-back interval 30 days
697614 26857 Salerno, IT 2012	sugar beets  Winter barley/ Margaret	4)26/11/2012	1.0	200	0.5	30/05/2012	BBCH 16	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	335 350 410 410	LOQ: 0.05 mg/kg  Plant-back interval 180 days
697614 26857 Salerno, IT 2012	sugar beets  Spring barley/ Arda	4)30/04/2012	1.0	200	0.5	30/05/2012	BBCH 16	forage hay grain straw	<0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05	365 386 428 428	LOQ: 0.05 mg/kg  Plant-back interval 335 days