

# ***European Commission***



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

**ETHOFUMESATE**

**Volume 3 – B.6 (AS)**

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

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## Version History

When	What
1998	Initial DAR, RMS SE (no addenda to DAR containing additional information on toxicology and metabolism could be identified on CIRCABC or were provided by the original RMS SE)
2001	Final evaluation table toxicology and metabolism (Doc. 6487/VI/99 rev. 6 (05.02.2001))
2015/01	DRAR

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## **B.6. TOXICOLOGY AND METABOLISM DATA**

In the last evaluation table (Doc. 6487/VI/99 rev. 6 (05.02.2001)) of first approval of active substance ethofumesate, for which the TaskForce was the only notifier with the complete dossier, no open points for mammalian toxicity remained.

For the renewal of approval of ethofumesate, two notifiers, TaskForce Ethofumesate (Bayer CropScience and Adama Deutschland GmbH (former Feinchemie Schwebda)) and UPL (United Phosphorus Limited), submitted complete dossiers by the deadline of January 31, 2014. The dossiers for the renewal contain very few new studies (based on e.g. new data requirements like phototoxicity), the major part of the dossier is the same as it was for the first approval of ethofumesate (*Commission Directive 2002/37/EC of 3 May 2002 amending Council Directive 91/414/EEC to include ethofumesate as an active substance*).

Regarding the old studies, originally evaluated in the DAR (RMS Sweden), mostly re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines was proven.

Regarding the originally derived reference values the RMS followed the original outcome (Review report for the active substance ethofumesate, SANCO/6503/VI/99-final, 15 May 2002) regarding the AOEL (2.5 mg/kg bw/d) and could confirm the non-necessity for the ARfD. However, the RMS derived the new ADI (1 mg/kg bw/d) different than the original one (0.07 mg/kg bw/d) based on the re-evaluation of the same study (chronic rat toxicity study).

The RMS also paid special attention to new criteria for classification and labelling according to Regulation (EC) 1272/2008. The outcome of the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances Pesticides, ECB Ispra, 19-21 May 1999 (ECBI/43/99 Rev. 2), that no classification and labelling for ethofumesate is necessary for human health, could be supported.

In almost none of the toxicity studies conducted with ethofumesate for the first approval the content of batches is known, only purity grades could be identified in the study reports. Therefore, the RMS requested from the TaskForce the QSAR analysis of currently present impurities in ethofumesate technical. This evaluation can be found in Volume 4 of the respective notifier.

Search of the scientific peer reviewed open literature was conducted by both notifiers, covering a period from 2003 to 2013. Both notifiers stated that their literature search was conducted according to EFSA Guidance “Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009” (EFSA Journal 2011;9(2):2092. [49 pp.]).

Both TaskForce and UPL used different bibliographic databases but at least some of the databases were considered appropriate by RMS for search on effects on human health (e.g. MEDLINE). While TaskForce

conducted the search using only the name of active substance, known metabolites and its trade names without considering any toxicological keywords, UPL included in the search only the active substance name combined by Boolean operators with some toxicological keywords which they considered relevant to address data requirements. The RMS concluded that in case of ethofumesate, where all detected metabolites are unique ethofumesate metabolites, the metabolites would be captured in the search also by using only the key word ethofumesate. Regarding the inclusion of the trade names in the search it is considered that this should be done case by case (if it is known that the formulation has a higher toxicity than the active substance) since trade names might tremendously increase the “background noise” (amount of information not related to the topic) in the search.

After the rapid and the full-text assessment the TaskForce concluded that none of the articles retrieved was relevant. However, in its dossier, two metabolism studies from the scientific peer reviewed open literature were included and summarised. UPL identified in its search three relevant metabolism studies, two of them being the same as studies included in the dossier by the TaskForce.

After detailed assessment of the chosen approaches for the literature search, the RMS concluded that both notifiers, although having different approaches, appropriately addressed the scientific peer reviewed open literature. The RMS made a short search in the PubMed using solely the key word “ethofumesate” (for ethofumesate no synonym is known) and did not identify any other potentially relevant article than the notifiers did.

RMS concluded that three metabolism studies from the scientific peer reviewed open literature are of questionable relevance; however they were included in the DRAR as some additional information (to be found under B.6.1.3 Other ADME studies). The RMS did not prove these studies upon their reliability since no OECD Guideline for this type of studies is available at present.

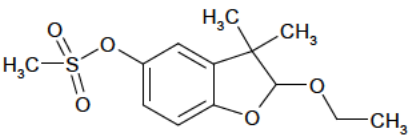
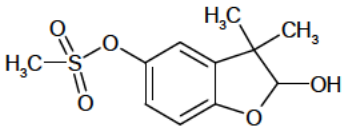
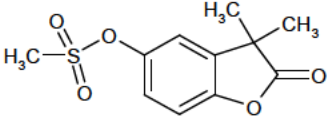
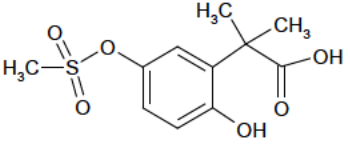
### **B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS**

#### **B.6.1.1. Absorption, distribution, metabolism and excretion by oral route**

No new ADME studies by oral route were submitted for the renewal of ethofumesate (not considered necessary). The evaluations of studies presented below were included in the original DAR (1998). Only re-wording was conducted and additional information or tables were included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guideline 417 (2010) was proven.

The metabolism studies in hen, cow and sheep, originally evaluated in the DAR B5 (toxicology) were excluded from the DRAR B6. These studies can be found in the DRAR B7 (residue behaviour).

For better understanding the RMS included below the structure, codes and synonyms of the active substance and the metabolites identified in rat metabolism studies. The expressions in bold were used throughout the DRAR B6.

Expressions used in the B6 DRAR	IUPAC name	Structure
<b>Ethofumesate</b>  Synonyms: a.s. NC 8438, AE B049913	(RS)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate	
<b>Ethofumesate-2-hydroxy</b>  Synonyms: NC 8493, AE C508493, BCS-BB94377, hydroxy-derivative, 2-hydroxy-ethofumesate, Fumesate	2,3-dihydro-2-hydroxy-3,3-dimethylbenzofuran-5-yl methane-sulfonate	
<b>Ethofumesate-lactone</b>  Synonyms: NC 9607, AE C509607, 2-keto-Ethofumesate, Ethofumesate-2-keto, Oxo-derivative, Fumesate lactone	2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methanesulfonate	
<b>Ethofumesate-carboxylic acid</b>  Synonyms: NC 20645, AE C520645, BCS-AV65501, RO 9607 ("ring-open 9607"), "Hydrolyzed AE C509607" [res. method no. 01116/M001], Ethofumesate-γ-hydroxy-carboxylic acid, open-ring-2-keto-ethofumesate, ring opened lactone ----- AE C639175 (potassium salt) BCS-CU88901 (sodium salt)	2-(2-hydroxy-5-methanesulfoxyphenyl)-2-methyl propionic acid	

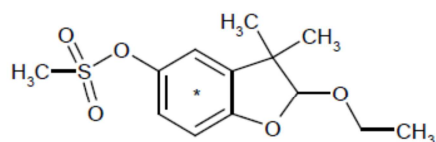
<b>Reference:</b>	Metabolism of ethofumesate in rat
Author(s), year:	1991
Report/Doc. number:	A87552 / M-161454-01-1

Guideline(s):	OECD 417 (1984)
GLP:	Yes
Deviations from OECD 417 (2010):	Minor (partially) reporting bias: - administration site for intravenous route not specified - age of animals not given (however, based on the body weight information, the animals were between 6 and 12 weeks old, which is compliant to the OECD 417 (2010)) - the weight variation of animals per test group not given - tissue to plasma (blood) ratio not reported Major shortcoming of the metabolism part of the study: - huge amount of “polar material” not identified in the metabolite profiling
Acceptability:	Yes for absorption, distribution and excretion; metabolites identification not sufficient

### Material and methods

The study was designed to examine the rates and extent of absorption, distribution and excretion of [ $^{14}\text{C}$ ]-ethofumesate in the rat, following oral and intravenous administration.

The absorption, distribution, metabolism and excretion of ethofumesate were studied in male and female rats (Sprague Dawley). The  $^{14}\text{C}$  ethofumesate was uniformly labelled on the phenyl ring. The radiochemical purity was originally 89% (measured by thin layer chromatography (TLC)). After repurification 99.6% radiopurity was measured by TLC.



\* = phenyl-UL- $^{14}\text{C}$ -label

Four groups of Sprague –Dawley rats (180 – 350 g body weight), each consisting of five male and five female animals were dosed with the radioactive test substance as follows:

#### Group A - single intravenous dose (low dose): 1 mg/kg bw

- administered dose volume: ca.250  $\mu\text{l}$  ( $< 1\text{ mL/kg bw}$ )
- ethofumesate dissolved in ethanol (1 ml), followed by water (1 ml) for injection
- no site of application stated in the report

#### Group B - single oral dose (low dose): 1 mg/kg bw

- administered dose volume: 1 - 1.1 ml ( $< 10\text{ mL/kg bw}$ )
- ethofumesate grinded and diluted with 1% carboxymethylcellulose (CMC) solution
- homogeneity proven



**Group C – repeated oral dose (low dose): 1 mg/kg bw**

- 14 daily oral doses of non-radiolabelled ethofumesate, followed by a single radioactive oral dose on day 15
- administered dose volume: 1.3 - 1.6 ml (< 10 mL/kg bw)
- ethofumesate grinded and diluted with 1% carboxymethylcellulose (CMC) solution
- homogeneity proven

**Group D - single oral dose (high dose): 100 mg/kg bw**

- administered dose volume: 1 ml (< 10 mL/kg bw)
- ethofumesate grinded and diluted with 1% carboxymethylcellulose (CMC) solution
- homogeneity proven

Following the administration of the radioactivity, animals were placed in metabolism cages (12 h light/dark cycle) and the urine and the faeces were collected for up to 168 hours post dosing, at which time the animals were sacrificed (CO<sub>2</sub> narcosis) and the levels of radioactivity in selected tissues and organs (bone, brain, fat, heart, skeletal muscle, uterus, ovaries, testes, liver, lung, spleen, kidney, stomach (including contents), large intestine (including contents) and residual carcass) and body fluids were analysed. Expired air was collected in a trapping solution consisting of 30% ethanolamine in 2-ethoxyethanol. For metabolite profiling sex specific proportionally pooled samples of urine and faeces were investigated. The samples were co-chromatographed (TLC) with ethofumesate and standard compounds and run in toluene-ethyl acetate 4:1 v/v solvent system.

Sample collection times (for results on excretion):

urine: 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h;

faeces: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h;

expired air: 8, 24, 48 h

Sample collection times (for metabolite profiling):

urine: 0-12, 12-24 h post dose

faeces: 0-24 h post dose

**Results**

Radioactivity in the expired air was extremely low (0.01% in group A, < 0.2% in group B, < 0.1% in group C and < 0.05% in group D).

Following both single and multiple oral administrations at the low and high dose level, most radioactivity was absorbed and eliminated rapidly (already from 0-24 hours), mainly via urine. Following intravenous administration of the low dose level, radioactivity was rapidly excreted mainly via urine, but with a proportion also being excreted via faeces, indicating some biliary excretion. Based on the results of this experiment, biliary

excretion after intravenous administration of the low dose level accounted for 13% in the males and 7% in the females.

**Table B.6.1.1-1: Mean % of dose recovered in excreta**

		<b>Group A</b> <b>single intravenous</b> <b>dose (low dose) 1</b> <b>mg/kg bw</b>		<b>Group B</b> <b>single oral dose</b> <b>(low dose) 1 mg/kg</b> <b>bw</b>		<b>Group C</b> <b>repeated oral</b> <b>dose (low dose)</b> <b>1 mg/kg bw</b>		<b>Group D</b> <b>single oral dose</b> <b>(high dose) 100</b> <b>mg/kg bw</b>	
		male	female	male	female	male	female	male	female
0-168 h	Urine	80 %	83 %	83 %	85 %	85 %	87 %	77 %	76 %
	Faeces	13 %	7 %	17 %	9 %	13 %	10 %	18 %	14 %
	total <sup>1</sup>	94 %	93 %	101 %	97 %	98 %	100 %	98 %	96 %

<sup>1</sup>includes cage wash and tissues

In all treatment groups the elimination of radioactivity was rapid and at 168 h post dosing, the levels of radioactivity in the tissues were approaching or had already reached limit of reliable detection (< 0.5%). Tissues containing radioactivity (slightly) above background levels were: the gastro-intestinal tract, liver, kidneys and lungs.

The metabolite profile of the urine was very similar in all the treatment groups: 96-98% of the radioactivity in the urine was associated with very polar material which was retained at the origin of the TLC plates. 1-4% of the radioactivity in the urine was linked with the metabolite ethofumesate-lactone. Trace amounts of radioactivity in the urine were associated with the reference compound Y5496-01 of which the chemical structure was not reported in the study report. Unchanged parent compound was not found.

In the faeces a clear difference was noted between the high dose (oral administration) and low dosed groups (both oral and intravenous administration). In the low dose groups the main metabolite, accounting for 74-89% of the radioactivity in the faeces was associated with very polar material which was retained at the origin of the TLC plates. A further metabolite, accounting for 7-15% of the radioactivity in faeces of low dosed groups, was ethofumesate-lactone. The reference compound Y5496-01 accounted for 2-4% of the low dose faecal radioactivity. Several additional polar metabolites were noted, none of them accounting for more than 2-3% of the administered radioactivity. Unchanged parent compound was associated with 1-3% of the low dose faecal radioactivity.

At the high dose level the very polar material accounted for 61% of the faecal radioactivity in males and for 43% in females. Ethofumesate-lactone was associated with 5% of the faecal radioactivity. The reference compound Y5496-01 accounted for 2-3% of the high dose faecal radioactivity. Several additional polar metabolites were noted, none of them accounting for more than 3% of the administered radioactivity. Unchanged parent compound accounted for 25% of the high dose faecal radioactivity in males and for 48% in females. The latter

findings indicate that absorption at the high dose level is saturated. This is well in line with the data from the kinetic part of the study which demonstrated an increase in excretion via the faeces at higher dose levels.

**Table B.6.1.1-2: % of total radioactivity on plate**

<b>Ethofumesate and metabolites</b>	<b>Group A single intravenous dose (low dose) 1 mg/kg bw</b>	<b>Group B single oral dose (low dose) 1 mg/kg bw</b>	<b>Group C repeated oral dose (low dose) 1 mg/kg bw</b>	<b>Group D single oral dose (high dose) 100 mg/kg bw</b>
<b>0-12 h pooled urine (range; males and females combined)</b>				
Ethofumesate	0.00	0.00	0.00	0.00
Reference compound Y5496-02	0.00	0.00	0.00	0.00
Ethofumesate-lactone	0.9-1.1	1.1-1.4	0.9-1.5	0.8-0.9
Ethofumesate-2-hydroxy	0.0-1.2	0.00	0.00	0.00
Reference compound Y5496-01	0.0-0.4	0.0-0.2	0.00	0.00
Not identified metabolites (no single component)	0.5	0.3-0.6	0.4-0.6	0.3-0.4
Origin	96.8-98.6	98.0-98.1	97.9-98.2	98.8
<b>12-24 h pooled urine (range; males and females combined)</b>				
Ethofumesate	0.00	0.00	0.00	0.00
Reference compound Y5496-02	0.00	0.00	0.00	0.00
Ethofumesate-lactone	3.1-3.6	3.4-3.6	3.6-3.9	3.3-3.5
Ethofumesate-2-hydroxy	0.00	0.00	0.00	0.00
Reference compound Y5496-01	0.00	0.00	0.00	0.00
Not identified metabolites (no single component)	0.0-0.7	1.0-1.4	0.0-0.5	0.0-0.7
Origin	96.2-96.4	95.2-95.4	95.8-96.1	96.1-96.5
<b>0-12 h pooled faeces (range; males and females combined)</b>				
Ethofumesate	0.0-1.2	0.7-2.9	1.2-1.7	25.2-48.3
Reference compound Y5496-02	0.0	0.0-0.6	0.0	0.0-0.7
Ethofumesate-lactone	8.2-14.9	12.2-13.5	6.6-8.9	4.9-5.1
Ethofumesate-2-hydroxy	0.0-1.8	0.0-0.8	0.0-1.1	0.0-0.7
Reference compound Y5496-01	2.2-3.1	2.0-4.1	3.0-3.2	2.2-2.6
Origin	73.8-88.7	78.8-79.7	82.6-88.5	42.5-61.3

## Conclusion

Following both single and multiple oral administrations at the low and high dose level, most radioactivity was absorbed and eliminated very rapidly (already from 0-24 hours), mainly to very polar species via urine.

By 168 h post dose < 0.5 % of the dose remains in the body of the animal. Multiple administrations at this dose level have no effect on the pattern of absorption, metabolism or elimination.

Following intravenous administration of the low dose level, radioactivity was very rapidly excreted mainly in the urine, but with a proportion also being excreted in the faeces, indicating some biliary excretion. Based on the results of this experiment, biliary excretion after intravenous administration of the low dose level accounted for 13% in the males and 7% in the females.

Oral administration at the high dose level (100 mg/kg bw) appears to saturate the absorption pathway of ethofumesate. A higher proportion of the dose is eliminated in the faeces at this dose level, a large proportion of which consists of parent compound. The dose is, however, still very rapidly eliminated and 0.5 % remains in the body of the animal at 168 h post dose. Data indicates that elimination of the test substance is almost complete within 8 days. Only trace amounts were found in the organs and tissues.

The metabolite profile of the urine was very similar in all the treatment groups: 96-98% of the radioactivity in the urine was associated with very polar material (not further identified) which was retained at the origin of the TLC plates. 1-4% of the radioactivity in the urine was linked with the metabolite ethofumesate-lactone.

In the faeces a clear difference was noted between the high dose and low dosed groups.

In the low dose group the main metabolite, accounting for 74-89% of the radioactivity in the faeces was associated with very polar material which was retained at the origin of the TLC plates. The second metabolite, accounting for 7-15% of the radioactivity in the low dose faeces was ethofumesate-lactone.

At the high dose level the very polar material accounted for 61% of the faecal radioactivity in males and for 43% in females. Ethofumesate-lactone was associated with 5% of the faecal radioactivity. Several additional polar metabolites were noted, none of these accounting for more than 3% of the administered radioactivity. Unchanged parent compound accounted for 25% of the high dose faecal radioactivity in males and for 48% in females. The latter findings indicate that absorption at the high dose level is saturated. This is well in line with the data from the kinetic part of the study which demonstrated an increase in excretion via the faeces at higher dose levels.

<b>Reference:</b>	ADME study in rats
Author(s), year:	██████████ 1993
Report/Doc. number:	8210/1/93 / M-351968-01-1
Guideline(s):	OECD 417 (1984)
GLP:	Yes
Deviations from OECD 417 (2010):	Minor (partially) reporting bias: - administration site for intravenous route not specified - tissue to plasma (blood) ratio not reported - impurities > 2% not identified - radioactivity in residual carcass not measured Major shortcoming of the study: - low recovery of radioactivity in excreta after oral administration
Acceptability:	Yes (limited conclusion possible regarding absorption and excretion)

### Material and methods

The absorption, distribution, metabolism and excretion of ethofumesate was studied in male and female rats (Sprague Dawley ; 6-8 weeks old ; 184 – 203 g males and 181 – 205 g females). The  $^{14}\text{C}$  test material was labelled on the phenyl ring. The radiochemical purity ranged from 84.3% (group IV) to 100.8% (group II) measured by thin layer chromatography (TLC).

Three groups of rats, consisting of 4 male and 4 female animals and a fourth group consisting of 12 females were included in the study. The rats were dosed with the radioactive test substance as follows:

**Group I - single intravenous low dose: 0.33 mg/rat (approx. 1 mg/kg bw.)**

- administered dose volume: 500  $\mu\text{l}$
- ethofumesate dissolved in ethanol, followed by water
- no site of application stated in the report
- urine and faeces sampling (absorption, excretion)

**Group II - single oral low dose: 0.33 mg plus 2 mg/rat unlabelled substance (approx. 10mg/kg bw)**

- administered dose volume: 3 ml
- ethofumesate dissolved in aqueous methylcellulose and ethanol
- urine and faeces sampling (absorption, excretion)

**Group III - single oral high dose: 0.33 mg plus 40 mg unlabelled substance (approx. 200 mg/kg bw)**

- administered dose volume: 3 ml
- ethofumesate dissolved in aqueous methylcellulose and ethanol
- urine and faeces sampling (absorption, excretion)

**Group IV - single intravenous high dose: 1 mg/rat (approx. 5 mg/kg bw).**

- administered dose volume: 100  $\mu\text{l}$
- ethofumesate dissolved in ethanol
- no site of application stated in the report
- blood and organ sampling (distribution) and urine (metabolites identification)

Sample collection times:

Urine and faeces (for excretion estimation): seven continuous days at 24 h-fractions per animal

Blood and organ sampling (for distribution estimation): 3 females each were sacrificed after 1, 4 and 24 hours and 7 days after the administration (brain, spinal cord, fat (1 sample), kidneys, liver, heart and ovaries investigated)

Total radioactivity was measured by liquid scintillation counting (LSC). For urine metabolites profiling, the samples were run in A) cyclohexane: ethyl acetate 3:1 v/v solvent system and B) ethyl acetate: ethanol 7:3 v/v solvent system and analysed by radio-TLC on silica gel plates. The structures of the metabolites were deduced from the  $^1\text{H-NMR}$  in methanol- $\text{d}_4$

## Results

Following single oral administrations at the low and high dose level (group II and III) and single intravenous administrations at the low dose (group I), most radioactivity was absorbed and eliminated rapidly (already from 0-24 hours), mainly via urine. Since no cage wash values were reported only urine and faeces values were summed. The recovery ranged from 72% (group II males) to 98% (group I males). Regarding oral administration, radioactivity excreted by females after 7 days was higher than excreted by males, both via urine and faeces. In group III (high oral dose) amount excreted via faeces by both males and females was higher than in the group II (low oral dose) indicating that the ethofumesate administered at high dose might not have been completely absorbed. After intravenous administration males excreted more via faeces than females but the total excretion was for both sexes comparable.

**Table B.6.1.1-3: Mean cumulative % of applied radioactivity in excreta after 7 days**

		<b>Group I</b> <b>single intravenous low</b> <b>dose 1 mg/kg bw</b>		<b>Group II</b> <b>single oral low dose 10</b> <b>mg/kg bw</b>		<b>Group III</b> <b>single oral high dose 200</b> <b>mg/kg bw</b>	
		male	female	male	female	male	female
0-168 h	Urine	82	90	66	79	62	72
	Faeces	16	6	6	9	17	21
	total <sup>1</sup>	98	96	72	88	79	93

<sup>1</sup> no tissue (no radioactivity expected there according to tissue distribution measurement) or cage wash included

One hour after intravenous administration (group IV) 0.25% of the radioactivity was found in the fat (not specified) and 0.29% in the kidneys. After 24 hours 0.04% was found in fat and 0.01% in kidneys and liver. Seven days after application a very small fraction of radioactivity (< 0.01% of the dose) was still detectable only in fat.

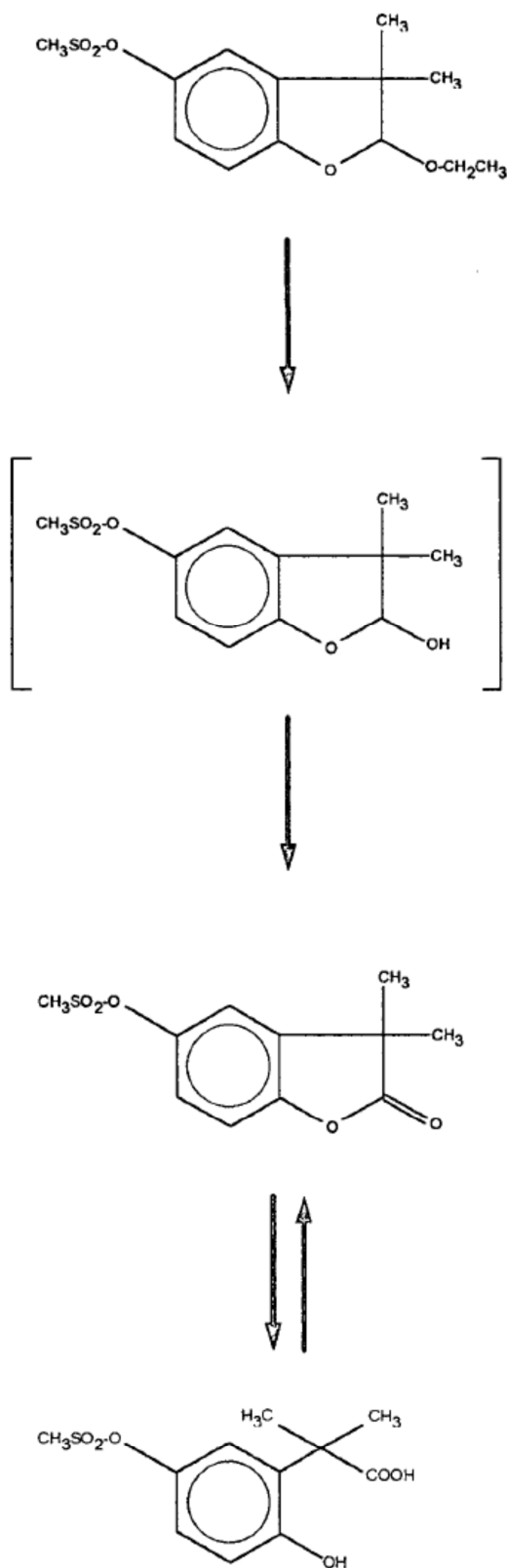
**Table B.6.1.1-4: Radioactivity distribution in organs (mean of three females per timepoint)**

Time post application	% of applied radioactivity (per gram organ)							
	Blood	Liver	Kidneys	Fat	Heart	Brain	Spinal cord	Ovaries
1 hour	0.03	0.06	0.29	0.25	0.04	0.03	0.05	0.07
4 hours	0.02	0.06	0.15	0.35	0.02	0.01	0.01	0.04
24 hours	0.00	0.01	0.01	0.04	0.00	0.00	0.00	0.00
7 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

No parent compound could be detected in the urine sampled during the first 24 hours after intravenous administration of ethofumesate to group IV.

The major urine metabolite (93% - 97% of total radioactivity) was identified as ethofumesate-carboxylic acid, and the minor urine metabolite (0-3%) was found to be ethofumesate-lactone. There was a third (minor) metabolite but it could not be extracted from the TLC. The study author suggested that this metabolite might be most likely a conjugate of ethofumesate-carboxylic acid as it could be deliberated from the plate by applying acid containing eluent only.

Based on the results of the study, following metabolic pathway of ethofumesate in rat was proposed:





## Conclusion

When administered to rats ethofumesate is rapidly absorbed and metabolised. Elimination, mainly via urine, is almost complete within 7 days of the dose administration. The highest amounts of radioactivity were found in the urine of the first 24 h period. Amount excreted via faeces increased with increasing oral doses. After intravenous administration males excreted more via faeces than females but the total excretion was for both sexes comparable. There were no signs of accumulation of ethofumesate in the animals. Seven days after application a very small fraction of radioactivity (< 0.01% of the dose/gram organ) was still detectable only in fat. The major urine metabolite (93% - 97% of total radioactivity) was identified as ethofumesate-carboxylic acid, and the minor urine metabolite (0-3%) was found to be ethofumesate-lactone.

<b>Reference:</b>	The metabolism of $^{14}\text{C}$ -ethofumesate in rats
Author(s), year:	██████████ 1992
Report/Doc. number:	TOX/92/136-133 / M-155244-01-1
Guideline(s):	OECD 417 (1984)
GLP:	Yes
Deviations from OECD 417 (2010):	Minor (partially) reporting bias: - tissue to plasma (blood) ratio not reported
Acceptability:	Yes (no exact figure for metabolite identification (% of recovered radioactivity))

## Material and methods

The extent of absorption and rates and routes of excretion and the distribution of radioactivity in the tissues were studied in rats following oral administration.

Rats (Sprague-Dawley, CrI:CD<sup>®</sup>Br) were randomly assigned to study groups (5/sex/group). The rats were 5 week (males) and 9 weeks (females) old, with the weight range of 180-220 grams. The  $^{14}\text{C}$  test material was labelled on the phenyl ring.

$^{14}\text{C}$ -ethofumesate (radiochemical purity > 98%, measured in TLC.) was administered according to the following scheme:

### Group I: single oral low dose: 10mg/kg bw,

- administered dose volume: 0.5 mL
- ethofumesate diluted with ethanol and suspended in 1% carboxymethylcellulose (CMC)

### Group II : single oral high dose : 500mg/kg bw

- administered dose volume: 0.5 mL
- ethofumesate diluted with ethanol and suspended in 1% carboxymethylcellulose (CMC)
- based on low recovery of administered radioactivity in these animals further 10 animals (5 males, 5 females) were dosed and excreta and tissues collected

### Group III : single oral dose at 10 mg $^{14}\text{C}$ -ethofumesate /kg bw after 14 daily doses at 10 mg/kg bw with unlabelled ethofumesate

- administered dose volume: 0.5 mL
- ethofumesate diluted with ethanol and suspended in 1% carboxymethylcellulose (CMC)
- based on low recovery of administered radioactivity in these animals further 4 animals (2 males, 2 females) were dosed and excreta and tissues collected

Sample collection times:

Urine: 0-6, 6-24 and 24-hour intervals during 5 days

Faeces: 24-hours intervals during 5 days

Expired air: not measured in the main study since less than 2% of the total radioactivity was measured in the traps in the pilot study with administration of 500 mg/kg bw

At 120 hours after dosing the animals were anaesthetised with halothane and a blood sample withdrawn by cardiac puncture, a portion of which was taken for analysis (plasma as well). The rats were sacrificed by cervical dislocation. Liver, kidneys, brain, lungs, spleen, heart, gonads, gastro-intestinal tract and samples of bone, muscle and fat were analysed for radioactivity.

Radioactivity was measured by liquid scintillation counting. For metabolites profiling urine and faecal samples were run on TLC in A) toluene: ethyl acetate 3:1 v/v solvent system, D) dichloromethane solvent system E) toluene : ethyl acetate : acetic acid : water (25.50:20:3 v/V) solvent system and analysed. Urine and faecal metabolites were resolved and confirmed by HPLC.

## Results

The excretion results show that oral doses of  $^{14}\text{C}$ -ethofumesate were quickly absorbed and eliminated (mainly via urine) at both high and low dose levels and after repeat dosing with unlabelled ethofumesate followed by a single oral dose of  $^{14}\text{C}$ -ethofumesate. The urinary and faecal radioactivity was mainly excreted during 0-6 hours. The results indicated that ethofumesate was absorbed to approximately the same extent at the low and high dose levels and therefore, that absorption was not dose-dependent. The results obtained from the repeated dose study indicated that repeated daily dosing for 14 days with unlabelled ethofumesate had no significant effect on the absorption and distribution of a subsequent single radiolabelled dose of ethofumesate.

**Table B.6.1.1-5: Excretion and retention of radioactivity during 5 days post dosing, mean values (% dose)**

	<b>Group I</b> single oral low dose 10 mg/kg bw		<b>Group II</b> single oral high dose 500 mg/kg bw		<b>Group III</b> single oral dose at 10 mg $^{14}\text{C}$ -ethofumesate /kg bw after 14 daily doses at 10 mg/kg bw (unlabelled material)	
	males	females	males	females	males	females
<b>Tissues</b>	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>

Urine (0-6 h)	72.6	78.0	45.4	43.7	64.5	74.7
Urine (6-24 h)	8.1	7.8	22.6	32.4	14.6	14.8
Urine (24-48 h)	0.9	1.7	1.2	2.4	1.1	1.3
Urine (48-72 h)	0.4	0.7	0.6	0.8	0.5	0.4
Urine (72-96 h)	0.2	0.4	0.3	0.6	0.3	0.3
Urine (96-120 h)	0.1	0.3	0.2	0.2	0.2	0.2
<b>Total urine</b>	<b>82.4</b>	<b>88.9</b>	<b>70.4</b>	<b>80.2</b>	<b>81.3</b>	<b>91.7</b>
<b>Cage wash</b>	<b>0.2</b>	<b>0.5</b>	<b>0.4</b>	<b>0.4</b>	<b>0.3</b>	<b>0.2</b>
Faeces (0 – 24 h)	12.2	4.6	25.6	13.7	14.2	3.9
Faeces (24 – 48 h)	0.8	1.4	1.6	2.7	1.4	1.5
Faeces (48 – 72 h)	0.2	0.2	0.2	0.5	0.3	0.2
Faeces (72 – 96 h)	0.1	0.1	0.1	0.1	0.1	0.1
Faeces (96 – 120 h)	0.1	0.1	0.1	0.1	0.1	< 0.1
<b>Total faeces</b>	<b>13.3</b>	<b>6.2</b>	<b>27.6</b>	<b>17.1</b>	<b>16.1</b>	<b>5.8</b>
<b>Total recovery</b>	<b>96.0</b>	<b>95.7</b>	<b>98.6</b>	<b>97.8</b>	<b>97.9</b>	<b>97.9</b>

Tissues with the highest concentrations of radioactivity (above limit of detection), five days after termination of dosing at all three dose regimes were liver, gastro-intestinal tract and carcass, however, the amount of radioactivity in tissues was very low.

**Table B.6.1.1-6: Amounts of radioactivity in tissues (µg ethofumesate equivalents/g)**

	<b>Group I</b> single oral low dose 10 mg/kg bw		<b>Group II</b> single oral high dose 500 mg/kg bw		<b>Group III</b> single oral dose at 10 mg <sup>14</sup> C-ethofumesate /kg bw after 14 daily doses at 10 mg/kg bw	
	males	females	males	females	males	females
Muscle	< 0.011	< 0.011	< 0.305	< 0.304	< 0.009	< 0.010
Fat	< 0.011	0.016	< 0.336	0.382	< 0.010	< 0.010
Spleen	0.009	< 0.012	0.216	0.271	< 0.007	< 0.010
Brain	< 0.011	0.072	< 0.304	< 0.302	< 0.009	< 0.010
Lungs	< 0.012	0.013	< 0.305	< 0.310	< 0.010	< 0.010
Ovaries	-	< 0.027	-	< 0.452	-	< 0.019
Testes	< 0.011	-	< 0.307	-	< 0.010	-
Bone	< 0.007	< 0.009	0.232	0.247	0.005	0.006
Heart	< 0.011	0.018	< 0.307	< 0.305	< 0.010	< 0.010
Whole	0.005	< 0.005	0.665	0.703	0.006	0.006

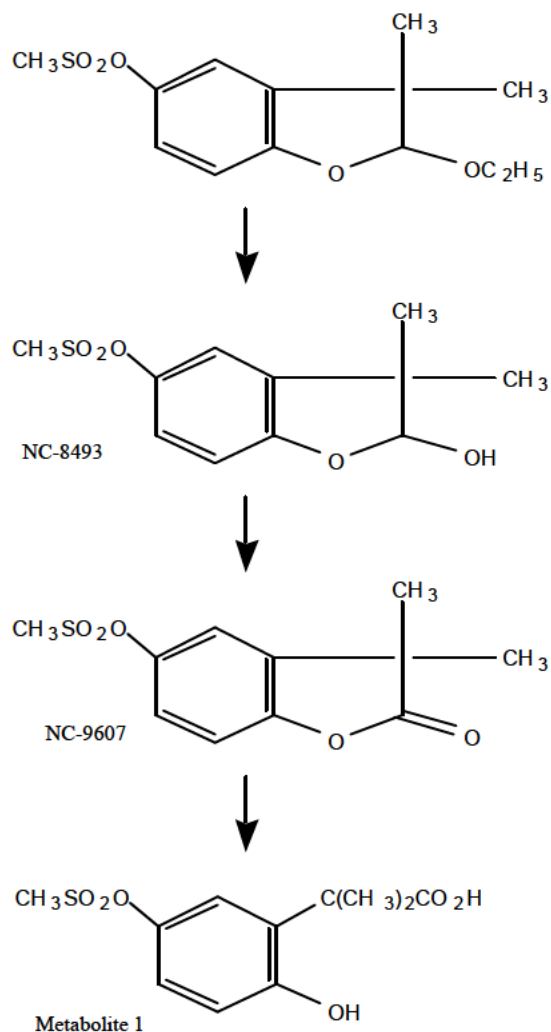
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blood						
Plasma	0.005	< 0.004	< 0.115	0.167	0.004	< 0.004
Kidneys	< 0.011	0.012	0.506	0.542	0.012	0.010
Liver	0.015	0.007	1.5	0.891	0.026	0.011
GI tract	0.045	0.066	7.43	4.32	0.216	0.124
Carcass	0.008	0.018	< 0.343	0.753	< 0.010	0.013

The “major component” in the urine at all dose levels accounted for approximately 74% of the dose (TLC). Unchanged ethofumesate accounted for less than 1% of the dose. In the faecal extracts of the low dose and repeated low dose animals the “major component” was the same as for the urine. In high dose faecal extracts the “major component” was unchanged ethofumesate.  $\beta$ -glucuronidase treatment had no effect on the number or proportion of radioactive components in the urine or faecal extracts. The “major component” in urine (low and high dose animals) and faeces (low dose) was further identified by GC-MS to be ethofumesate-carboxylic acid. Minor identified metabolites (urine and faeces) were ethofumesate-lactone and ethofumesate-2-hydroxy. Based on the different subsequent steps in identification procedure no exact amount of ethofumesate-lactone and ethofumesate-carboxylic acid in excreta was reported.

The following metabolic pathway was proposed in the study:

**Based on the results of the study, following metabolic pathway of ethofumesate in rat was proposed:**



### Conclusion

In the rat, ethofumesate is rapidly absorbed, metabolised and excreted, predominantly in the urine during 0-6h. Tissues with the highest concentrations of radioactivity (above limit of detection), five days after termination of dosing at all three dose regimes were liver, gastro-intestinal tract and carcass, however, the amount of radioactivity in tissues was very low. Unchanged ethofumesate was the major compound of the high dose group (faeces) only. For low dose animals (urine and faeces), repeated dose animals (urine and faeces) as well as high dose animals (urine) ethofumesate-carboxylic acid was identified as major metabolite (exact figure in % of recovered radioactivity not given). Minor metabolites were shown as ethofumesate-lactone and ethofumesate-2-hydroxy.

<b>Reference:</b>	Further mammalian metabolism studies on NC 8438
<b>Author(s), year:</b>	1974
<b>Report/Doc. number:</b>	A82951 / M-155228-01-1
<b>Guideline(s):</b>	No OECD Guideline available 1974
<b>GLP:</b>	No (the study predates the development of Good Laboratory Practices)
<b>Deviations:</b>	Study not conducted according to OECD 417 (1984, 2010)
<b>Acceptability:</b>	Supporting information

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## Material and methods

The study was conducted in rats and cows in order to estimate absorption and metabolism in rats and accumulation in tissues in cow. Only the rat part of the study is included here in the evaluation.

Male rats (Wistar Albino rats) were randomly assigned to study groups (3 / group for oral, intravenous and biliary excretion and 1 animal for the incubation of ethofumesate with stomach contents). The rats had the weight range from 200-300 grams.

<sup>35</sup>S-ethofumesate (no radiochemical purity stated) was administered according to the following scheme:

### **Group I: single oral dose: 30 mg/kg bw (3 animals)**

- ethofumesate suspended in 1% mucilage of tragacanth

### **Group II : single intravenous dose : 3 mg/kg bw (3 animals)**

- ethofumesate dissolved in glycerol and water

### **Group III : single oral dose for bile excretion: two animals 30 mg/kg bw and one animal 10 mg/kg bw**

- ethofumesate suspended in 1% mucilage of tragacanth (for 30 mg/kg bw group)

- ethofumesate dissolved in glycerol (for 10 mg/kg bw animal)

### **Group IV : incubation of ethofumesate with stomach contents (1 animal)**

- 75 µg of <sup>35</sup>S-ethofumesate dissolved in 50 µl glycerol

- three samples from one male rat incubated for 1, 2 or 3 hours

Sample collection times (groups I, II and III):

Blood and plasma (from the retro-orbital sinus) after oral dose: 30 minutes, 1, 2, 3, 4, 5, 6 and 8 hours after dosing

Blood and plasma (from the retro-orbital sinus) after intravenous dose: 2, 5, 10, 20, 30 minutes and 1, 2 and 3 hours after dosing

Bile: hourly, for up to 7 hours

Radioactivity was measured by liquid scintillation counting. For metabolites profiling (1 animal of group III; 30 mg/kg bw) the samples were analysed by TLC (no solvent system specified in the study).

## Results

Ethofumesate was rapidly absorbed following the oral administration to rats, with maximum blood levels occurring after 1 hour. According to the study author, ethofumesate was rapidly and almost completely

metabolised to ethofumesate-carboxylic acid within 2 hours of absorption. The terminal plasma half-life of ethofumesate-carboxylic acid was calculated as 1.6 hours in the rat.

Between 7-9% of the applied radioactivity was excreted within 7 hours post-dosing in the bile from three cannulated rats (no dose-dependent difference in amount of bile excretion). Determination of metabolites from the bile of one male rat (30 mg/kg bw dose) showed that main metabolites were ethofumesate-carboxylic acid (35.7% - 47.6% of recovered radioactivity) and ethofumesate-2-hydroxy-conjugate (49.1% – 61.5%).

No significant breakdown of ethofumesate to metabolites occurred in the presence of stomach content for up to 3 hours.

### Conclusion

The study is considered to be only of supporting information, based on many deviations from the OECD guideline, not available at the time the study was conducted. However, the study gives supporting evidence that the ethofumesate is rapidly absorbed and that the main metabolite is ethofumesate-carboxylic acid. Additionally, the study gives supporting evidence that a portion of radioactivity excreted via faeces (as estimated in the above evaluated OECD guideline conform studies) was absorbed and excreted via bile.

<b>Reference:</b>	Investigation of residue accumulation in the rat following repeated administration of $^{14}\text{C}$ ethofumesate
Author(s), year:	██████████ 1977
Report/Doc. number:	A82962 / M-155239-01-1
Guideline(s):	No OECD Guideline available 1977
GLP:	No (the study predates the development of Good Laboratory Practices)
Deviations:	Study not conducted according to OECD 417 (1984, 2010)
Acceptability:	Supporting information

### Material and methods

The study was conducted in rats to determine whether residues accumulate in tissues following repeated administration of  $^{14}\text{C}$  ethofumesate.

Six male and six female rats (ca. 100 g body weight, CFY - remote Sprague-Dawley) were fed on a diet containing 200 ppm (approximately 8 mg/kg bw)  $^{14}\text{C}$ -ethofumesate (radiochemical purity 98%) for ten days. Two male and two female animals served as control. At the end of the treatment period, the rats were fed on an untreated diet until the excretion of radioactivity in the urine was complete (6 days). The animals were then sacrificed and samples of the brain, muscle, liver, kidney and mobile fat were taken for analysis.

Sample collection times:

Urine and faeces: “at regular intervals” (not specified)

Radioactivity was measured by liquid scintillation counting. For metabolites profiling urine samples were analysed by TLC using 4 different solvent systems: 1) toluene – ethyl acetate – acetic acid – water (25:50:20:3), 2) methanol, 3) toluene – ethyl acetate (4:1) and 4) dichloromethane.

## Results

Residues at the limit of detection of 0.05 ppm were present in the muscle samples from two rats and the kidney samples from three rats. In the samples of liver from nine rats, residues of up to 0.15 ppm were detected. In all other samples, the residues were below the limit of detection (0.25 ppm equivalent to  $^{14}\text{C}$ -ethofumesate for fat; 0.05 ppm for all other tissues).

The major metabolite accounting for >90% of recovered radioactivity in urine was shown to be ethofumesate-carboxylic acid. A smaller amount of ethofumesate lactone was also identified.

## Conclusion

The study is considered to be only of supporting information, based on many deviations from the OECD guideline, not available at the time the study was conducted. However, the study gives supporting evidence that the ethofumesate main metabolite in urine is ethofumesate-carboxylic acid. Additionally, the study gives supporting evidence that there is no accumulation of ethofumesate residues in selected tissues after repeated dosing on 10 subsequent days.

Two additional ADME studies, conducted in dogs (██████████ 1977) and hamsters (██████████, 1977) were also evaluated in the original DAR. In the DRAR some additional information from the study reports was added but as for rat studies, no deviating conclusions in comparison to DAR were drawn.

<b>Reference:</b>	The pharmacokinetics and metabolism of $^{14}\text{C}$ -ethofumesate in the dog
Author(s), year:	██████████ 1977
Report/Doc. number:	A82960 / M-155237-01-1
Guideline(s):	No OECD Guideline available 1977
GLP:	No (the study predates the development of Good Laboratory Practices)
Deviations:	Study not conducted according to OECD 417 (1984, 2010)
Acceptability:	Supporting information

## Material and methods

Dogs (Beagle) were randomly assigned to study groups (1/sex/group).  $^{14}\text{C}$ -ethofumesate was administered as single oral doses (gelatine capsule) at 10, 50 or 250 mg/kg bw. Blood (1/2, 1, 2, 3, 4, 6, 8, 10 and 12 hours post dosing), urine and faeces were collected at different intervals for up to 48 hours and analysed. Total radioactivity of samples was measured using LSC. Ethofumesate in plasma was analysed using GC-MS. Urine and faeces were examined for metabolite identification by TLC using different solvent systems.

## Results

The fraction of the ethofumesate absorbed decreased with the increase in the dose level, decreasing from 84% at 10 mg/kg bw to 62% at 250 mg/kg as measured in urine. Faecal excretion increased from 10 mg/kg bw (11%) to 250 mg/kg bw (28.6%). Elimination of the administered dose in the urine and faeces was almost complete within 24 hours for all the dose levels examined. The main route of elimination was in the urine. Only



unmetabolised ethofumesate was detected in the faeces, indicating a reduction in the amount of ethofumesate absorbed at high dose levels rather than an increase in biliary excretion. .

Once absorbed, ethofumesate was very rapidly metabolised. Only very low levels of ethofumesate could be detected in the plasma (less than 0.1% of circulating radioactivity), even before absorption was complete. This suggests that a very high proportion of ethofumesate is metabolically converted on its initial pass through the liver.

The metabolites were rapidly cleared from the plasma. The plasma half-life for total radioactivity was neither sex nor dose dependent and ranged from 2.0-2.6 hours. No reduction in the whole body clearance of radioactivity was observed with an increase in the dose rate. These calculations indicate that no major changes in pharmacokinetics occurred with dose levels of up to 250 mg/kg.

Ethofumesate-carboxylic acid and ethofumesate-2-hydroxy-glucuronide accounted for over 90% of the radioactive material detected in the urine with ethofumesate-carboxylic acid being slightly more abundant. The proportions of metabolites found were neither dose nor sex dependent.

## Conclusion

The results of absorption, metabolism and excretion study in dogs (1 animal per group and dose) indicate the comparable picture to results from rat studies. The main route of excretion was via urine and excretion was almost complete within 24 hours for all the dose levels examined. The increase in faecal excretion of radioactivity with increasing doses was observed in rat studies as well indicating a reduction in the amount of ethofumesate absorbed at high dose levels. Once absorbed, ethofumesate was very rapidly metabolised also in dogs. The plasma half-life for total radioactivity was neither sex nor dose dependent and ranged from 2.0-2.6 hours in dogs. Ethofumesate-carboxylic acid and ethofumesate-2-hydroxy-glucuronide accounted for over 90% of the radioactive material detected in the urine with Ethofumesate-carboxylic acid being slightly more abundant.

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<b>Reference:</b>	The metabolism of <sup>14</sup> C ethofumesate in the hamster
Author(s), year:	1977
Report/Doc. number:	A82961 / M-155238-01-1
Guideline(s):	No OECD Guideline available 1977
GLP:	No (the study predates the development of Good Laboratory Practices)
Deviations:	Study not conducted according to OECD 417 (1984, 2010)
Acceptability:	Supporting information

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## Material and methods

Male Syrian hamsters (ca. 100 g bw) were randomly assigned to study groups (4/group). <sup>14</sup>C-ethofumesate (radiochemical purity > 95%) was administered as single oral doses at 20, 100 or 500mg/kg bw. Urine and faeces were collected after 24 and 48 hours and analysed. Radioactivity was measured by liquid scintillation counting. For metabolites profiling the samples were analysed by TLC using toluene : ethyl acetate : ethanol: acetic acid (80:10:5:0.5) solvent systems:

## Results

Ethofumesate was well absorbed by the hamster, between 80-99% of the administered radioactivity was eliminated in the urine within the first 24 hours after dosing. Excretion in the faeces was minor (<3%). The rate and route of elimination were not dose dependent within the range of the doses examined.

Over 90% of the radioactivity eliminated in the urine in the first 24 hours was present as ethofumesate-carboxylic acid. Small amounts of ethofumesate-2-hydroxy and an unidentified component were detected, probably occurring as conjugates. The metabolism of ethofumesate was not dose dependent within the range of the doses examined.

### Conclusion

The results support the conclusion that following oral administration of ethofumesate most of the substance is rapidly excreted. As for rats and dogs, excretion via urine was the main route of elimination. The main metabolite in urine (> 90%) was ethofumesate-carboxylic acid, as minor metabolite ethofumesate-carboxylic acid was identified.

#### B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

Several ADME studies (■■■■■ 1974; ■■■■■ 1991; ■■■■■ 1993; ■■■■■ 1994) were conducted also including intravenous route. These studies are described under B.6.1.1 since the investigations on oral route were always done in parallel.

#### B.6.1.3. Other ADME studies

Notifier TaskForce submitted two *in vitro* studies in order to identify if any unique human metabolite is built. No study on this new data requirement was provided by the notifier UPL. For this kind of studies no OECD Guideline is available at present.

Since according to *Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) 283/2013 and Regulation (EU) No 284/2013* (SANCO/10181/2013–rev. 2, May 2013) it is stated that “*In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02*”, these studies were included in the DRAR as supplementary information only. The waiver from the notifier UPL is considered acceptable.

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	rats and humans for inter-species comparison
Author(s), year:	██████ 2013
Report/Doc. number:	S45316 / M-471058-01-1
Guideline(s):	No OECD Guideline available (US EPA OCSPP 870.SUPP;not specified)
GLP:	Yes
Deviations:	-
Acceptability:	Yes (supplementary information)

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## Executive Summary

The comparative metabolism of [Phenyl-UL-<sup>14</sup>C]Ethofumesate (<sup>14</sup>C-Ethofumesate) was investigated in animal *in-vitro* systems by incubating the test item with liver microsomes from male Wistar rats (RLM) and humans (HLM) in the presence of NADPH cofactor. The test item concentration was 15 µM and the protein concentration 1 mg/mL. The 15 µM test item concentration was chosen in order to have enough sample material for possible identification of metabolites by chromatographic or spectroscopic methods. The sampling times were 0 and 1 hour after test start. The test duration of 1 hour for the test item was considered as reasonable because positive results were obtained from the enzymatic reaction of Testosterone to Hydroxy-Testosterone already after 10 minutes. Samples were analyzed following protein precipitation by reversed phase HPLC with radiochemical detection (HPLC-RAD).

The recovery of radioactivity was measured in the microsome incubations and amounted to >95.9% for the 1 hour samples.

The metabolic activity of the microsomes was clearly demonstrated by determining 6β-hydroxytestosterone that was formed from testosterone by testosterone 6β-hydroxylase. This biochemical reaction is a well-known marker for the CYP3A microsomal enzyme.

The results of the tests indicated that the *in-vitro* metabolism of <sup>14</sup>C-Ethofumesate when incubated with liver microsomes was different between rats and humans.

In rat microsomal incubations, <sup>14</sup>C-Ethofumesate was totally transformed towards a single metabolite, namely metabolite E-1 (ethofumesate-carboxylic acid).

The human microsomal incubations produced three further metabolites in addition to E-1 (ethofumesate-carboxylic acid), namely E-2 (ethofumesate-2-hydroxy), E-3 (not determined) and E-4 (ethofumesate-lactone). Metabolites ethofumesate-carboxylic acid, ethofumesate-2-hydroxy and ethofumesate-lactone accounted for 17.2%, 74.2% and 8.6% of the relative percentage (calculated from peak area values), respectively. Metabolite E-3 was only detected in trace amounts below the lower limit of quantitation.

## Test system

Pooled liver microsomes from male Wistar rats (RLM, batch 1010126, pool of 200 individuals) and humans (HLM, batch 1110189, pool of 50 donors from both genders) were purchased from Xenotech, LLC (USA).

### Sample Preparation and Incubation

<sup>14</sup>C-Ethofumesate was incubated separately with RLM and HLM (n=3) at 37 ± 1°C in a final volume of 500 µL. Incubations were performed in a thermomixer (Eppendorf) with shaking at 1000 rpm. The final incubation volume was 500 µL.

### Sample Processing for Analysis

The microsomal incubates were centrifuged at approximately 16.000 x g for 15 minutes at ca. 20 °C. After centrifugation, 150 µL of each supernatant were diluted with 225 µL of HPLC mobile phase A. The samples were directly analysed by HPLC-RAD without any further extraction procedure.

### Conclusion

From the results of the present study, the following conclusions can be drawn:

- The *in-vitro* metabolite profile of <sup>14</sup>C-Ethofumesate when incubated with liver microsomes was different between rats and humans.
- In rat microsomal incubations, <sup>14</sup>C-Ethofumesate was totally transformed towards ethofumesate-carboxylic acid. The human microsomal incubations produced beside ethofumesate-carboxylic acid two further main metabolites (ethofumesate-2-hydroxy and ethofumesate-lactone).
- The abundance of metabolites ethofumesate-2-hydroxy and ethofumesate-lactone could suggest an important role for the formation of these metabolites in the overall disposition of ethofumesate in human microsomes that is not likely present in rat microsomes.

<b>Reference:</b>	[Phenyl-UL-14C]ethofumesate: Isolation and identification of metabolite(s) from an in-vitro study with rat and human liver microsomes
Author(s), year:	██████████ 2013
Report/Doc. number:	EnSa-13-0841 / M-469296-01-1
Guideline(s):	No OECD Guideline available (US EPA OCSPP 870.SUPP;not applicable)
GLP:	Yes
Deviations:	-
Acceptability:	Supplementary information

In this subsequent study, parent compound and metabolites ethofumesate-carboxylic-acid, ethofumesate-2-hydroxy and ethofumesate-lactone were identified by spectroscopic methods (LC-MS and GC-MS) in the incubations with RLM and HLM.

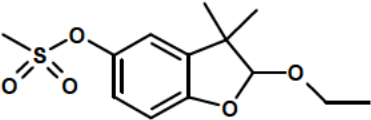
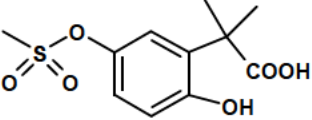
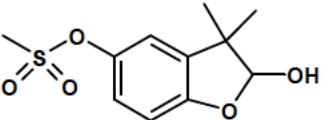
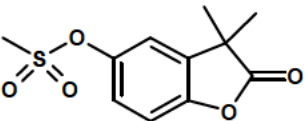
### Sample Preparation

The samples of the tests with **rat** liver microsomes (see previous study) from time zero (R-0-1, R-0-2, R-0-3) and time 60 min incubations (R-60-1, R-60-2, R-60-3) were thawed, centrifuged and afterwards combined to one

sample each (sample ID's: LAGO0367A, time 0 min; LAGO0367B, time 60 min). Aliquots thereof were taken for spectroscopic investigations.

The samples of the tests with **human** liver microsomes (see previous study) from time zero (H-0-1, H-0-2, H-0-3) and time 60 min incubations (H-60-1, H-60-2, H-60-3) were thawed, centrifuged and afterwards combined to one sample each (sample ID's: LAGO0367C, time 0 min; LAGO0367D, time 60 min). Aliquots thereof were taken for spectroscopic investigations. In sample LAGO0367D, compounds 1 and 2 were examined by LC-MS and compound 3 by GC-MS.

## Results

Chemical structure	Report name	Metabolite ID-no.	Comment
	Ethofumesate	Parent compound	Detected and identified by LC-MS in all time zero incubations with RLM and HLM
	Ethofumesate-carboxylic acid	E-1	Detected and identified by LC-MS as the only radioactive compound in the 60 min incubations with RLM Detected and identified by LC-MS as minor metabolite in the 60 min incubations with HLM
	Ethofumesate-2-hydroxy	E-2	Detected and identified by LC-MS as major metabolite in the 60 min incubations with HLM
	Ethofumesate-lactone	E-4	Detected and identified by GC-MS as the lowest metabolite in the 60 min incubations with HLM

## Conclusion

The observed differences in metabolism between the rat and human liver microsome incubations are presumably due to dissimilarities of phase I biotransformation reactions in the tested species. The activities of the enzymes in rat microsomes were obviously higher than in human microsomes. It can be assumed that shorter incubation periods with rat liver microsomes would lead to a more similar metabolic pattern.

A qualitative different biotransformation pathway between rat and human microsomal in-vitro incubations can therefore be excluded.

### Information on ADME from the scientific peer reviewed open literature search

Both notifiers, TaskForce and UPL, identified two studies (one *in vivo* and one *in vitro* study) on stereo-selective metabolism of ethofumesate and its enantiomers in the scientific peer reviewed open literature which they included in the dossier as supplementary information. Additionally, UPL identified a study on enantioselective metabolism of ethofumesate in rat and chicken hepatocytes which was also included in the dossier.

The RMS concluded that none of the three studies identified in the scientific peer reviewed open literature has any implication on the risk assessment and that their relevance is questionable. Since no major differences in the toxicity profile between rats and rabbits were seen in the regulatory standard toxicology studies and since the toxicology studies are mainly performed in rats as standard species, a relevance of the observation for toxicology is not obvious. Additionally, ethofumesate is a stable racemic mixture and all toxicity studies were conducted with this technical material. No study (not considered necessary) is available in the dossier to prove the toxicity of the single enantiomer.

The studies were included in the DRAR as supplementary information and briefly summarised; however, since no OECD Guideline is available for this kind of studies, no check for reliability was conducted by RMS.

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<b>Reference:</b>	Zhu W, Dang Z, Qiu J, Lv C, Jia G, Li L, Zhou Z. (2007)
<b>Title:</b>	Stereoselective toxicokinetics and tissue distribution of ethofumesate in rabbits
<b>Source:</b>	Chirality, 19, 632-637
<b>Guidelines:</b>	not applicable
<b>GLP/GEP:</b>	no

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### Abstract

The stereo-selective toxicokinetics behaviour of ethofumesate enantiomers following a single intravenous (i.v.) administration at doses of 30 mg/kg were investigated in rabbits. Plasma concentrations of (+)- and (-)-ethofumesate were analysed by a validated chiral HPLC method that involved extraction of plasma with organic solvent followed by separation on a cellulose-tris-(3,5-dimethylphenylcarbamate)-based chiral column and quantification by UV absorbance at 230 nm. Plasma concentration-time curves after i.v. administration were best described by an open two-compartment model. In plasma the concentration of the (-)-enantiomer decreased more rapidly than that of the (+)-enantiomer. Significant differences in toxicokinetic parameters between the two enantiomers indicated a stereo-selective behaviour with the (-)-enantiomer being preferentially metabolized and eliminated. As in the plasma, the concentration of the (+)-enantiomer was higher in kidney, liver and fat. No stereo-selective degradation was observed in lung, heart, muscle and spleen.

### Material and methods

#### **1. Test material**

Test item:	Racemic ethofumesate (1:1)
Active substance(s):	Ethofumesate
Chemical state and description:	Technical grade substance

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Source of test item:	Institute for the Control of Agrochemicals, Ministry of Agriculture (Beijing, China)
Batch number:	None given
Purity:	98%
Storage conditions:	Stock solution in 2-propanol at -20 °C
Water solubility:	Not stated

**2. Vehicle:**

Racemic ethofumesate was dissolved in 10% (w/v) alcohol and then diluted with normal saline

**3. Test animals:**

Species:	Rabbit
Strain:	Japanese white rabbit
Age:	Not given
Sex:	male
Weight at dosing:	2.0-2.25 kg
Source:	Experimental Animal Research Institute of China Agriculture University
Diet:	Rabbits were kept on fast for 12 h before administration
Water:	Free access to water, <i>ad libitum</i>
Housing:	Animals were housed under a 12 h light/12 h dark cycle at 22 °C

**Study design****1. Dosing and sample collection**

Racemic ethofumesate was administered at 30 mg/kg body weight by intravenous (i.v.) injection in the ear vein. Plasma samples were collected and stored immediately in heparinized tubes at 5, 15, 30, 60, 90, 120, and 240 min after administration, each data point is the mean of six replicates. Blank blood samples were collected before drug administration. After blood sample collection, the animals were killed at 5, 15, 30, 60, 90, 120, and 240 min after being anesthetized. The heart, kidney, liver, lung, fat, muscle, spleen, and brain of each rabbit were excised and weighed separately. Plasma and tissue samples were stored at -80 °C for later analysis.

**2. Preparation of samples**

Aliquots (1 mL) of the rabbit plasma or of the homogenized tissue matrix (1 g) were weighed into a 15 mL polypropylene centrifuge tube. About 5 mL of ethyl acetate was added, and the sample was vortexed for 5 min. After centrifugation at 4000 rpm for 5 min, the clear solution was decanted into a test tube. The extraction and centrifuge steps were repeated with another 5 mL of ethyl acetate. The organic phases were combined and evaporated to dryness under a stream of nitrogen, the residue of the fat was dissolved in 4 mL acetonitrile, then partitioned three times with hexane, and the acetonitrile phase was evaporated to dryness under a stream of nitrogen. The resulting residue was redissolved in 0.2 mL of 2-propanol, and this solution was vortexed. A 20-μL aliquot was injected into the HPLC.

**3. Toxicokinetic analysis**

Individual toxicokinetic parameters of ethofumesate enantiomers were determined using standard compartmental analysis methods and calculated with the Drug and Statistics computer program (Section of Quantitative Pharmacology, Chinese Pharmacological Society). An open two-compartment model best described the plasma concentration versus time data for both enantiomers after i.v. administration based on the Akaike's Information

Criterion (AIC). For the estimation of toxicokinetic parameters, distribution and elimination half-lives ( $t_{1/2\alpha}$  and  $t_{1/2\beta}$ ), the plasma clearance (CL) was calculated according to standard pharmacokinetic equations. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule. Mean residence time (MRT) was calculated by dividing the area under first-moment curve AUMC by AUC. The enantiomeric ratio (= enantiomer fraction EF) was used as a measure of the enantio-selectivity of the two isomers in animal kinetic analysis. The EF is defined by equation 1.

$$EF = \frac{(+)}{(+)+(-)} \quad (1)$$

where (+) and (-) are the concentrations of the first eluted (+)- and the second eluted (-)-enantiomer according to the determination result of polarimeter in previous work. The EF for a racemate is 0.5 [(+) = (-)]. A paired *t*-test was used to test the significance of stereo-selective differences in toxicokinetic parameters.

#### 4. Analytical methods

Samples were analysed by HPLC-UV. Enantiomers were separated on a cellulose-tris(3,5-dimethylphenylcarbamate) chiral stationary phase (CDMPC-CSP; provided by the Department of Applied Chemistry, China Agricultural University, Beijing). The chromatographic separation was conducted at room temperature. The mobile phase was made up of 95% hexane and 5% 2-propanol with a flow rate of 1.0 mL/min. The eluates were monitored at 230 nm.

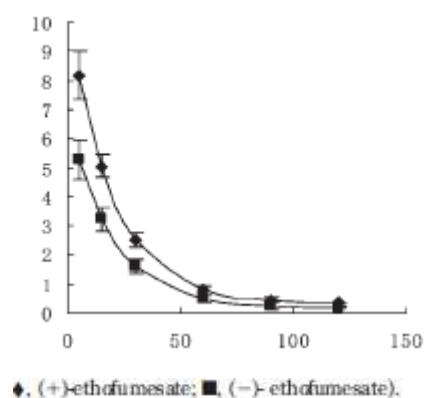
Calibration samples were prepared by spiking control plasma samples (1 mL) with working standards of racemic ethofumesate (*rac*-ethofumesate). Linear calibration curves were obtained over the concentration range of 0.20–25 µg/mL for both (+)-ethofumesate ( $y = 125.01x + 14.5950$ ,  $R^2 = 0.9983$ ) and (-)-ethofumesate ( $y = 124.96x + 8.9545$ ,  $R^2 = 0.9991$ ). The accuracy and precision of the assay for both enantiomers are suitable with the coefficients of variation (CV) from 2.7% to 11.2%. Recoveries of each enantiomer fortified at 0.25, 2.5, and 10.0 µg/mL ranged from  $(89.1 \pm 2.8)\%$  to  $(98.1 \pm 6.8)\%$ . The LOD was 0.025 µg/mL and the LOQ was 0.1 µg/mL plasma.

## Results

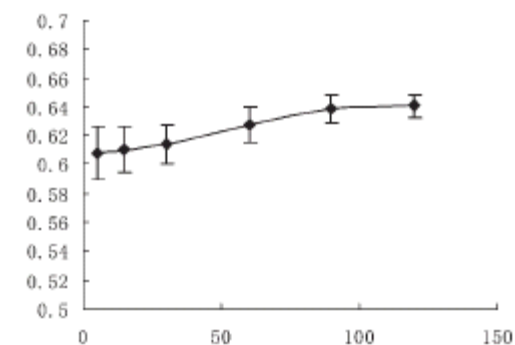
### A. Toxicokinetics in rabbit plasma

Plasma concentration-time curves of (+)- and (-)-ethofumesate after i.v. administration of 30 mg/kg of racemic ethofumesate to the rabbits are shown in figure below (concentration vs. time in min). The mean plasma concentrations of the two enantiomers were different from each other at each time point. The plasma concentration of the (-)-ethofumesate was significantly lower than that of its antipode. The preferential disappearance of the (-)-enantiomer from plasma was evident from the chromatograms of plasma extracts at 5 min after administration. Compartmental toxicokinetic analysis and *t*-test results showed significant differences between the principal toxicokinetic parameters of the two enantiomers (Table below).





**Figure 6.1.3-1: Plasma concentration-time curves of ethofumesate enantiomers in rabbits following administration of *rac*-ethofumesate at 30 mg/kg bw**



**Figure 6.1.3-2: EF (= enantiomeric ratio) of ethofumesate enantiomers in rabbits following administration of *rac*-ethofumesate at 30 mg/kg bw**

**Table 6.1.3-1: Toxicokinetic parameters of ethofumesate enantiomers in rabbits following administration of *rac*-ethofumesate at 30 mg/kg bw (n = 6)**

Toxicokinetic parameter	(+)-Ethofumesate	(-)-Ethofumesate
$T_{1/2\alpha}$ (min)	13.53	13.44
$T_{1/2\beta}$ (min)	69.32	69.31
CL (L/min/kg)	0.09	0.14
$AUC_{0-120 \text{ min}}$ (mg/L $\times$ min)	273.15	172.27
$AUC_{0-\infty}$ (mg/L $\times$ min)	333.49	24.56

## B. Toxicokinetics in rabbit tissue

As in the plasma, in liver, kidney and fat the concentration of the (+)-enantiomer was higher than that of its antipode. In the liver, especially the concentration of the (+)-enantiomer was more than 3-fold higher than that of the (-)-enantiomer, while both enantiomers were no longer detectable at 60 min. In the brain, the concentration of the (-)-enantiomer was much higher at earlier time points but was no longer detectable at 90 min. Nevertheless, both enantiomers were detected and thus could penetrate the blood-brain barrier. No obvious stereo-selective degradation was observed in lung, heart, muscle and spleen.

## Conclusion

Toxicokinetics analysis showed evidence of stereo-selective disposition of the two enantiomers of ethofumesate in rabbit. The (-)-enantiomer was preferentially eliminated from plasma compared with its antipode. Also in liver, kidney and fat an increased concentration of the (+)-enantiomer was detected, whereas no obvious stereo-selective degradation was observed in lung, heart, muscle and spleen. The higher concentration of the (+)-enantiomer in the metabolizing organs liver and kidney suggests a preferred degradation of the (-)-enantiomer. In addition, renal excretion may have been stereo-selective, as well – but no data were collected to support this assumption and generally passive processes like absorption, distribution and excretion do not differentiate between enantiomers. Overall, the concentrations of both enantiomers decreased with time and both enantiomers were generally no longer detectable 240 min after the administration, indicating a fast degradation and/or a fast elimination of both enantiomers.

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<b>Reference:</b>	Zhu, W., Dang, Z., Qiu, J. Liu, Y., Lv, C., Diao, J., Zhou, Z (2009)
Title:	Species differences for stereoselective metabolism of ethofumesate and its enantiomers <i>in vitro</i>
Source:	Xenobiotica, 39, 649-655
Guidelines:	not applicable
GLP/GEP:	no

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## Abstract

The stereo-selective metabolism of the enantiomers of ethofumesate in rabbit and rat liver microsomes has been studied by HPLC using a chiral stationary phase (HPLC-CSD). Two metabolites were detected in liver microsomes of rat and rabbit in the presence of  $\beta$ -nicotinamide adenine dinucleotide phosphate (NADPH). A stereo-selective formation was detected in liver microsomes of rabbits, but stereo-selectivity was not evident in liver microsomes of rats. In rabbit microsomes, the half-life of the (+)-enantiomer was significantly higher indicating a preferred depletion of the (-)-enantiomer due to the preferred degradation. This stereo-selectivity was also detected in an *in vivo* study with rabbits.

There was also no chiral inversion from the (+)-ethofumesate to (-)-ethofumesate or inversion from (-)-ethofumesate to (+)-ethofumesate in both rabbit and rat liver microsomes.

## Material and methods

### 1. Test material

Test item:	Racemic ethofumesate (1/1)
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:	$\geq 99\%$
Water solubility:	Not stated

## **2. Test animals for preparation of microsomes:**

Species:	Rabbit and rat
Strain:	Japanese white rabbits and Sprague-Dawley rats
Age:	Not given
Sex:	male
Weight at sacrifice:	2.0-2.25 kg (rabbit) and 200-250 g (rat)
Source:	Experimental Animal Research Institute of China Agriculture University
Acclimation:	1 week
Diet:	Rodent feed, <i>ad libitum</i>
Water:	Water, <i>ad libitum</i>
Housing:	Animals were housed in solid bottom cages with hardwood chips under a 12 h light/12 h dark cycle

## **3. Preparation of microsomes**

- After sacrifice of animals, the liver was removed, blotted, weighed and placed in ice-cold 1.15% KCl solution
- Tissue was minced with scissors and washed with 1.15 KCl solution to remove blood
- After draining of the KCl solution, individual livers were homogenized in ice-cold SET solution (1 mM EDTA and 50 mM Tris-HCl, pH 7.4
- The homogenate was centrifuged at 10 000g for 20 min at 4°C and the pellet was discarded
- The supernatant was centrifuged at 108 000g for 60 min at 4°C The supernatant (cytosol) was decanted
- The pellet was washed with 50 mM Tris-HCl and the homogenate was centrifuged at 108 000g for 60 min at 4°C
- The pellet was re-suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 20% glycerol
- The microsomes were stored at -80 °C until used
- .

## **Study design**

### **1. Microsomal incubation**

*In vitro* substrate-depletion studies (to determine the half-lives of *rac*-ethofumesate and its individual enantiomers) were performed by incubation of *rac*-ethofumesate or its enantiomers with 1.0 mg microsomal protein in 50 mM Tris-HCl buffer (pH 7.4) with 5.0 mM MgCl<sub>2</sub>. Ethofumesate or its enantiomers were prepared in methanol and added to the incubation solutions. The volume of methanol added to each incubate was less than 1.0% v/v. All reaction mixtures were pre-incubated in a heated water bath at 37°C for 5 min before initiation of the reaction with the addition of NADPH at a final reaction concentration of 1.0 mM, the final total reaction volume was 1.0 mL. After incubation in a water bath (37°C) for 5–30 min, the reactions were terminated by adding 5.0 mL of ice-cold diethyl ether to the sample. After centrifugation at 3500 rpm for 5 min, the clear solution was decanted into a test tube. The extraction and centrifugation steps were repeated with another 5 mL of diethyl ether. The organic phase was combined and evaporated to dryness under a stream of nitrogen, the resulting residue was re-dissolved for HPLC analysis.

The formation kinetics of the metabolites were investigated on the basis of ethofumesate-2-hydroxy (NC 8493), the metabolite formed in the first degradation step by desalkylation. 10.0-200 µM of *rac*-ethofumesate or its individual enantiomers were incubated for 20 minutes as described above and analysed by HPLC.

## 2. Analytical procedure

Samples were analysed by HPLC-UV. Enantiomers were separated on a cellulose-tris(3,5-dimethylphenylcarbamate) chiral stationary phase (CDMPC-CSP; provided by the Department of Applied Chemistry, China Agricultural University, Beijing). The chromatographic separation was conducted at room temperature. The mobile phase was made up of 90% hexane and 10% 2-propanol with a flow rate of 1.0 mL/min. The eluates were monitored at 230 nm.

Microsomes (1.0 mg) in 50 mM Tris-HCl buffer (pH 7.4) were spiked with standard solutions of ethofumesate enantiomers or the metabolite. Samples were prepared as described above and calibration curves were generated by plotting the concentration of each enantiomer or of ethofumesate-2-hydroxy (NC8493) in the spiked samples versus the peak area of each compound. Linear regression analysis was performed using Microsoft Excel 2003. The precision and accuracy of the assay were obtained by comparing the predicted concentration (obtained from the calibration curve) with the actual concentration of each enantiomer fortified in drug-free microsomes.

There were no endogenous interference peaks detected at the retention times of the enantiomers of parent or the metabolite in all samples. Linear calibration curves were obtained over the concentration range of 2-200  $\mu\text{M/mL}$  in microsomes for

(+)-ethofumesate ( $y = 35.06x - 39.46$ ,  $R^2 = 0.9985$ ) and  
(-)-ethofumesate ( $y = 35.40x - 43.87$ ,  $R^2 = 0.9987$ ) and  
ethofumesate-2-hydroxy ( $y = 31.03x + 112.72$ ,  $R^2 = 0.9984$ ).

The lowest recovery rate was > 80%. The accuracy and precision of the assay over the entire calibration range can satisfy the requirement of quantitative analysis.

## 3. Data analysis

The degradation of *rac*-ethofumesate or its enantiomers appeared to follow a first-order kinetic reaction, and the degradation rate constants were derived from “ $\ln(C_0/C)$  versus  $t$ ” plots by regression analysis (Excel 2003, Microsoft, Inc.). The starting point was the maximum concentration. The *in vitro* elimination half-life ( $T_{1/2}$ ) was determined by the following equation (Obach 1999):

$$T_{1/2} = 0.693/k \quad (1)$$

The enantiomer fraction (EF) was used as a measure of the enantio-selectivity of the ethofumesate enantiomers *in vitro*:

$$EF = (+)/[(+) + (-)] \quad (2)$$

For the racemate the ratio is  $EF = 0.500$ , whereas preferential degradation of the (+)- or (-)-enantiomer yields an  $EF$  of < 0.500 or > 0.500, respectively.

Non-linear regression of substrate concentration versus reaction velocity curves were analysed using Origin 8.0 software by fitting experimental data to the Michaelis–Menten equation. The formation of a metabolite from ethofumesate enantiomers by rat and rabbit liver microsomes was fitted to equation (3), and the  $K_m$  and  $V_{max}$  values were calculated by the following equation:

$$V_0 = V_{max} \times S / (K_m + S) \quad (3)$$

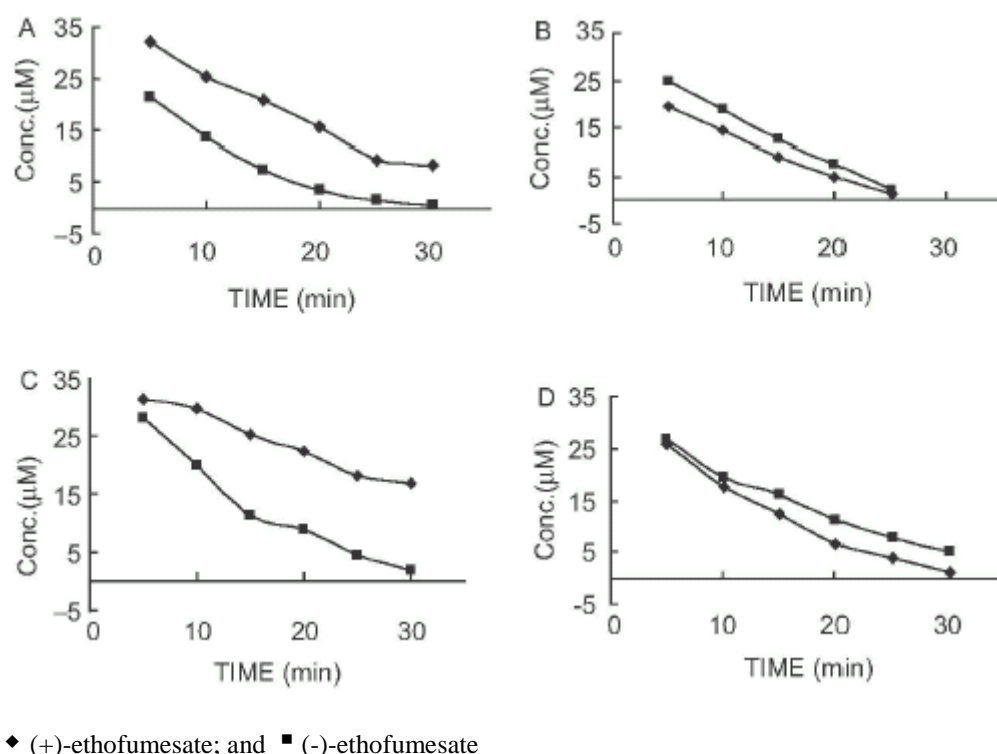
where  $V_0$ ,  $V_{max}$ ,  $S$ , and  $K_m$  represent the initial rate of metabolism, maximum rate of metabolism, substrate concentration, and Michaelis constant, respectively. Intrinsic metabolic clearance ( $CL_{int}$ ) was calculated by the following equation:

$$CL_{int} = V_{max} / K_m \quad (4)$$

## Results

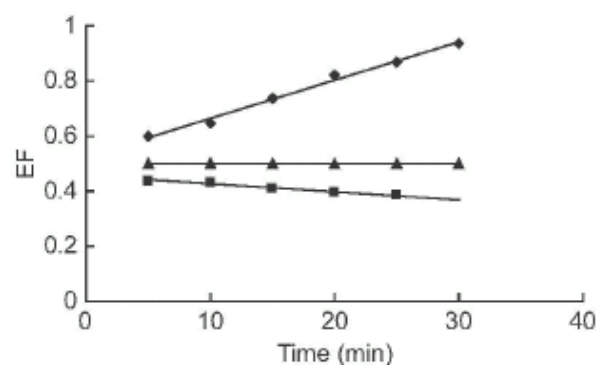
### A. Kinetic degradation in liver microsomes

Liver microsomes concentration time curves of (+)- and (-)-ethofumesate after incubation of liver microsomes of rabbit with *rac*-ethofumesate at 80  $\mu$ M are shown in figure below. The mean concentrations of the two enantiomers were different from each other at each time point. The half-lives ( $T_{1/2}$ ) of (+)-ethofumesate and (-)-ethofumesate were 12.2 and 4.7 min, when evaluated separately. The result indicated that the (-)-ethofumesate degraded faster than its antipode in liver microsomes of rabbit. Results of the incubation with the individual enantiomers (+)- and (-)-ethofumesate, each at 40  $\mu$ M, indicated that similar to *rac*-ethofumesate, the (-)-ethofumesate also degraded faster than its antipode.  $T_{1/2}$  of (+)-ethofumesate and (-)-ethofumesate were 25.9 and 6.7 min, respectively. However, after incubation in liver microsomes of rat with *rac*-ethofumesate at 80  $\mu$ M, the concentration of the (+)-ethofumesate was lower than the one of its antipode. Concentration time curves of (+)- and (-)-ethofumesate after incubation of rat liver microsomes with *rac*-ethofumesate (80  $\mu$ M) or its individual enantiomers (each 40  $\mu$ M) are shown in Figure 6.1.3-3 B and D, respectively. The  $T_{1/2}$  of (+)-ethofumesate and (-)-ethofumesate were 5.3 and 5.9 min after incubation with *rac*-ethofumesate and 7.8 and 10.6 min after incubation with the individual enantiomers. The preferential disappearance of the (-)-enantiomer as determined for the rabbit liver microsomes was not evident for the rat liver microsomes.



**Figure 6.1.3-3: Concentration-time curves of the enantiomers after 20 min incubation of (A) *rac*-ethofumesate (80  $\mu\text{M}$ ) in rabbit liver microsomes, (B) *rac*-ethofumesate (80  $\mu\text{M}$ ) in rat liver microsomes, (C) individual ethofumesate enantiomers (each 40  $\mu\text{M}$ ) in rabbit liver microsomes, and (D) individual ethofumesate enantiomers (each 40  $\mu\text{M}$ ) in rat liver microsomes**

The enantiomer fraction (EF) was 0.50 after incubation of *rac*-ethofumesate in liver microsomes without NADPH. With NADPH, the EFs changed with time. The EF in rabbit liver microsomes increased with time indicating a preferred degradation of (-)-ethofumesate, whereas the EF in rat liver microsomes decreased only slightly with time indicating - more or less - no difference in the degradation of the enantiomers (Figure 6.1.3-4). These results indicated an enantio-selective degradation of ethofumesate in rabbit, but not in rat liver microsomes. Thus, the stereo-selectivity was shown to be different between the two species.

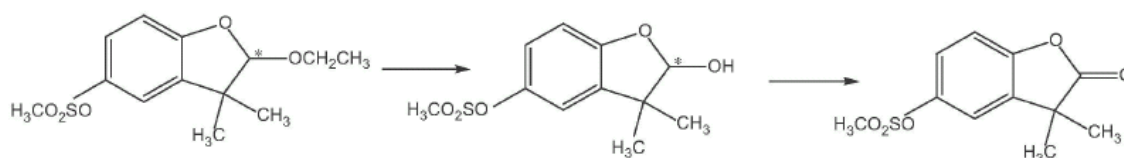


◆ rabbit liver microsomes; ■ rat liver microsomes; ▲ rabbit liver microsomes without NADPH

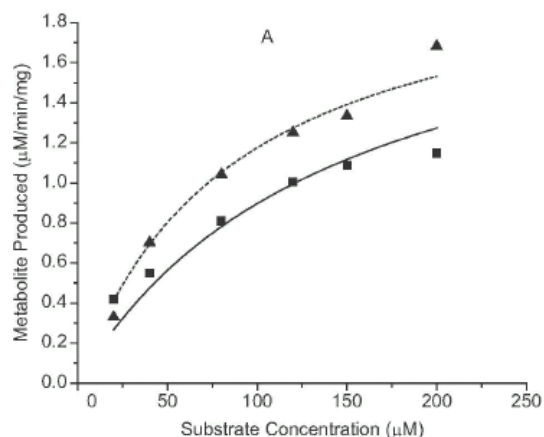
**Figure 6.1.3-4: Enantiomer fraction (EF) of ethofumeste enantiomers in liver microsomes after incubation of *rac*-ethofumesate administration at 40  $\mu$ M**

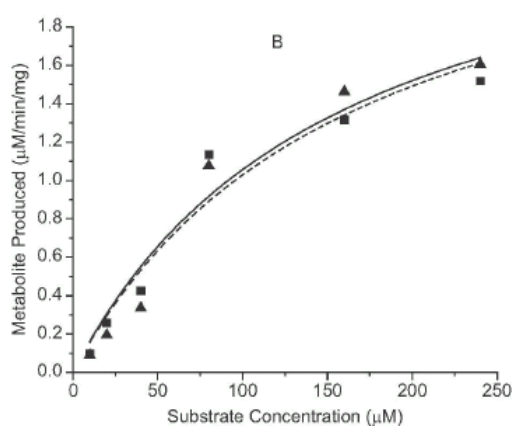
### B. *In vitro* biotransformation and enzyme kinetic analysis

Both ethofumesate enantiomers were transformed by rat or rabbit liver microsomes to two detectable metabolites, ethofumesate-2-hydroxy and ethofumesate-lactone. The metabolites are formed by desalkylation and oxidation (Figure 6.1.3-5). The conversion to ethofumesate-2-hydroxy and ethofumesate-lactone was NADPH-dependent and increased in a time-dependent manner. Metabolic rate constants (apparent  $K_m$  and  $V_{max}$ ) were determined after a 20 min incubation period for species comparison. The formation rates of ethofumesate-2-hydroxy and ethofumesate-lactone increased with increasing concentration of parent ethofumesate. Enantio-selective degradation of ethofumesate to ethofumesate-2-hydroxy was determined in liver microsomes of rabbit, whereas no different degradation of ethofumesate enantiomers was detected in the rat (Figure 6.1.3-6).



**Figure 6.1.3-5: Metabolic degradation of ethofumesate to its major metabolites ethofumesate-2-hydroxy and ethofumesate-lactone in rabbit and rat liver microsomes**





▲ (-)-ethofumesate; ■ (+)-ethofumesate

**Figure 6.1.3-6: Formation rate of ethofumesate-2-hydroxy in rabbit (A) and rat (B) liver microsomes after 20 min incubation of (+) and (-)-ethofumesate administration at 40  $\mu$ M**

**Table 6.1.3-2: Apparent kinetic constants of Ethofumesate-2-hydroxy formation in rabbit and rat in liver microsomes *in vitro***

Toxicokinetic parameter	(+)-Ethofumesate	(-)-Ethofumesate
<b>Rabbit</b>		
$V_{Max}$ (nmol/min/mg protein)	1526	2591
$K_M$ ( $\mu$ M)	64.6	122.6
CL (mL/min/mg protein)	23.6	21.1
<b>Rat</b>		
$V_{Max}$ (nmol/min/mg protein)	2401	3051
$K_M$ ( $\mu$ M)	129.4	193.3
CL (mL/min/mg protein)	18.6	15.8

## Conclusion

In this *in vitro* study, differences were found for the stereo-selective degradation of ethofumesate in the two different species under investigation (rabbit and rat), although the same metabolites (ethofumesate-2-hydroxy and ethofumesate-lactone) were detected as primary degradation products.

In liver microsomes of rabbits, an enantio-selective degradation of ethofumesate was detected. The half-life ( $T_{1/2}$ ) of (+)-ethofumesate was nearly three to four times higher than the one determined for (-)-ethofumesate, indicating a concentration of the (+)-enantiomer in the liver. This finding is in line with the results of an *in vivo* study conducted in rabbits to determine the stereo-selective toxicokinetics and tissue distribution of



ethofumesate. In this study (-)-ethofumesate was preferentially eliminated from plasma compared with its antipode and also in liver, kidney and fat an increased concentration of the (+)-enantiomer was detected.

In contrast to the findings in rabbit liver microsomes, no enantio-selective depletion of ethofumesate was detected in liver microsomes of rats. The half-lives of the individual enantiomers were nearly identical after incubation with *rac*-ethofumesate and showed a slightly higher  $T_{1/2}$  for the (-)-enantiomer after incubation of the individual enantiomers. This is reflected in an enantiomer factor of  $< 0.5$ . Thus, a slight concentration of the (-)-enantiomer (not the (+)-enantiomer, as detected in rabbits) can be presumed, however a preferred metabolism of the (+)-enantiomer to metabolite ethofumesate-hydroxy was not detected. Thus the preferred excretion of the enantiomer could be an explanation, however in general passive processes like absorption, distribution and excretion do not differentiate between enantiomers. Therefore it is more likely that no stereo-selective depletion occurs.

A chiral conversion of the enantiomers (conversion from the (+)-enantiomer to the (-)-enantiomer or conversion from the (-)-enantiomer to the (+)-enantiomer) was also not detected in this study, neither in rat nor in rabbit liver microsomes.

In summary no significant changes in the ratio of the racemate (1:1) were observed in the present *in vitro* study with liver microsomes of rats. The degradation and distribution of both enantiomers is assumed to be identical in all rat matrices.

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<b>Reference:</b>	Xu X, Diao J, Wang X, Dang Z, Zhang P, Li Y, Zhou Z. (2012)
Title:	Enantioselective metabolism and cytotoxicity of the chiral herbicide Ethofumesate in rat and chicken hepatocytes
Source:	Pesticide Biochemistry and Physiology 103, 62-67
Guidelines:	not applicable
GLP/GEP:	no

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### Abstract

The metabolic kinetics and toxicity of Ethofumesate (ETO) in rat and chicken hepatocytes using a chiral high-performance liquid chromatographic (HPLC) method was investigated. The metabolic [degradation] of ETO in rat hepatocytes was enantioselective, whereas it was not in chicken hepatocytes. The  $T_{1/2}$  of (-)-ETO was about two times longer than that of (+)-ETO after the rat hepatocytes had been incubated with 20  $\mu$ M *rac*-ETO. There was no chiral conversion or transformation during their incubation with the hepatocytes. Cytotoxicity differences were observed between the two enantiomers of ETO, reflected in their  $EC_{50}$  values in rat and chicken hepatocytes. The stereoselective cytotoxicity of the two enantiomers was opposite in rat and chicken hepatocytes. A method of studying the toxicokinetics and cytotoxicity of chiral agrochemicals in hepatocytes isolated from mammals (rats) and chicken was developed.

### Material and methods

<b>1. Test Material:</b>  Purity: Source:   Purity: Source:   Purity: Source:	(±) Ethofumesate  98%  Institute for the Control of Agrochemicals, Ministry of Agriculture, Beijing, China  (+) Ethofumesate  >99%  Prepared by chiral HPLC  (-)Ethofumesate  >99%  Prepared by chiral HPLC
<b>2. Test animals</b>  Species Strain: Sex: Body weight: Source:	Rat Spargue-Dawley Male 200 – 250 g Experimental Animal Research Institute, Agricultural University of China, Beijing
<b>2. Test animals</b>  Species Strain: Age: Body weight: Source:	Chicken SPF 5 weeks 450 - 600 g Beijing Laboratory Animals Research Center, Beijing
<b>4. HPLC Apparatus and Conditions:</b>  Apparatus: Column:  Mobile phase:  Temperature Detection	Agilent 1100 CDMPC-CSP (Dept. Applied Chemistry, China Agricultural University, Beijing) 95% -n-hexane 5% - 2-propanol Room temperature 230 nm (G1314 VWD detector)

### Test performance

Experiments were conducted at the Department of Applied Chemistry, China Agricultural University, Beijing, China.

### 1. Isolation of primary hepatocytes

Rat and chicken hepatocytes were obtained by a two-step collagenase perfusion method following published procedures.

Viability of hepatocyte preparation was 95% measured by Trypan blue exclusion.

### 2. *In vitro* incubation - degradation

Hepatocyte monolayers ( $5 \times 10^6$  cells) were incubated with 20  $\mu$ M test substance. After 0.1, 1, 2, 3, 4, and 7 h an aliquot of 1 mL of medium was isolated and extracted with diethyl ether. Five replicate samples were collected per time point.

### 3. Cytotoxicity assay

The test substance was added as a DMSO solution at concentration of 0 – 800  $\mu$ M with the final DMSO concentration remaining below 1%. Cytotoxicity was determined by assessing mitochondrial function using an MTT assay with formazan concentrations determined spectrometrically.

## Results

### A. Analytical determinations – calibration and method validation

There were no endogenous interferences, recoveries were at least 82% and linear calibration curves were obtained.

### B. Kinetic degradation in hepatocytes

#### Rat hepatocytes

When racemic Ethofumesate was incubated the (+) Ethofumesate was degraded more rapidly with  $T_{1/2}$  of 2.0 h as compared to the (-) Ethofumesate ( $T_{1/2} = 4.1$  h).

#### Chicken hepatocytes

The degradation was similar for both enantiomers upon incubation of racemic Ethofumesate (2.2 min and 2.3 h for (+) and (-) Ethofumesate).

**Table 6.1.3-3** Calculated half life of Ethofumesate enantiomers in rabbit and chicken hepatocytes *in vitro*

	Racemic Ethofumesate	(+)-Ethofumesate	(-)-Ethofumesate
Rat hepatocytes	Not reported	2.1 h	4.1 h
Chicken hepatocytes	Not reported	2.2 h	2.3 h

**C. Cytotoxicity**

While in rat hepatocytes the (+) enantiomer had a lower EC<sub>50</sub> the EC<sub>50</sub> was lower for the (-) enantiomer in chicken hepatocytes.

**Table 6.1.3-4** Calculated EC<sub>50</sub> values (μM) of racemic Ethofumesate and Ethofumesate enantiomers in rabbit and chicken hepatocytes *in vitro*

	Racemic Ethofumesate	(+)-Ethofumesate	(-)-Ethofumesate
Rat hepatocytes	535 ± 11	370 ± 16	570 ± 12
Chicken hepatocytes	681 ± 21	>800	316 ± 9

**Conclusion**

While some differences were noted in the half-life of Ethofumesate in rat hepatocytes *in vitro* no difference was noted in chicken hepatocytes. Generally cytotoxicity was rather low with minor stereoselectivity and species differences.

**Overall conclusion on potential stereo specific effects**

Three published studies reported on stereo selectivity of ethofumesate toxicokinetics in rabbits, metabolism and degradation in rabbit and rat liver microsomes and the degradation and cytotoxicity in rat and chicken hepatocytes.

While some differences were observed both between enantiomers and also among species the stereo selectivity was minor and observations were somewhat inconsistent:

In the first study conducted only in the rabbit (-) enantiomer was degraded more rapidly than its antipode. In the second study degradation of the (-) enantiomer was again more rapid in rabbit liver microsomes and no stereo selectivity was observed in rat liver microsomes. In the third study the (+) enantiomer was more rapidly degraded in rat hepatocytes than (-) enantiomer while no stereo selectivity was noted in hepatocytes from chicken liver.

Since no OECD Guideline is available for this kind of studies and each study had its specifics the RMS concluded that there is only limited confidence in the observed effects.

### B.6.2. ACUTE TOXICITY

One acute inhalation toxicity study (“Acute (4-hour) inhalation toxicity of Ethosat in rats”; [REDACTED] 1993) was not evaluated in DAR, but was included as an “old” study in the supplementary dossier for renewal of ethofumesate. In the study report was stated that “Ethosat” was applied and that “Ethosat” is a mixture of 500 g/l in water. However, since “Ethosat” is neither the active substance nor the representative formulation and no composition of this mixture is given (ethofumesate is not soluble in water, therefore, it is assumed that some co-formulants must be in the formulation as well), there is no justification to include the study evaluation in the DRAR. Since the notifier did not claim adverse data for this study according to Article 56 (Information on potentially harmful or unacceptable effects) of Regulation (EC) No 1107/2009, this study was not evaluated in the DRAR (2014).

One acute oral study in rats ([REDACTED], 1988), one acute oral toxicity study in mice ([REDACTED], 1988) and one acute inhalation study in rats ([REDACTED] 1986) were originally submitted by Barclay Chemicals R&D Limited and were evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies, these studies were not subject for re-wording or inclusion of additional information. The study summaries were copied from the DAR (1998) in the DRAR (2014).

The evaluations of all studies presented below were already included in the original DAR (1998). Only re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for acute toxicity was proven. The RMS did not derive in the DRAR any different general conclusion on acute toxicity than originally derived in the DAR.

#### B.6.2.1. Oral

##### *B.6.2.1.1. Rat*

<b>Reference:</b>	ETHOFUMESATE: ACUTE ORAL TOXICITY (LIMIT TEST) IN THE RAT
Author(s), year:	[REDACTED] 1992
Report/Doc. number::	A87559 / M-161466-01-1
Guideline(s):	OECD 401 (1987)
GLP:	Yes
Deviations from OECD 401 (1987):	No
Acceptability:	Yes

#### **Material and Methods**

Ethofumesate (purity: not stated in the study report) was administered by gavage to five male and five female albino rats of the Sprague-Dawley strain. The test material (10 ml/kg bw) was administered in peanut oil at the dose level of 2000 mg/kg bw. The observation period was 14 days. Gross pathological examination (external examination and necropsy) was performed in all animals that died during study procedure or were sacrificed at study termination. No tissue was retained.

#### **Results**

No mortalities were observed. The LD<sub>50</sub> for male and female rats is concluded to be > 2000 mg/kg. Hunched posture and tremor were observed at the beginning of the dosing but disappeared one to two days later. All

animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, oral LD<sub>50</sub> in male and female rats was above 2000 mg/kg bw.

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<b>Reference:</b>	Acute oral toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats
Author(s), year:	██████████, 1991
Report/Doc. number::	OFC00004836 / M-351974-01-1
Guideline(s):	OECD 401 (1987)
GLP:	Yes
Deviations from OECD 401 (1987):	No
Acceptability:	Yes

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### Material and Methodes

Ethofumesate (purity: 98%) was administered by gavage to five male and five female Wistar rats. The test material was administered as a suspension in peanut oil at the dose levels of 2500, 5000 and 7500 mg/kg bw (6.25 ml/kg bw, 12.5 ml/kg bw and 18.75 ml/kg bw). The observation period was 15 days. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination.

### Results

No mortalities were observed. The LD<sub>50</sub> for male and female rats is concluded to be > 7500 mg/kg. No clinical signs were observed during the study. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, oral LD<sub>50</sub> in male and female rats was above 7500 mg/kg bw.

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<b>Reference:</b>	Ethofumesate technical powder - Acute oral toxicity (limit test) in the rat
Author(s), year:	██████████, 1988
Report/Doc. number::	A83223 / M-155494-01-1
Guideline(s):	OECD 401 (1987)
GLP:	Yes
Deviations from OECD 401 (1987):	No
Acceptability:	Yes

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### Material and Methodes

Ethofumesate (purity: not stated in the study report) was administered by gavage to five male and five female Sprague-Dawley rats. The test material (10 ml/kg bw) was administered in peanut oil at the dose level of 5000

mg/kg bw. The observation period was 14 days. Gross pathological examination (external examination and necropsy) was performed in all animals that died during study procedure or were sacrificed at study termination. No tissue was retained.

### Results

No mortalities were observed. The LD<sub>50</sub> for male and female rats is concluded to be > 5000 mg/kg. No clinical signs were observed during the study. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, oral LD<sub>50</sub> in male and female rats was above 5000 mg/kg bw.

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*The study below (██████, 1988) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).*

### Acute oral toxicity in rats (██████ 1988)

#### Experimental design

Ethofumesate technical was administered orally, by stomach tube, to five male and five female albino rats of the CD strain. The test material was administered as a suspension in corn oil and the dose levels were 6000, 7000 and 8000 mg/kg body weight. The observation period was 14 days.

#### Results

LD<sub>50</sub> for rats was greater than 8000 mg/kg for both males and females.

Clinical signs: decreased activity, irritability and abnormal body posture.

There were no gross changes noted at necropsy in any animal.

#### Comments

The study follows the OECD guideline. There is a QA statement and a statement of compliance with GLP standards. Ethofumesate has a low acute oral toxicity.

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#### B.6.2.1.2. Mouse

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<b>Reference:</b>	ETHOFUMESATE: ACUTE ORAL TOXICITY (LIMIT TEST) IN THE MOUSE
Author(s), year:	██████ 1992
Report/Doc. number::	A87560 / M-161468-01-1
Guideline(s):	OECD 401 (1987)

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GLP:	Yes
Deviations from OECD 401 (1987):	No
Acceptability:	Yes

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### Material and Methods

Ethofumesate (purity: not stated in the study report) was administered by gavage to five male and five female CD1 mice. The test material (10 ml/kg bw) was administered in peanut oil at the dose level of 5000 mg/kg bw. The observation period was 14 days. Gross pathological examination (external examination and necropsy) was performed in all animals that died during study procedure or were sacrificed at study termination. No tissue was retained.

### Results

No mortalities were observed. The LD<sub>50</sub> for male and female mice is concluded to be > 5000 mg/kg. No clinical signs were observed during the study. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, oral LD<sub>50</sub> in male and female mice was above 5000 mg/kg bw.

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<b>Reference:</b>	Acute oral toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Swiss albino mice
Author(s), year:	██████████ 1991
Report/Doc. number::	OFC00004837 / M-351978-01-1
Guideline(s):	OECD 401 (1987)
GLP:	Yes
Deviations from OECD 401 (1987):	No
Acceptability:	Yes

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### Material and Methods

Ethofumesate (purity: 98%) was administered by gavage to five male and five female Swiss Albino mice. The test material (10 ml/kg bw) was administered in peanut oil at the dose level of 2500, 5000 and 7500 mg/kg bw. The observation period was 15 days. Gross pathological examination (external examination and necropsy) was performed in all animals that died during study procedure or were sacrificed at study termination.

### Results

There was no mortality observed at 2500 mg/kg bw. One male mouse died within 48 hours in 5000 mg/kg bw dose group and two female mice died within 72 hours in 7500 mg/kg bw dose group post administration. LD<sub>50</sub> for mice was above 7500 mg/kg for both males and females.

All mice from 2500 mg/kg bw dose group had gained body weight at day 14. In 5000 mg/kg bw dose group few mice had maintained and all others had gained body weight at day 14. In 7500 mg/kg bw dose group both death



mice had lost body weight while the surviving mice had maintained or marginally lost body weight at day 7 and 14.

There were no toxicity symptoms observed in 2500 mg and 5000 mg dose groups while in 7500 mg dose group few mice showed lethargy, mild clonic convulsions and ataxia.

No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, oral LD<sub>50</sub> in male and female rats was above 7500 mg/kg bw.

*The study below (██████ 1988) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).*

### Acute oral toxicity in mice (██████ 1988)

#### Experimental design

Ethofumesate technical was administered orally, by stomach tube, to five male and five female albino mice of the CD strain. The test material was administered as a suspension in corn oil and the dose levels were 3000, 6000 and 8000 mg/kg body weight. The observation period was 14 days.

#### Results

LD<sub>50</sub> for mice was greater than 8000 mg/kg for both males and females.

Clinical signs: decreased activity, irritability and abnormal body posture.

Necropsy findings: swollen intestine.

#### Comments

The study follows the OECD guideline. There is a QA statement and a statement of compliance with GLP standards. Ethofumesate has a low acute oral toxicity.

## B.6.2.2. Dermal

### B.6.2.2.1. Rat

<b>Reference:</b>	ETHOFUMESATE: ACUTE DERMAL TOXICITY (LIMIT TEST) IN THE RAT
Author(s), year:	██████ 1992
Report/Doc. number::	A87561 / M-161469-01-1
Guideline(s):	OECD 402 (1987)

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GLP:	Yes
Deviations from OECD 402 (1987):	No
Acceptability:	Yes

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### Material and Methods

Approximately 24 hours prior to the application of the test substance ethofumesate (purity: not stated in the study report), five Sprague-Dawley rats of each sex were shaven closely with electric clippers. The solid test material was moistened with peanut oil and a dose level of 2000 mg/kg was applied topically to the shaven intact skin (approximating to 10% of the total body surface area) of all the rats. A piece of surgical gauze was placed over the treatment area and semi-occluded with a piece of self-adhesive bandage. The test substance remained in contact with the skin of each animal for 24 hours. Afterwards the bandage was carefully removed and the treated skin and surrounding hair wiped with cotton wool moistened with peanut oil to remove any residual test material. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination.

### Results

One female was found dead 3 days after dosing. LD<sub>50</sub> for male and female rats was above 2000 mg/kg.

Isolated incidents of systemic toxicity noted were lethargy, hunched posture, decreased respiratory rate, laboured respiration, ataxia and pallor of the extremities. No signs of skin irritation were noted during the study.

Surviving animals showed expected gain in body weight during the study.

Abnormalities noted at necropsy of the female that died during the study were haemorrhagic lungs, dark liver, patchy pallor of the spleen, dark kidneys, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine. No abnormalities were noted at necropsy of animals that were killed at the end of the study.

### Conclusion

Under the conditions of the study and based on the information given in the study report, dermal LD<sub>50</sub> in male and female rats was above 2000 mg/kg bw.

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<b>Reference:</b>	TECHNICAL ETHOFUMESATE POWDER: ACUTE DERMAL TOXICITY (Limit Test) IN THE RAT
Author(s), year:	██████████ 1988
Report/Doc. number::	A83224 / M-155495-01-1
Guideline(s):	OECD 402 (1981)
GLP:	Yes
Deviations from OECD 402 (1987):	No
Acceptability:	Yes

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### Material and Methods

Approximately 24 hours prior to the application of the test substance ethofumesate (purity: not stated in the study report), five Sprague-Dawley CFY rats of each sex were shaven closely with electric clippers. The solid test material was moistened with peanut oil and a dose level of 2000 mg/kg was applied topically to the shaven intact skin (approximating to 10% of the total body surface area) of all the rats. A piece of surgical gauze was placed over the treatment area and semi-occluded with a double layer of elastic adhesive bandage wrapped around the trunk of the rat. The test substance remained in contact with the skin of each animal for 24 hours. Afterwards the bandage was carefully removed and the treated skin and surrounding hair wiped with moist cotton wool to remove any residual test material. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination. No tissues were retained.

### Results

No mortalities were observed. The dermal LD<sub>50</sub> for male and female rats is concluded to be > 2000 mg/kg. No clinical signs were observed during the study. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, dermal LD<sub>50</sub> in male and female rats was above 2000 mg/kg bw.

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<b>Reference:</b>	Acute dermal toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats
Author(s), year:	██████████ 1991
Report/Doc. number::	OFC00004838 / M-351980-01-1
Guideline(s):	OECD 402 (1987)
GLP:	Yes
Deviations from OECD 402 (1987):	No
Acceptability:	Yes

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### Material and Methods

Approximately 24 hours prior to the application of the test substance ethofumesate (purity: 98%), five Wistar rats of each sex and dose group (2500 and 5000 mg/kg) were shaven closely with electric clippers. The test material was made into a slurry with distilled water on aluminium foil and was applied topically to the shaven intact skin (approximating to 10% of the total body surface area) of all the rats. The foil with slurry was fixed with an adhesive tape USP. The test substance remained in contact with the skin of each animal for 24 hours. Afterwards the dressing was removed and the skin was flushed with luke warm water. Then the rats were washed with 1% Labklin and one more time with luke warm water. Washed rats were wiped dry with a cotton hand towel. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination.

### Results

No mortalities were observed. The dermal LD<sub>50</sub> for male and female rats is concluded to be > 5000 mg/kg. No clinical signs were observed during the study.

All rats gained weight at day 14 except one female rat in 2500 mg dose group and one female rat in 5000 mg dose group which had lost body weight.

No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, dermal LD<sub>50</sub> in male and female rats was above 5000 mg/kg bw.

#### *B.6.2.2.2. Rabbit*

<b>Reference:</b>	Ethofumesate technical CR 4805/4 acute dermal toxicity study in rabbits
Author(s), year:	██████████ 1979
Report/Doc. number::	A83173 / M-155447-01-1
Guideline(s):	US EPA (1978); the study predates OECD Guideline for acute dermal toxicity
GLP:	No
Deviations from OECD 402 (1987):	-Abrasion of stratum corneum before application of test substance was conducted additionally to shaving of fur
Acceptability:	Yes

### Material and Methods

Prior to the application of the undiluted test substance ethofumesate (purity: 97.8%), the entire trunk of five New Zealand White Rabbits of each sex and dose group (0 (vehicle control), 2000 and 20050 mg/kg) was shaven and the skin was abraded (stratum corneum but not dermis). The test material, moistened in 0.9% NaCl physiological saline, was applied topically on a piece of gauze to the shaven skin (approximating to 10% of the total body surface area) of all dosed animals. The gauze was covered with an impervious covering of sleek occlusive tape. The test substance remained in contact with the skin of each animal for 24 hours. Afterwards the dressing was removed and the skin was wiped to remove any remaining test material. Deaths and signs of toxicity were recorded frequently during the day of administration and thereafter twice daily during 14 days. Individual body weights were recorded on the day of dosing and weekly thereafter. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination. Furthermore two skin samples and all kidney samples were taken and examined histologically.

### Results

No mortalities were observed. The dermal LD<sub>50</sub> for male and female rabbits is concluded to be > 20050 mg/kg. Increased activity was observed in all groups up to one hour after dosing. Since the same effect was observed in control animals, this effect is rather contributable to handling than to ethofumesate treatment.

All animals gained weight during the study.

The marginal skin lesions that were detected were considered to be incidental findings.

The widespread kidney lesions that were detected were considered to be incidental findings and not chemical related since they were observed in all three groups including control. Such lesions are often associated with the condition of Nosematosis but sections stained by Gram failed to reveal the presence of the causal organism *Nosema cuniculi*.

## Conclusion

Under the conditions of the study and based on the information given in the study report, dermal LD<sub>50</sub> in male and female rabbits was above 20050 mg/kg bw.

### B.6.2.3. Inhalation

#### B.6.2.3.1. Rat

<b>Reference:</b>	Acute inhalation toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats
Author(s), year:	1991
Report/Doc. number::	OFC00004840 / M-351989-01-1
Guideline(s):	OECD 403 (1981)
GLP:	Yes
Deviations from OECD 403 (2009):	-Particle size distribution not reported, however median of the particle size between 1 and 2 µm, measured after 1, 2 and 3 hours of exposure
Acceptability:	Yes

## Material and Methods

Four groups of 5 male and 5 female Wistar rats received a 4-hour whole-body exposure to the wet aerosol of the test material ethofumesate (purity: 98%). Ethofumesate was dissolved in xylene. The aerosol was generated using a glass atomiser which delivers wet aerosol with a size of less than 2 µm. The aerosol concentration in the chamber atmosphere was measured with a HPLC. The particle size distribution was not reported, however, the median of the particle size was between 1 and 2 µm, measured after 1, 2 and 3 hours of exposure. The concentrations of ethofumesate were 0, 0.05, 0.085 and 0.16 mg/L. 0.16 mg/L was the maximal concentration that could be used for aerosol generation (as determined in previous studies) based on limitations of physical and chemical properties of ethofumesate and solvent used. Gross pathological examination was performed in all animals that died during study procedure or were sacrificed at study termination.

## Results

No mortalities were observed. The inhalative LC<sub>50</sub> for male and female rats is concluded to be > 0.16 mg/L, the highest attainable concentration. No clinical signs were observed during the study. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

## Conclusion

Under the conditions of the study and based on the information given in the study report, inhalative LC<sub>50</sub> in male and female rats was above 0.16 mg/L when exposed to the test material for four hours whole-body.

<b>Reference:</b>	ETHOFUMESATE: ACUTE INHALATION TOXICITY STUDY IN RATS 4 HOUR EXPOSURE
Author(s), year:	1989
Report/Doc. number::	A87562 / M-161471-01-1
Guideline(s):	OECD 403 (1981)
GLP:	Yes

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Deviations from OECD 403 (2009):	-Only one concentration applied
Acceptability:	Yes; limited information since only 48.7% of particles had aerodynamic diameter < 5.5 µm (respirable size)

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### Material and Methods

Two groups of 5 male and 5 female Wistar albino rats received a 4-hour whole-body exposure to the dust aerosol of the test material ethofumesate (purity: 97%). The aerosol concentration in the chamber atmosphere was measured gravimetrically five times during the exposure period. The particle size distribution was measured twice during the exposure period using an Andersen sampler. The concentrations of ethofumesate were 0 and 0.3 mg/l (highest attainable concentration). Only 48.7% of particles had aerodynamic diameter < 5.5 µm (respirable size). Gross pathological and microscopic examinations were performed in all animals that died during study procedure or were sacrificed at study termination. The lungs were weighed and together with liver and kidney preserved for microscopic investigation.

### Results

No mortalities were observed. The inhalative LC<sub>50</sub> for male and female rats is concluded to be > 0.3 mg/L, the highest attainable concentration. However, only 48.7% of particles had aerodynamic diameter < 5.5 µm (respirable size).

Reduced respiratory rate was observed during exposure. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, inhalative LC<sub>50</sub> in male and female rats was above 0.3 mg/L when exposed to the test material for four hours whole-body. However, only 48.7% of particles had aerodynamic diameter < 5.5 µm (respirable size), allowing limited conclusion on true available concentration and on acute inhalative toxicity of ethofumesate.

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<b>Reference:</b>	ETHOFUMESATE TECHNICAL: ACUTE INHALATION TOXICITY STUDY FOUR-HOUR EXPOSURE IN THE RAT
Author(s), year:	██████████ 1988
Report/Doc. number::	A83217 / M-155489-01-1
Guideline(s):	OECD 403 (1981)
GLP:	Yes
Deviations from OECD 403 (2009):	-Only one concentration applied
Acceptability:	Yes; limited information since only 5.8% of particles had aerodynamic diameter < 4 µm (respirable size)

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### Material and Methods

5 male and 5 female Sprague-Dawley rats received a 4-hour nose-only exposure to the dust aerosol of the test material ethofumesate (purity: not stated in the study report). The aerosol was generated using a Wright dust generator. The aerosol concentration in the chamber atmosphere was measured gravimetrically 17 times during the exposure period. The particle size distribution was measured once per hour during the exposure period using

a cascade impactor. The concentration of ethofumesate was 3.97 mg/l (maximum attainable mean concentration). The mean mass median aerodynamic diameter (MMAD) was 25 µm. The respiratory fraction (particle size < 4 µm) was 5.8%. Gross pathological and microscopic examinations were performed in all animals that died during study procedure or were sacrificed at study termination.

### Results

No mortalities were observed. The inhalative LC<sub>50</sub> for male and female rats is concluded to be > 3.97 mg/L, the highest attainable concentration. However, only 5.8% of particles had aerodynamic diameter < 4 µm (respirable size).

Reduced respiratory rate was observed during exposure. All animals gained weight during the study. No treatment related macroscopic pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, inhalative LC<sub>50</sub> in male and female rats was above 3.97 mg/L when exposed to the test material for four hours nose-only. However, only 5.8% of particles had aerodynamic diameter < 4 µm (respirable size), allowing limited conclusion on true available concentration and on acute inhalative toxicity of ethofumesate.

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*The study below [REDACTED] et al., 1986) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).*

### Acute inhalation toxicity in rats [REDACTED] et al., 1986)

#### *Experimental design*

Two groups of five male and five female rats (Wistar) received a 4-hour head-only exposure to the wet aerosol of ethofumesate technical. All the animals were observed for signs of intoxication for 14 days following exposure.

The exposure chamber was a "Buntschuh"-inhalator. The aerosol was generated using a nebulizer and the dosages per hour were 10 and 60 ml. The aerosol concentration and the particle size distribution in the chamber were not determined.

#### *Results*

No mortality and no determination of aerosol concentration and the particle size distribution led to LC<sub>50</sub> not being evaluated.

Clinical signs: decreased activity and apathy.

Necropsy: red to brownish coloured lungs with multiple dark to black points.

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*Comments*

The study does not follow the OECD guideline. There are no statements concerning GLP and there were no QA inspections. The report is very short and incomplete.

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**B.6.2.4. Skin irritation*****B.6.2.4.1. Rabbit***

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<b>Reference:</b>	Ethofumesate: Acute dermal irritation test in the rabbit
Author(s), year:	██████████, 1992
Report/Doc. number::	A87563 / M-161472-01-1
Guideline(s):	OECD 404 (1981)
GLP:	Yes
Deviations from OECD (2002):	No
Acceptability:	Yes

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**Material and Methods**

One day prior to the application of the test substance ethofumesate (purity: not stated in the study report), three New Zealand White rabbits (two females and one male) were clipped free of fur from the dorsal flank area using veterinary clippers. 0.5 g of the test material was moistened with 0.5 ml distilled water and introduced under a gauze patch and placed in position on the shorn skin. The patch was secured in position with a strip of surgical adhesive tape. The test substance remained in contact with the skin of each animal for 4 hours. Afterwards the patches were removed and any residual test material removed by gentle swabbing with cotton wool soaked in distilled water. Approximately one hour following the removal of the patches and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored.

**Results**

The overall mean erythema and oedema scores from the 24-, 48- and 72-hour observations were both 0.00. Ethofumesate is classified as non-irritant to skin.

**Conclusion**

Under the conditions of the study and based on the information given in the study report, rabbits exposed dermally to the test material for four hours did not develop any sign of skin irritation.

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<b>Reference:</b>	Primary skin irritation study with ethofumesate technical (FSG 03189 H/27 Feb.90) in new zealand white rabbits
Author(s), year:	██████████, 1991
Report/Doc. number::	OFC00004841 / M-351993-01-1
Guideline(s):	OECD 404 (1981)
GLP:	Yes
Deviations from OECD (2002):	No
Acceptability:	Yes

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### Material and Methods

Approximately 24 hours prior to the application of the test substance ethofumesate (purity: 98%), the dorsal fur (10 x 10 cm) of three New Zealand White rabbits (two males and one female) was clipped using an electric clipper. 0.5 g of the test material was moistened with distilled water on an aluminium foil and applied to the prepared skin. Bare aluminium foil was also applied to the prepared skin posterior to the test patch to act as control patch. The patches were secured in position by wrapping an elastic bandage around the abdomen. The test substance remained in contact with the skin of each animal for 4 hours. Afterwards the patches were removed and the skin was flushed with distilled water. One hour following the removal of the patches and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored.

### Results

The overall mean erythema and oedema scores from the 24-, 48- and 72-hour observations were both 0.00. Ethofumesate is classified as non-irritant to skin.

### Conclusion

Under the conditions of the study and based on the information given in the study report, rabbits exposed dermally to the test material for four hours did not develop any sign of skin irritation.

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<b>Reference:</b>	TECHNICAL ETHOFUMESATE: RABBIT SKIN IRRITANCY STUDY
Author(s), year:	██████, 1991
Report/Doc. number::	A83207 / M-155479-01-1
Guideline(s):	OECD 404 (1981)
GLP:	Yes
Deviations from OECD (2002):	-The solid test substance was not moistened -First observation after 30 to 60 minutes instead of 1 hour
Acceptability:	Yes

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### Material and Methods

One day prior to the application of the test substance ethofumesate (purity: 98 %), three female New Zealand White rabbits were clipped free of fur on the dorso-lumbar region (10 x 10 cm) using veterinary clippers. 0.5 g of the solid test material was applied to the shaved skin under a gauze pad. The patch was secured in position by adhesive semi-occlusive plaster. The test substance remained in contact with the skin of each animal for 4 hours. Afterwards the patches were removed and the treatment site was washed with soap and water to remove residual test material, rinsed and dried. About 30 to 60 minutes after the end of the exposure and daily for up to three days post application the test sites were examined for evidence of primary irritation and scored.

### Results

The overall mean erythema and oedema scores from the 24-, 48- and 72-hour observations were both 0.00. Ethofumesate is classified as non-irritant to skin.

### Conclusion

Under the conditions of the study and based on the information given in the study report, rabbits exposed dermally to the test material for four hours did not develop any sign of skin irritation.

### B.6.2.5. Eye irritation

#### B.6.2.5.1. Rabbit

<b>Reference:</b>	Ethofumesate: Acute eye irritation test in the rabbit
Author(s), year:	██████████ 1993
Report/Doc. number::	A87564 / M-161473-01-1
Guideline(s):	OECD 405 (1987)
GLP:	Yes
Deviations from OECD (2012):	- It is not reported in the study if the eyes were rinsed with saline or distilled water
Acceptability:	Yes

### Material and Methods

On the day of dosing, 0.1 ml of the test material ethofumesate (purity: not stated in the study report) was applied into the conjunctival sac of one eye of three New Zealand White rabbits. The lower eyelid was held open momentarily during dosing. The eyelids were then gently held together for about one second and released to allow the animal to blink freely. The other eye of each rabbit was not treated and served as a control. It is not reported in the study if the eyes were rinsed with saline or distilled water after 24 hours. The cornea, iris, and conjunctiva of the treated and control eyes were examined with a standard ophthalmoscope prior to the test and at 1, 24, 48, and 72 hours after treatment.

### Results

Mild conjunctival irritation was observed in all the rabbits 1 hour after treatment. All treated eyes appeared normal 24 hours after treatment. The overall mean scores for redness, chemosis, corneal opacity and iritis from the 24-, 48- and 72-hour observations were all 0.00. Ethofumesate is classified as non-irritant to eyes.

### Conclusion

Under the conditions of the study and based on the information given in the study report, ethofumesate is not an eye irritant.

<b>Reference:</b>	Primary eye irritation study with ethofumesate technical (FSG 03189 H/27 Feb.90) in New Zealand white rabbits
Author(s), year:	██████████ 1991
Report/Doc. number::	OFC00004842 / M-351996-01-1
Guideline(s):	OECD 405 (1987)
GLP:	Yes
Deviations from OECD (2012):	None
Acceptability:	Yes

### Material and Methods

On the day of dosing, 0.1 mg of test material ethofumesate (purity: 98%) was applied into the conjunctival sac of one eye of three New Zealand White. The lower eyelid was held open during dosing. The eyelids were then gently held together for about one second and released to allow the animal to blink freely. The other eye of each rabbit was not treated and served as a control. After 24 hours contact period the treated eye and the control eye were irrigated with distilled water to remove the residual test compound. The cornea, iris, and conjunctiva of the treated and control eyes were examined with a hand-held light prior to the test and at 1, 24, 48, and 72 hours after treatment.

### Results

Mild conjunctival irritation was observed in all the rabbits 1 hour after treatment. All treated eyes appeared normal 24 hours after treatment. The overall mean scores for redness, chemosis, corneal opacity and iritis from the 24-, 48- and 72-hour observations were all 0.00. Ethofumesate is classified as non-irritant to eyes.

### Conclusion

Under the conditions of the study and based on the information given in the study report, rabbit eyes exposed to the test material did not develop any sign of eye irritation.

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<b>Reference:</b>	TECHNICAL ETHOFUMESATE: RABBIT EYE IRRITANCY STUDY
Author(s), year:	██████████ 1991
Report/Doc. number::	A83208 / M-155480-01-1
Guideline(s):	OECD 405 (1987)
GLP:	Yes
Deviations from OECD (2012):	- It is not reported in the study if the eyes were rinsed with saline or distilled water
Acceptability:	Yes

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### Material and Methods

On the day of dosing, 0.1 ml of test material ethofumesate (purity: 98%) was applied into the conjunctival sac of one eye of three New Zealand White rabbits. One rabbit was treated initially and the remaining two animals were treated 6 days later. The lower eyelid was held open during dosing. The eyelids were then gently held together for about one second and released to allow the animal to blink freely. The other eye of each rabbit was not treated and served as a control. The cornea, iris, and conjunctiva of the treated and control eyes were examined with a hand-held light prior to the test and at 1, 24, 48, and 72 hours after treatment. The degree of eye irritation was evaluated according to the criteria of Draize *et al.*

### Results

Mild conjunctival irritation was observed in all the rabbits 1 hour after treatment and in two animals 24 hours post-instillation. All eyes were normal by 48 hours. The overall mean scores for redness, from the 24-, 48- and 72-hour observations were 0, 0.3 and 0.3. The overall mean scores for chemosis, corneal opacity and iritis from the 24-, 48- and 72-hour observations were all 0.00. Ethofumesate is classified as non-irritant to eyes.

### Conclusion

Under the conditions of the study and based on the information given in the study report, rabbit eyes exposed to the test material did not develop any sign of eye irritation.

#### B.6.2.6. Skin sensitization

##### *B.6.2.6.1. Magnusson and Kligman*

<b>Reference:</b>	ETHOFUMESATE: SENSITISATION TEST IN THE GUINEA PIG (MAGNUSSON AND KLIGMAN MAXIMISATION METHOD)
Author(s), year:	██████████, 1989
Report/Doc. number::	A87565 / M-161474-01-1
Guideline(s):	OECD 406 (1981)
GLP:	Yes
Deviations from OECD (1992):	- The concentration of test substance used for each induction exposure was well-tolerated but did not show any signs of irritation, however, it is stated that it was the highest practical concentration (in the skin irritation studies ethofumesate did not produce any kind of skin reaction) - No positive control included; no information on any positive control study conducted in that time period
Acceptability:	Yes; limited reliability since no information on positive controls

#### Material and Methods

The substance ethofumesate (purity: not stated in the study report) was tested according to GPMT (Magnuson and Kligman) on female guinea pigs of the Dunkin-Hartley strain. A 5% concentration was chosen for the main study injection stage, this being the maximum injectable concentration and one that caused an acceptable minimum localised response during the observation period. For the topical irritancy ranging study four dilutions of the test material were prepared in ethanol (30%, 20%, 10% and 5%).

Twenty guinea pigs were treated by using an intradermal injection (6 injections per animal) in the shoulder region with 1) Freund's Complete Adjuvant, 2) the test material (5% w/v in Alembicol D) and 3) 10% w/v test material in Freund's Complete Adjuvant emulsified with distilled water to give a 5% concentration of test material. Seven days later this induction procedure was boosted by topical application of the test material (30% w/v in ethanol) over the injection sites. A second group of twenty guinea pigs was similarly treated but the vehicle Alembicol D Fractionated Coconut oil was substituted for the test material at the injection stage and ethanol at the topical application stage. Two weeks after this induction phase all the animals from both the test and control groups were challenged with two concentrations of the test material (20 and 30% w/v in ethanol) which were applied topically to the flanks. The 30% challenge concentration used in this study was the highest practical concentration. No positive control was included in the test and no information was given in the study report on any positive control study conducted in that time period.

#### Results

No response was exhibited by any animal in the test or control group when challenged with either concentration of the test material. However, since no information on any kind of positive control is given, the reliability of the study might be questioned.

## Conclusion

Under the conditions of the study and based on the information given in the study report, none of the treated guinea pigs reacted upon dermal challenge, however, no dermal reactions were observed during induction phase and no positive control was mentioned in the study report.

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<b>Reference:</b>	Technical ethofumesate CR 4805. Delayed contact hypersensitivity in the guinea-pig – and Amendment 1
Author(s), year:	██████████, 1984
Report/Doc. number::	M-155461-02-1
Guideline(s):	OECD 406 (1981)
GLP:	Yes
Deviations from OECD (1992):	- According to the study report, the irritant test substance concentrations suitable for the induction phase of the main study and non-irritant concentrations by the topical route of administration for the challenge phase was estimated in the irritation preliminary study and applied in the main test. However, this is somehow doubted since in the skin irritation studies and other skin sensitization studies ethofumesate did not produce any kind of skin irritation
Acceptability:	Yes;

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## Material and Methods

The substance ethofumesate (purity: 96.8%) was tested according to GPMT (Magnuson and Kligman) test on guinea pigs of the Dunkin-Hartley strain. Twenty guinea pigs were treated using an intradermal injection in the shoulder region with 1) Freund's Complete Adjuvant, 2) the test material (10% w/w in Alembicol D) and 3) the test material and a mixture of Freund's Complete Adjuvant. Seven days later this induction procedure was boosted by topical application of the test material (25% w/w in Alembicol D) over the injection sites. A second group of twenty guinea pigs was similarly treated with the exception that the test compound was omitted from the intradermal injections and topical application. Two weeks after this induction phase all the animals from both the test and control groups were challenged with two concentrations of the test material (5 and 10% w/w in Alembicol D) which were applied topically to the flanks. Prior to the main test the intradermal and topical irritancy of a range of dilutions of ethofumesate in Alembicol D was investigated to identify (a) irritant test substance concentrations suitable for the induction phase of the main study and (b) non-irritant concentrations by the topical route of administration for the challenge phase. According to the study report the appropriate concentrations were estimated and applied in the main test. The positive control (formalin) was not included in the study but was part of the concurrent studies.

## Results

Very slight localised erythema was observed in six of twenty test animals. This effect was considered to be nonspecific and not indicative of a sensitisation response. No response was seen in the negative control animals. The positive control group gave adequate response.

## Conclusion

Under the conditions of the study and based on the information given in the study report, none of the treated guinea pigs reacted upon dermal challenge, however, no dermal reactions were observed during induction phase.

**B.6.2.6.2. Buehler test**

<b>Reference:</b>	Skin sensitization study with ethofumesate technical (FSG 03089 H/27 Feb. 90) in Guinea Pigs (Buehler Test)
Author(s), year:	██████████ 1991
Report/Doc. number::	OFC00004843 / M-351999-01-1
Guideline(s):	OECD 406 (1981)
GLP:	Yes
Deviations from OECD (1992):	-Only 10 animals in treatment group - The concentration of test substance used for each induction exposure was well-tolerated but did not show any signs of irritation (however, it has to be considered that in the skin irritation studies ethofumesate did not produce any kind of skin reaction)
Acceptability:	Yes; limited value of the study since too low number of animals used for the treatment group

**Material and Methods**

The substance ethofumesat (purity: 98%) was tested in the Buehler test on albino guinea pigs. 25 guinea pigs (10 treated group, 10 negative control, 5 positive control) were treated with a dermal application on the shoulder region with 500 mg of ethofumesate made into a slurry with normal saline. The procedure was repeated once a week for 3 weeks. Two weeks after the last induction all animals were challenged with 500 mg of the test material which was applied topically to the flanks. The induction and challenge period lasted for 6 hours. The positive control was 1-Chloro-2,4-di-Nitrobenzene (DNCB).

**Results**

No response was exhibited by any animal in the treated or negative control group. The positive control group gave adequate response. The results are only of limited value since too low number of animals (for the Buehler test) in the treated group was used.

**Conclusion**

Under the conditions of the study and based on the information given in the study report, none of the treated guinea pigs reacted upon dermal challenge, however, too low number of animals were used for the treated group and no dermal reactions were observed during induction phase.

**B.6.2.7. Phototoxicity**

According to new data requirements for active substances (Regulation (EU) No 283/2012) an *in vitro* phototoxicity study is required where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , no toxicity testing is required.

Ethofumesate absorbs electromagnetic radiation mostly below 290 nm. However, at 290 nm the extinction coefficient of ethofumesate is still  $> 1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , which is above the trigger of  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ . Therefore, testing ethofumesate for phototoxicity is justified.

UPL conducted a phototoxicity *in vitro* study to which the TaskForce has a Letter of access.

<b>Reference:</b>	Cytotoxicity assay In vitro with BALB/c 3T3 cells: Neutral red (NR) test with Ethofumesate during simultaneous irradiation with artificial sunlight
Author(s), year:	Heppenheimer, A, 2012
Doc. number:	1474000
Guideline(s):	OECD 432 (2004)
GLP:	Yes
Deviations from OECD 432 (2004):	No
Acceptability:	Yes

### Material and methods

<b>1. Test Material:</b>  Description: Lot/Batch #: Purity: Stability: Storage conditions  Solvent used:	Ethofumesate  No data AI02ETH040 99.8% Expiry date 26.10.2013 at room temperature protected from sunlight  Dimethylsulfoxid (DMSO)
<b>2. Control Materials:</b>  Negative: Solvent/final concentration: Positive: Chlorpromazine	Negative and solvent control DMSO in EBSS at 1% (with and without irradiation)  Without irradiation: 6.25, 12.5, 25, 37.5, 50, 75, 100, 200 µg/mL With irradiation: 0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0 µg/mL
<b>3. Irradiation</b>  Apparatus: Filter Produced wavelength	Dr Hönle Sol 500 H1 > 320 nm
<b>4. Test system</b>  Cells: Incubation medium:  Temperature Atmosphere	BALB/c 3T3 clone 31 Dulbecco's Minimal Essential Medium, supplemented with 10% NCS 37 ± 1.5 °C 7.5% ± 0.5% carbon dioxide
<b>5. Test concentrations:</b>	1.95, 3.91, 7.81, 15.83, 31.65, 62.5, 125, 250 µg/mL (higher concentrations resulted in precipitation)

The test was conducted according to OECD TG 432 as follows:

$2 \times 10^4$  cells per well were seeded in 100  $\mu$ l culture medium (two 96-well plates, one exposed to artificial sunlight one kept in the dark).

After 24 h cells were washed with EBSS and test item in EBSS/1% DMSO added at eight dilutions.

After pre-incubation for one hour in the dark one plate was irradiated through the lid at 1.7 mW/cm<sup>2</sup> (5 J/cm<sup>2</sup>) for  $50 \pm 2$  min at 20-30 °C, one plate was stored for  $50 \pm 2$  min at 20-30 °C in the dark.

Plates were washed twice with EBSS and incubated with culture medium overnight.

Cytotoxicity was determined by Neutral Red Uptake determination.

EC<sub>50</sub> values, Photo-Irritancy-Factor (PIF) and Mean Phototoxic Effect (MPE) are calculated if possible.

If PIF < 2 or MPE < 0.1: no phototoxic potential is predicted

If PIF > 2 and < 5 or MPE > 0.1 and < 0.15 probable phototoxic potential is predicted

If PIF > 5 or MPE > 0.15 a phototoxic potential is predicted.

## Results

Precipitation of the test item was observed at >250  $\mu$ g/mL.

No cytotoxicity was observed either with or without irradiation.

Based on the results of the preliminary test, the test concentrations of 0 (vehicle DMSO), 1.95 - 250  $\mu$ g /mL, with and without irradiation, were chosen. Cytotoxicity was not observed, either in the presence or in the absence of irradiation.

With the positive control chlorpromazine cytotoxicity was noted in the absence of irradiation and at lower concentrations in the presence of irradiation, allowing determination of EC<sub>50</sub> values and the calculation of a Photo-Irritancy-Factor (PIF) which were within the range of historical control values as was the Mean Phototoxic Effect (MPE). MPE for the test substance was well below the cut-off value of 0.15. The results are summarized in below.



Table B.6.2.7: Summary of results

	Test substance	ED <sub>50</sub> (+ UV) [µg/mL]	ED <sub>50</sub> (- UV) [µg/mL]	PIF	MPE	% viability of solvent control of irradiated versus non irradiated plate
Range finding experiment	Ethofumesate	-	-	-	0.013	97.6
	Positive control	0.36	11.36	32.16	0.527	98.7
Main experiment	Ethofumesate	-	-	-	0.079	91.8
	Positive control	0.19	17.74	92.74	0.627	90.4

### Conclusion

Ethofumesate did not have any phototoxic effects on BALB/c 3T3 cells when being irradiated with wavelengths mainly in UVA spectrum (> 320 nm).

The RMS agrees that ethofumesate was not phototoxic to BALB/c 3T3 cells under conditions of the study.

However, some doubts remain on the suitability of the used electromagnetic spectrum in case of ethofumesate. Since ethofumesate absorbs electromagnetic radiation at 290 nm wavelength (UVB) above the trigger of  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$  (and also above  $1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ ) but shows no absorption at or above 320 nm (UVA), the results of the study do not allow a definite conclusion on the suitability of the test in the spectral range relevant for ethofumesate (mainly 290 nm) even if the solar simulator with H1 filter allows UVB to some extent. On the other hand, two of the positive controls from the ring test validation (amiodarone and chlorpromazine) have their absorption peaks near to that of ethofumesate (amiodarone: 300 nm; chlorpromazine: 309 nm) and showed clear positive results in the ring test, also with H1 filter.

RMS contacted the laboratory that conducted this study in order to elucidate if the BALB/c 3T3 test would provide any additional information with higher amount of UVB. The Harlans Laboratories Germany responded that the used solar filter H1 is the only recommended and validated filter for BALB/c 3T3 cells according to OECD 432 since it can suitably attenuate the highly cytotoxic UVB wavelengths. They added that solar simulator and the filter H2 would allow only marginally higher piece of UVB wavelengths which would not substantially change the irradiation spectrum.

In the ICH harmonised tripartite guideline S10 (Photosafety evaluation of pharmaceuticals) from November 2013 also the following is stated:

“The BALB/c 3T3 cell line is sensitive to UVB and the initially recommended irradiation conditions (Ref. 6) involve the use of filters to attenuate wavelengths below 320 nm. However, depending on the light source and filters used, the ratio of UVB to UVA can be adjusted such that it is possible to assess UVB-induced phototoxicity in this test. UVB-induced phototoxicity is rarely a problem for pharmaceuticals with systemic exposure since UVB minimally penetrates beyond the epidermis. However, UVB-induced phototoxicity is more relevant for topical products. For components of topically applied products that absorb predominately in the UVB range, and where in vitro assessment is desired, the use of the 3T3 NRU-PT with modified irradiation

conditions (see above) can be considered. Alternatively, in vitro skin models, which better tolerate UVB, could be considered. Reconstructed human skin models, with the presence of a stratum corneum, permit testing of various types of topically applied materials ranging from neat chemicals to final clinical formulations. The assays developed with reconstructed human skin to date measure cell viability with and without irradiation. These assays appear to be capable of detecting known human acute dermal phototoxicants”.

At the time it is concluded that based on the negative results of 3T3 NRU-PT, which is currently the only listed test for addressing phototoxicity (Commission Communications 2013/C 95/01), ethofumesate is not considered to be phototoxic.

In the absence of any other validated method (reconstructed human skin model is not yet validated for phototoxicity), better suitable for testing phototoxicity of substances being UVB absorbers (like ethofumesate), the RMS concluded that the notifiers fulfilled their duty to address phototoxicity of ethofumesate.

*According to Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) 283/2013 and Regulation (EU) No 284/2013 (SANCO/10181/2013– rev. 2, May 2013) it is stated that “In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02”.*

The waiving of the more suitable phototoxicity test is considered acceptable as long as no alternative test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.

### **B.6.3. SHORT-TERM TOXICITY**

One new short-term study (90 days study in Beagle dogs; [REDACTED] 1999) was, by mistake, included in the dossier for purpose of re-newal of ethofumesate. The notifier thought that this study was evaluated in the original DAR (1998) but it was not submitted at that time. Since the notifier did not claim adverse data for this study according to Article 56 (*Information on potentially harmful or unacceptable effects*) of Regulation (EC) No 1107/2009, this study was not evaluated in the DRAR (2014).

One 90 days study in rats ([REDACTED], 1990) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998) in the DRAR.

The evaluations of all studies presented below were already included in the original DAR (1998). Only re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for short-term toxicity was proven. The RMS did not derive in the DRAR any different general conclusion on short-term toxicity than originally derived in the DAR.

**B.6.3.1. Oral 28-day study*****B.6.3.1.1. Rat***

<b>Reference:</b>	Desmedipham / ethofumesate; dietary dose range finding study in rats
Author(s), year:	██████████ 1989
Report/Doc. number::	A63715 / M-147174-01-1
Guideline(s):	OECD 407 (1981)
GLP:	No
Deviations from OECD 407 (2008):	- Only very limited information like body weight and body weight gain as well as food consumption was obtained from the study
Acceptability:	Yes (limited information); range finding test

**Material and methods**

Groups of 10 male and 10 female Sprague-Dawley rats were dosed with ethofumesate (no purity given) via the diet at constant concentrations of 10000, 20000 and 30000 ppm ethofumesate (approximately 1000, 2000 and 3000 mg/kg bw/d according to the conversion factor of 0.1 for young rats of ca. 100 g weight). A further group of 10 male and 10 female rats were given untreated diet as control. During week 3, due to unavailability of ethofumesate, these groups were given a control diet for a period of one week after which time they were given ethofumesate. After a total period of 5 weeks all the animals were sacrificed.

**Results**

There were no deaths during the study. All treated animals showed food consumption similar to that of control animals. All females receiving ethofumesate showed a moderate to marked reduction in body weight gain (1000 mg/kg bw/d: 12%, 2000 mg/kg bw/d: 10%, 3000 mg/kg bw/d: 19%). All males receiving ethofumesate showed body weight gain comparable to or higher than that of control animals).

**Conclusion**

The study was conducted to provide a basis for selecting dose levels in later studies. The NOAEL was considered to be 20000 ppm (2000 mg/kg bw/d) based on reduction of body weight gain of 19% in females dosed with 30000 ppm (3000 mg/kg bw/d). At lower doses no dose-response for this effect was evident.

<b>Reference:</b>	ETHOFUMESATE: PREMILINARY DOSE RANGE FINDING STUDY IN RATS BY DIETARY ADMINISTRATION FOR 4 WEEKS
Author(s), year:	██████████ 1988
Report/Doc. number::	A87566 / M-161475-01-1
Guideline(s):	OECD 407 (1981)
GLP:	No; however it was stated in the report that that the study and the records were made as required by GLP
Deviations from OECD 407 (2008):	- No weight of epididymis, prostate and seminal vesicles, thymus and brain recorded - No histopathological investigation of any organ conducted - no haematology and clinical biochemistry investigated - no FOP conducted
Acceptability:	Yes (limited information); range finding test

**Material and methods**

Three groups of 5 male and 5 female CD rats (approximately 28 days old) received the test material ethofumesate (purity: 98.9%) by dietary administration for a period of 4 weeks. Dietary inclusion levels remained constant at nominal levels of 300, 3000 and 30000 ppm (30.5 mg/kg bw/d, 297 mg/kg bw/d and 3081 mg/kg bw/d in males and 33.1 mg/kg bw/d, 320 mg/kg bw/d and 3219 mg/kg bw/d in females) and a fourth group of rats of identical size received an untreated diet for control purposes. Individual animals were observed at least once daily for any signs of behavioural changes, reaction to treatment or ill health. The weight of each rat was recorded at the time of the allocation to group, on the day of commencement of treatment and once a week thereafter. After 4 weeks all surviving rats were sacrificed. All tissues were examined macroscopically either visually or by palpation. Adrenals, liver, heart, spleen, lung and testes were dissected free of fat and weighed. Samples of all other tissues from all animals were routinely preserved in formalin to satisfy any possible future requirement for examination of tissues. No histopathological investigation of any organ was conducted.

## Results

There were no deaths and no clinical signs related to the treatment during the study.

Slightly lower body weight gains were recorded among the male and female rats which were given 30000 ppm (19% in males and 10% in females) and males given 3000 ppm (9% lower body weight gain) in comparison to the control rats. Slightly smaller food intakes were recorded among male and female rats given 30000 or 3000 ppm.

The macroscopic pathology investigation revealed no findings. Increased absolute liver weights were noted among males (+ 18%) and females (+ 14%) treated with 30000 ppm ethofumesate.

## Conclusion

The study was conducted to provide a basis for selecting dose levels in later studies. Based on the decreased body weight gain in males and increased absolute liver weight in males and females dosed with 30000 ppm ethofumesate, the NOAEL of the study is set at 3000 ppm (297 mg/kg bw/d in males and 320 mg/kg bw/d in females).

<b>Reference:</b>	28 day dietary study in Wistar rats - Ethofumesate technical
Author(s), year:	██████████ 1991
Report/Doc. number::	OFC00004844 / M-352006-01-1
Guideline(s):	OECD 407 (1981)
GLP:	Yes
Deviations from OECD 407 (2008):	- No weight of epididymis, prostate and seminal vesicles, thymus, spleen, heart and brain recorded - no FOP conducted
Acceptability:	Yes (limited information)

## Material and methods

Rats (Wistar) were given ethofumesate (purity: 98%) by dietary administration at 0 (control), 200, 2000, and 20000 ppm (approximately 20, 200 and 2000 mg/kg bw/d according to the conversion factor of 0.1 for young rats of ca. 100 g weight). The treatment lasted for 28 days and there were 5 animals/sex/group. An additional

high dose recovery group was included in the study where 28 days medication period was followed by 14 days recovery period. Mortality, clinical signs, body weight, food and water consumption, haematology, blood chemistry, gross pathology and histopathology (of the control and high dose groups) were investigated.

## Results

All animals survived the dosing period. Body weight gain and food consumption were unaltered.

There was a reversible significant decrease in the neutrophils at the high dose level and a decrease in the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations at the mid and high dose levels. Relative liver and kidney weights were reversibly increased at the high dose level. There were no histopathological lesions which were attributable to treatment. The NOAEL in this study was 2000 ppm based on statistically significantly increased relative liver and kidney weights, significant decrease in the neutrophils and a decrease in the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations at 20000 ppm. All values were evaluated for combined sexes.

## Conclusion

The NOAEL in this study was 2000 ppm (approximately 200 mg/kg bw/d) in males and females based on statistically significantly increased relative liver and kidney weights, significant decrease in the neutrophils and a decrease in the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations at 20000 ppm.

### *B.6.3.1.2. Dog*

<b>Reference:</b>	ETHOFUMESATE: ORAL (CAPSULE/GAVAGE) MAXIMUM TOLERATED DOSE (MTD) AND 28 DAY REPEAT DOSE RANGE FINDING STUDY IN THE DOG
Author(s), year:	1994
Report/Doc. number::	A87567 / M-161477-01-1
Guideline(s):	No OECD guideline for 28 days studies with non-rodents available
GLP:	Yes
Deviations from OECD:	No OECD guideline for 28 days studies with non-rodents available but the number of animals was anyhow too low for any other information but estimation of approximate dose for further testing
Acceptability:	Yes (limited information); range finding test

## Material and methods

Eight Beagle dogs (4 males and 4 females) were allocated to the study. The study consisted of two phases; an increasing dose level phase of 24 days duration and a 28 day fixed dose phase. No information on purity of the test material could be found in the study report.

The doses were: Phase 1 (increasing dose): 10-30-100-300-1000-1500-2000 mg/kg (capsule) and 2000 mg/kg (gavage). Phase 2 (fixed dose): 500; 1000 and 2 000 mg/kg (gavage).

Phase 1: increasing dose (every 3 days) according to the dosing scheme for 24 days. On days 1-21, the test article was administered orally in gelatine capsules. Group 1 animals received the same number of gelatine capsules as group 2 animals but empty and acted as controls. On days 22-24, the animals were dosed orally, by

gavage. On days 22-24 control animals received the vehicle, 1% methylcellulose alone. Group 1 animals were then allocated to phase 2.

Phase 2 (fixed dose): the animals were dosed according to the dosing scheme for 28 days. During phase 2 the test article was administered orally by gavage once daily.

**Table B.6.3.1.2-1: Allocation of animals according to phase 1**

Group number	Number of animals		Dose levels (mg/kg bw/d) on days :							
	Males	Females	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24*
1	3	3	0	0	0	0	0	0	0	0
2	1	1	10	30	100	300	1000	1500	2000	2000

\* animals dosed by gavage

**Table B.6.3.1.2-2: Allocation of animals according to phase 2**

Group number	Number of animals		Dose level (mg/kg bw/d)
	Males	Females	
1	1	1	500
2	1	1	1000
3	1	1	2000

During the treatment periods clinical signs and food consumption were recorded daily. Bodyweights were recorded daily during phase 1 and weekly during phase 2.

Blood samples for clinical pathology examination were obtained before the start of phase 1 and again at the end of phase 1 and 2.

At the end of each phase animals were subject to a necropsy and the weights of adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroids (incl. parathyroids) were recorded. Kidneys, liver, lungs and all gross lesions were subject to histopathological examination.

## Results

There were no deaths during any phase of the treatment. There were no clear treatment related clinical signs noted.

Body weight gains and food consumption were unaffected by treatment.

No treatment-related haematological changes were observed. No treatment related changes in clinic-chemical parameters were measured but the cholesterol levels were reduced in both, the male and female animals dosed at 2000 mg/kg/day during phase 2. The relevance of this finding is questionable.

Absolute and relative spleen weights were increased in one treated male animal at the end of phase 1 (dosed from 10 to 2000 mg/kg bw/d), and were also increased during phase 2 in the male animal dosed at 500 mg/kg/day and in both the male and female animals dosed at 2000 mg/kg/day. In the male animal of phase 1 no

gross lesion of the spleen was observed and no histopathological findings recorded. In the one male and one female of the phase 2 dosed at 2000 mg/kg bw/d the spleen had abnormal shape/colour but no abnormal histopathological observations were recorded.

Minimal glomerular vacuolar degeneration was observed in the kidneys of the male and female animals dosed at 2000 mg/kg/day during phase 2.

### Conclusion

The NOAEL of the study (conducted with the lowest number of animals possible) was considered to be 1000 mg/kg bw/d based on increased relative and absolute spleen weight and minimal glomerular vacuolar degeneration in the kidneys in both male and female animal dosed with 2000 mg/kg bw/d.

### B.6.3.2. Oral 90- day study

#### B.6.3.2.1. Rat

<b>Reference:</b>	ETHOFUMESATE TECHNICAL: NINETY-DAY (Dietary Administration) TOXICITY STUDY IN THE RAT
Author(s), year:	████████████████████ 1989
Report/Doc. number::	A83225 / M-155496-01-1
Guideline(s):	OECD 408 (1981)
GLP:	Yes
Deviations from OECD 408 (1998):	- no functional observations conducted, no histopathology of salivary gland, mammary gland, accessory sex organs and prostate
Acceptability:	Yes

### Material and methods

Rats (Sprague-Dawley), six to seven weeks old, were given ethofumesate (purity: 99.5%) by dietary administration at 0 (control), 1000, 5000, and 20000 ppm. The achieved mean test material daily intake was 78, 350 and 1449 mg/kg for males and 88, 410 and 1651 mg/kg for females. The treatment lasted for 90 days and 10 animals per sex were allocated to the control and treated groups. Mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy examination, haematology, and blood chemistry were investigated. Adrenals, kidneys, brain, pituitary, gonads, spleen, heart and liver were weighed. All tissues from high dose and control group animals were examined microscopically.

### Results

There were no treatment-related mortalities or clinical signs of toxicity.

Overall female body weight gain at 20000 ppm was reduced to 67% of the controls. Overall body weight gain in females at lower dose levels and in males was unaffected by treatment. Food consumption of females at 20000 ppm was consistently reduced compared with the controls. At the end of the study the high dose females had 16.2% lower body weight than the control female animals.

Ophthalmoscopic examination revealed no treatment related effects.

Total white blood cells were reduced in males from all treated groups but were within the normal range for rats of this strain and age and the findings were rather attributable to unusually high values in the control male

animals. Albumin levels were reduced in females of mid and high dose group with consequent reduction in albumin/globulin ratio. Additionally, blood urea levels were significantly reduced in high dose females. The study author explained these findings with reduced protein intake of high dose females which showed reduced food consumption during the study.

No treatment related macroscopic abnormalities were detected.

Statistically significant increases in the absolute and relative liver and kidney weight were seen in males at 20000 ppm. The relative liver weight of high dose males was 21% higher than in the control group, the relative kidney weight was 13% higher. In females of high dose group relative liver weight was 21% higher than in the control animals. The histopathological evaluation revealed no treatment related effects in high dose animals.

## Conclusion

The no observable effect level (NOAEL) was 5000 ppm which approximated to an achieved intake of 350 and 410 mg/kg/day for males and females respectively. The NOAEL is based on decreased body weight and body weight gain in females and on the increased relative weight of liver and kidneys in males and increased relative weight of liver in females, all findings observed at 20000 ppm.

<b>Reference:</b>	90 day oral toxicity study in Wistar rats - Ethofumesate technical
Author(s), year:	██████████ 1992
Report/Doc. number::	OFC00004845 / M-352012-01-1
Guideline(s):	OECD 408 (1981)
GLP:	Yes
Deviations from OECD 408 (1998):	- no functional observations conducted, - no ophthalmological examination conducted - no organ weight of thymus, spleen, brain and heart measured - no histopathology of mammary gland, prostate, thymus, spinal cord, trachea, aorta, peripheral nerve and bone marrow done
Acceptability:	Yes

## Material and methods

10 Wistar rats per sex and group, eight weeks old, were given ethofumesate technical (purity: 98%) by dietary administration at 0 (control), 200, 2000, and 20000 ppm corresponding to 0, 14, 140 and 1400 mg/kg bw/d in males and 18, 160 and 2000 mg/kg bw/d in females for 90 days. An additional recovery group (30 days no medication period) dosed with 20000 ppm (1400 mg/kg bw/d in males and 2000 mg/kg bw/d in females) was included in the study. Mortality, clinical signs, body weight, food and water consumption, haematology, and blood chemistry were investigated. Adrenals, kidneys, gonads, and liver were weighed. All selected tissues from high dose and control group animals were examined microscopically.

## Results

One male of the high dose group died one day prior to termination of the study. No clinical signs of toxicity were observed in the treated animals during the study.



No statistically significant differences in body weight and food consumption could be observed between control and treated males of any group. In females of the high dose group slightly lower body weight (7% less than in the control) was observed after 90 days but this turned to the values of the control group during the recovery period. No statistically significant differences in haematology parameters between control and treated animals (males and females) were observed.

Na<sup>+</sup>-levels in the blood were increased in males at the high and medium dose levels, these changes were not observed in the high dose recovery group. In females, none of the clinic chemical parameters showed significant intergroup differences.

The absolute (+14.9%) and relative (+10.2%) (reversible) liver weight in males was statistically significantly increased in the high dose group. In females of the mid and high dose groups, as well as in the recovery group, the relative weight of ovaries was statistically significantly increased (mid dose: + 18.5%, high dose: 14.8%, recovery group: 11.1%). However, it is recognised that organ-to body-weight ratio has no prediction for ovary weight<sup>1</sup>. The histopathological examination did not reveal differences between control and treated animals.

## Conclusion

The NOAEL was set at 20000 ppm (1400 mg/kg bw/d in males and 2000 mg/kg bw/d in females), the highest tested dose. The increased liver and ovary weight, both below 20% increase comparing to control and without any histopathological finding were not considered adverse effects.

<b>Reference:</b>	ETHOFUMESATE: TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS
Author(s), year:	1989
Report/Doc. number::	A89580 / M-165018-01-1
Guideline(s):	OECD 408 (1981)
GLP:	Yes
Deviations from OECD 408 (1998):	- no functional observations conducted, - no organ weight of thymus measured - no histopathological examination of eyes, mammary gland, prostate, salivary gland, seminal vesicles, skeletal muscle, skin and spinal cord done
Acceptability:	Yes

## Material and methods

Rats (CrI:CD (SD) BR), approximately 7 weeks old, were given ethofumesate (purity: 98.9%) by dietary administration at 0 (control), 300, 3000 and 30000 ppm, corresponding to 18.2, 190 and 1900 mg/kg bw/d in males and 23.4, 230 and 2309 mg/kg bw/d in females. The treatment lasted for 13 weeks and 10 rats per sex were allocated to treatment groups. Mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, and blood chemistry were investigated. Adrenals, liver, testes, brain, ovaries, thyroid, heart, pituitary, uterus, kidneys and spleen were weighed. All selected tissues from high dose and control group animals were examined microscopically. Additionally, livers and kidneys from male rats from the low and intermediate dosage groups were histopathologically examined.

<sup>1</sup> Bailey at al., 2004: Relationships Between OrganWeight and Body/BrainWeight in the Rat: What Is the Best Analytical Endpoint. Toxicologic Pathology, 32:448–466, 2004

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## Results

There were no deaths and no clinical findings considered attributable to treatment with ethofumesate.

At 30000 ppm, body weight gain and food consumption were significantly reduced in females. Body weight gain in these females was reduced to 53% of the control animals whilst the food consumption was 90% of the control animals. No comparable effects were observed in males.

Ophthalmoscopic examinations revealed no ocular changes.

Regarding the haematological parameters there were no significant differences between control and treated animals. A minor increase in the albumin concentration for males given 30000 ppm and a decrease in the GPT activity for females of this group was noted.

Significantly increased liver weights were apparent among females given 30000 ppm. The relative liver weight in females treated with 30000 ppm was 46% higher than in the control animals. In females at 3000 and 30000 ppm the absolute uterus weight (no adjusted organ weight reported) was statistically significantly higher than in control animals (+ 68% at 3000 ppm and +15.9% at 30000 ppm) but there was no dose response. The kidney weight in females of high dose group was not statistically significantly different but was 25% higher than in the controls.

In males of 30000 ppm group statistically significant difference was estimated for relative spleen (+ 24.3%) and kidney weight (+ 24.8%) than in the control animals. The liver weight was not statistically significantly higher but the relative liver weight was 19% higher than in control animals.

A microscopic examination revealed an increased number of male rats, given 30000 ppm, with irregular cortical scarring of the kidney, increased eosinophilia of periportal cells and minimal periportal hepatocyte fat deposition in the livers. None of these findings were observed in females at any dose level.

Areas of tubular basophilia, dilatation, inflammation and/or fibrosis were noted in the kidneys of 4/10 males given 30000 ppm. Areas of tubular basophilia were also noted of 2/10 males given 300 ppm. This effect was not seen in the 3000 ppm males or among the females. Since tubular basophilia in the kidneys is a relatively common finding in male rats and the alteration was not dose-related the effects at 300 ppm were not considered to be treatment related. In females of 30000 ppm no histopathological findings in spleen or uterus accompanied the increased organ weight at this dose.

## Conclusion

The NOAEL in this study was 3000 ppm (190 mg/kg bw/day in males and 230 mg/kg bw/d in females) based on reduced body weight gain in females, increased weight of liver (males and females) and kidneys (males) together with histological changes in these organs (males) and on increased weight of spleen in males and uterus in females, all effects observed at 30000 ppm.

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*The study below (██████, 1990) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was*

not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).

#### 90-day feeding study in rats [REDACTED], 1990)

##### *Experimental design*

Rats (CD) were given ethofumesate by dietary administration at 0 (control), 500, 1000, 1500, and 3000 ppm. The treatment lasted for 90 days and there were 10 animals/sex/group. An additional recovery group was included in the study. Mortality, clinical signs, body weight, food and water consumption, ophthalmoscopic investigation, haematology, blood chemistry and necropsy were investigated.

##### *Results*

One control animal died during the dosing period and one animal was mechanically injured and sacrificed for humane reasons.

The only effects seen in the animals were some significant differences in some clinical chemistry analyses. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound related adverse effect was seen.

The no observable effect level (NOEL) was 3000 ppm in the diet based on the lack of toxic effects.

##### *Comments*

There were no compound related adverse effects on the animals. There is a QA statement and a statement of compliance with GLP standards. The NOEL in this study was 3000 ppm, approximately comparable to 300 mg/kg bw/day.

#### **B.6.3.2.2. Mouse**

<b>Reference:</b>	ETHOFUMESATE 13 WEEK ORAL (DIETARY) DOSE RANGE FINDING STUDY IN THE MOUSE.
Author(s), year:	[REDACTED] 1990
Report/Doc. number::	A89579 / M-165016-01-1
Guideline(s):	OECD 408 (1981)
GLP:	Yes
Deviations from OECD 408 (1998):	- no functional observations conducted, - no clinic-chemical and haematological parameters investigated - no organ weights recorded - histopathological examinations conducted only on those tissues and organs showing macroscopic abnormalities
Acceptability:	Yes (limited information)

#### **Material and methods**

Mice (CrI :CD-1 (ICR)BR), approximately 6 weeks old, were given ethofumesate (purity: 97%) by dietary administration at 0 (control), 300, 3000 or 10000 ppm, corresponding to 45, 450 and 1500 mg/kg bw/d (using

conversion factor of 0.15). The treatment lasted for 13 weeks and 10 animals per sex were allocated to treatment groups. After 13 weeks of treatment all the surviving animals were sacrificed by carbon dioxide asphyxiation and subjected to a detailed necropsy. Only tissues or organs showing abnormalities were processed and examined microscopically.

## Results

One high dose group female animal died on day 90 of the study.

No treatment related clinical signs were observed during the study and there was no treatment related effect on the body weight gain. Food consumption of the treated groups was similar to that of the controls. There were no treatment related macroscopic post mortem findings or histological changes.

## Conclusion

There were no toxic effects in any dose group. The NOAEL in this study is 10000 ppm ethofumesate in the diet (corresponding to approximately 1500 mg/kg bw/d), the highest tested dose.

### *B.6.3.2.3. Dog*

<b>Reference:</b>	ETHOFUMESATE: 13 WEEK ORAL (GAVAGE) TOXICITY STUDY IN THE DOG.
Author(s), year:	1994
Report/Doc. number::	A87568 / M-161478-01-1
Guideline(s):	OECD 409 (1981)
GLP:	Yes
Deviations from OECD 408 (1998):	- no organ weights of epididymes and uterus recorded
Acceptability:	Yes

## Material and methods

Thirty-two (16 male and 16 female) pure bred beagle dogs, approximately 7 months old, were allocated to the study. They were divided into 4 groups, each consisting of 4 males and 4 females. Three groups received a suspension of the test article, ethofumesate (purity: 98.6%), orally (by gavage), once daily for 13 weeks, at dose levels of 250, 750 or 1500 mg/kg bw/d. The fourth group received the vehicle alone and acted as a control. Mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, and blood chemistry were investigated. Adrenals, liver, testes, brain, ovaries, thyroid, heart, pituitary, kidneys, spleen, thymus and lungs were weighed. All selected tissues from all groups were examined microscopically.

## Results

All animals survived the treatment period and there were no clinical signs noted which were considered to be attributable to administration of the test article.

Body weights and body weight gains were unaffected by the treatment.

There were no treatment related ocular or haematological changes.

Increases were noted in the alkaline phosphatase levels in one male dosed at 750 mg/kg/day and two males and one female dosed at 1500 mg/kg/day. Total protein and calcium levels were decreased in the males dosed at 1500 mg/kg/day.

The relative liver weight in males was 27% higher in animals dosed with 750 mg/kg bw/d and 32% higher in males dosed with 1500 mg/kg bw/d. In females, the relative liver weight was 14% higher at 750 mg/kg bw/d and 21% higher at 1500 mg/kg bw/d than in controls. The study author explained that this is normal in this laboratory for animals of this age and strain. The explanation is considered questionable. The weight changes in other organs showed no dose response relationships and were within normal range.

No treatment related macroscopic or histopathological abnormalities were noted in any organ.

## Conclusion

The NOAEL in 90 days study with Beagle dogs was 250 mg/kg bw/day based on an increase in relative liver weight in both males and females and increase in the alkaline phosphatase levels at 750 mg/kg bw/d in one male.

### B.6.3.3. Other routes

<b>Reference:</b>	TECHNICAL ETHOFUMESATE: RABBIT TWENTY ONE DAY DERMAL TOXICITY STUDY
Author(s), year:	[REDACTED] 1991
Report/Doc. number::	A83209 / M-155481-01-1
Guideline(s):	OECD 410 (1981)
GLP:	Yes
Deviations from OECD 419 (1981):	No
Acceptability:	Yes

## Material and methods

Groups of 5 male and 5 female New Zealand White rabbits were treated for 6 hours by a semi-occlusive dermal application of 0, 100, 300 or 1000 mg/kg bw/d ethofumesate (purity: 98.3%) once daily for 21 consecutive days. The test substance was wetted with methylcellulose in water. Animals were observed daily for clinical signs of toxicity and skin irritation. Body weight and food consumption were measured weekly whilst clinical chemistry and haematology determinations were conducted just prior to termination. At necropsy, selected organs (adrenals, liver, testes (with epididymis), kidneys and ovaries) were weighed, macroscopic abnormalities recorded and samples of treated and untreated skin from all animals and a range of tissues from the high dose and control rabbits was preserved. The skin samples from all animals and the liver and kidney of animals from the high dose and control groups were examined histopathologically.

## Results

There were no mortalities and no treatment related clinical signs. No dermal irritation was seen in animals treated with 1000 mg/kg bw/d. There were no significant differences between body weight gains recorded for control and treated animals. There were no treatment related differences between the groups in haematological

parameters and clinical chemistry. No significant differences in organ weights and no macroscopic findings were observed in treated and control animals.

## Conclusion

The NOAEL in 21 day dermal toxicity study in rabbits was 1000 mg/kg bw/d, the highest dose tested.

### B.6.4. GENOTOXICITY

Only one new AMES test (Sokolowski, 2013) was submitted for the re-newal of ethofumesate by the TaskForce (the justification was the new specification). The evaluations of all other studies presented below were already included in the original DAR (1998). Only re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for genotoxicity was proven. The RMS did not derive in the DRAR any different general conclusion on genotoxicity than originally derived in the DAR.

One AMES test (Thompson, 1992) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998) in the DRAR.

Two studies evaluated in the DAR (Wright, 1992: In vitro mammalian cytogenetic test and █████ 1992: In vivo mammalian bone marrow cytogenetic test – chromosomal analysis) were not listed in the reference lists of the DAR, either in Volume B5 (References relied upon) or in Volume 2 (all submitted studies). Since the new RMS does not have the complete original dossier from 1990-ies these two studies were not subject for re-wording or inclusion of additional information. The summaries of the studies were copied from the DAR (1998) in the DRAR.

#### B.6.4.1. In vitro studies

##### *B.6.4.1.1. Bacterial assay for gene mutation*

<b>Reference:</b>	Ethofumesate - Bacterial Mutation Assay
Author(s), year:	Gant, R., 1994
Report/Doc. number::	A83222 / M-155493-01-1
Guideline(s):	OECD 471 (1983), OECD 472 (1983)
GLP:	Yes
Deviations from OECD 471 (1997):	No
Acceptability:	Yes

## Material and Methods

### Test material:

Technical Ethofumesate

Batch No.: 19291/02/940701

Purity: 97.2%

Appearance: Off-white powder

Solvent/Vehicle: Dimethylsulphoxid (DMSO)

Positive Control:

In the absence of S-9 mix: N-Ethyl-N-nitro-N-nitrosoguanidin, 9-Aminoacridine, 2-Nitrofluorene

In the presence of S-9 mix: 2-Aminoanthracene

Bacterial strains:

The gene mutation test was performed on four different strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) and on two strains of *Escherichia coli* (CM 881 (WP2 with the addition of R factor pKM101 and CM 891 (WP2 with uvrA deletion and with the addition of A-factor pKM101)).

Test Procedure:

Preliminary Cytotoxicity test

Four concentrations of the test substance were assessed for toxicity using the six test strains. The highest concentration was 5000 µg/plate, followed by three 10-fold serial dilutions, with and without S9. The negative control was the chosen solvent, dimethyl sulphoxide.

Mutation Test

Five concentrations (15, 50, 150, 500, 1500 and 5000 µg/plate) of the test substance separated by half log<sub>10</sub> intervals were assessed using the six test strains. Aliquots of 0.1 ml of each bacterial strain dilution, containing approximately  $2 \times 10^9$  cells/ml, in both the presence (0.5 ml S-9 mix) and absence (0.5 ml 0.1 M phosphate buffer (pH 7.4)) of an exogenous metabolising system were placed in glass bottles. An aliquot of 0.1 ml of the test solution was added, followed by 2 ml of histidine/tryptophan deficient agar. The mixture was overlaid onto petri dishes containing 25 ml minimal agar. Three petri dishes were used for each dose level. The negative control was the chosen solvent, dimethyl sulphoxide. After incubation at 37°C for 3 days the number of revertant colonies was counted.

**Results**

Preliminary Cytotoxicity test

Toxicity was observed at 5000 µg/plate with S-9 mix and 0.1 M phosphate buffer towards TA 1535, TA 1537, TA 98 and TA 100. No substantial increases in revertant colony numbers of any of the test strains were observed following treatment with Technical Ethofumesate at any dose level, in the presence or absence of S-9 mix.

Mutation Test

Toxicity and precipitation were observed at 5000 µg/plate towards all the test strains and towards TA 1537, TA 98 and TA 100 at 1500 µg/plate. Toxicity was also observed in the absence of S-9 mix towards TA 1535, TA 1537 and TA 98 at 500 µg/plate.

No substantial increases in revertant colony numbers of any of the test strains were observed following treatment with Technical Ethofumesate at any dose level, in the presence or absence of S-9 mix.

The positive control gave a clear, positive result. The negative control (solvent) gave background levels.

### Conclusion

Ethofumesate was not mutagenic in the acceptable bacterial reverse mutation assay under the experimental test conditions.

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<b>Reference:</b>	Examination of ethofumesate for mutagenic activity in the ames test
Author(s), year:	Wilmer, J. W. G. M., 1987
Report/Doc. number::	A87570 / M-161481-01-1
Guideline(s):	OECD 471(1983)
GLP:	Yes
Deviations from OECD 471 (1997):	-no exogenous metabolizing system was used in the preliminary cytotoxicity test - no <i>E.coli</i> WP2 or <i>S.typhimurium</i> TA102 tested
Acceptability:	Yes

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### Material and Methods

#### Test material:

Ethofumesate

Batch No.: not stated

Purity: 98-99%

Appearance: white powder

Solvent/Vehicle: Dimethylsulphoxid (DMSO)

#### Positive Control:

In the absence of S-9 mix: Sodium azide with the strain TA 1535 and TA 100, 2-Nitrofluorene with the strains TA 1538 and TA 98, 9-aminoanthracene with the strain TA 1537

In the presence of S-9 mix: 2-Aminoanthracene with all strains

#### Bacterial strains:

The gene mutation test was performed on five different strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 1538, TA 98 and TA 100).

#### Test Procedure:



#### Preliminary Cytotoxicity test

Six concentrations of the test substance were assessed for toxicity using test strain TA 98. The highest concentration was 50000 µg/plate followed by five 10-fold serial dilutions. Aliquots of 0.1 ml of the appropriate bacterial strain dilution were placed to 2 ml molten agar. An aliquot of 0.1 ml of the test solution was added. The mixture was overlaid onto minimal glucose agar plates. A single plate was used for each dose level. The negative control was the solvent dimethylsulphoxide. After incubation at 37°C for 3 days the number of revertant colonies was counted.

#### Mutation Test

Five concentrations of the test substance (62, 185, 556, 1667 and 5000 µg/plate) were assessed using the five test strains. Aliquots of 0.1 ml of each bacterial strain dilution and an aliquot of 0.1 ml of the test solution were added to 2 ml molten top agar. Each concentration and test strain was tested in the presence and absence of a 0.5 ml exogenous metabolizing system (S-9 mix). The mixture was overlaid onto minimal glucose agar plates. Three plates were used for each dose level. The negative control was the solvent dimethylsulphoxide. After incubation at 37°C for 3 days the number of revertant colonies was counted.

### Results

#### Preliminary cytotoxicity test

Toxicity was not observed for TA 98 at any concentrations. Precipitation was observed for TA 98 at 5000 and 50000 µg/plate.

#### Mutation Test

No evidence of mutagenic activity was seen at any dose level. The positive controls (sodium azide, 2-nitrofluorene, 9-aminoacridine and 2-aminoanthracene) gave a clear, positive result. The negative control (solvent) gave background levels.

### Conclusion

Ethofumesate was not mutagenic in the acceptable bacterial reverse mutation assay under the experimental test conditions.

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<b>Reference:</b>	Mutagenicity – Salmonella Typhimurium Reverse Mutation Assay (AMES Test)
Author(s), year:	Suresh, T. P., 1993
Report/Doc. number::	904-MUT:AMES / M-359339-01-1
Guideline(s):	OECD 471(1983)
GLP:	Yes
Deviations from OECD 471 (1997):	- no <i>E.coli</i> WP2 or <i>S.typhimurium</i> TA102 tested
Acceptability:	Yes

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### Material and Methods

Test material:

Ethofumesate

Batch No.: 09/06/91

Purity: 98%

Appearance: solid white coloured crystals, odourless

Solvent/Vehicle: Dimethylsulphoxid (DMSO)

Positive Control:

In the absence of S-9 mix: Sodium azide with the strain TA 1535 and TA 100, 2-Nitrofluorene with the strains TA 1538 and TA 98, 9-aminoacridine with the strain TA 1537

In the presence of S-9 mix:

9-Aminoacridine with the strains TA 1537, 2-Aminofluorone with the strain TA 1538, TA 98 and TA 100 and Sodium azide with the strain TA 1535

Bacterial strains:

The gene mutation test was performed on five different strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 1538, TA 98 and TA 100).

Test Procedure:Preliminary Precipitation Test

Five concentrations (1, 3, 6, 8 and 10 mg/plate) of the test substance were tested for precipitation using the five test strains. The plates were read after 2 hours.

Preliminary Cytotoxicity test

Five concentrations (0.1, 0.5, 1, 5 and 10 mg/plate) of the test substance were assessed for toxicity using TA 100 test strain. The test compound was plated with an overnight TA 100 culture on selective minimal agar, both in the presence and absence of metabolic activation.

Mutation Test

Five concentrations (0.1, 0.5, 1, 5 and 10 mg/plate) of the test substance were assessed using the five test strains with and without metabolic activation. Aliquots of 0.1 ml of each bacterial strain dilution, containing approximately  $2 \times 10^9$  cells/ml, in both the presence (0.5 ml S-9 mix) and absence (0.5 ml 0.05 M phosphate buffer (pH 7.4)) of an exogenous metabolising system, an aliquot of 0.1 ml of the test solution and 2 ml soft agar were mixed. The mixture was overlaid onto 25 ml minimal bottom agar. Three petri dishes were used for each dose level. The negative control was the chosen solvent, dimethyl sulphoxide. After incubation at 37°C for 2 days the number of revertant colonies was counted.

**Results**

#### Preliminary Precipitation Test

The test compound precipitates at 3 mg/plate but the precipitation did not interfere with the counting of revertants.

#### Preliminary Cytotoxicity Test

At the highest concentration of 10 mg/plate a slight thinning of the bacterial background lawn was observed. At 5 and 10 mg/plate rough edged colonies were noticed.

#### Mutation Test

No evidence of mutagenic activity was seen at any dose level. The positive controls (2-aminofluorene, 2-nitrofluorene, sodium azide and 9AA) gave a marked, positive result. The negative control (solvent) gave background levels.

#### **Conclusion**

Ethofumesate was not mutagenic in the acceptable bacterial reverse mutation assay under the experimental test conditions.

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<b>Reference:</b>	Salmonella Typhimurium Reverse Mutation Assay with Ethofumesate, technical
Author(s), year:	Sokolowski, A., 2013
Report/Doc. number::	1523800 / M-449020-01-1
Guideline(s):	OECD 471(1997)
GLP:	Yes
Deviations from OECD Guideline:	No
Acceptability:	Yes

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#### **Material and Methods**

##### Test material:

Technical Ethofumesate

Batch No.: PFHCA-2012-11-12

Purity: 96.3% w/w

Appearance: not stated

Solvent/Vehicle: Dimethylsulphoxid (DMSO)

##### Positive Control:

In the absence of S-9 mix: Sodium azide with the strain TA 1535 and TA 100, 4-nitro-o-phenylene-diamine with the strains TA 1537 and TA 98, MMS with the strain TA 102

In the presence of S-9 mix:

2-aminoanthracene with all the strains used

##### Bacterial strains:

The gene mutation test was performed on five different strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 100 and TA 102)

#### Test Procedure:

##### Preliminary Cytotoxicity test

Eight concentrations (3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate) of the test substance were assessed for toxicity using the five test strains. Aliquots of 0.1 ml of each bacterial strain dilution, containing approximately  $2 \times 10^9$  cells/ml, in both the presence (0.5 ml S-9 mix) and absence (0.5 ml S-9 mix substitution buffer) of an exogenous metabolising system, were mixed with an aliquot of 0.1 ml of the test solution and 2 ml overlay agar. The mixture was overlaid onto selective agar plates. Three plates were used for each dose level. The negative control was the solvent dimethyl sulphoxide. After incubation at 37°C for at least 2 days the number of revertant colonies was counted.

##### Mutation Test

A plate incorporation and a preincubation assay was conducted. Both types of assay were conducted as follows. Eight concentrations (3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate) of the test substance were assessed using the five test strains. Aliquots of 0.1 ml of each bacterial strain dilution, containing approximately  $10^8$  -  $10^9$  cells/ml, in both the presence (0.5 ml S-9 mix) and absence (0.5 ml S-9 substitution buffer) of an exogenous metabolising system were mixed with an aliquot of 0.1 ml of the test solution and 2 ml of overlay agar. In the preincubation assay 2.0 ml overlay agar (45 °C) was added after preincubation at 37°C for 60 minutes. The mixture was overlaid onto selective agar plates. Three plates were used for each dose level. The negative control was the chosen solvent, dimethyl sulphoxide. After incubation at 37°C for at least 2 days the number of revertant colonies was counted.

#### **Results**

##### Preliminary Cytotoxicity test

Toxicity was observed at 5000 and 2500 µg/plate with and without S-9 mix towards TA 1537 and TA 102. Precipitation was observed for all strains at 5000 and 2500 µg/plate with and without S-9 mix in the overlay agar. Precipitation was observed for all strains at 5000 and 2500 µg/plate with and without S-9 mix in the overlay agar on the incubated agar plates.

##### Mutation Test

Toxicity was observed at 5000 and 2500 µg/plate with and without S-9 mix towards TA 1537 and TA 102. Precipitation was observed for all strains at 5 and 2.5 mg/plate with S-9 mix in the overlay agar. Precipitation was observed for all strains at 5 and 2.5 mg/plate with and without S-9 mix in the overlay agar on the incubated agar plates.

No evidence of mutagenic activity was seen at any dose level. The positive control gave a clear, positive result. The negative control (solvent) gave background levels.

## Conclusion

Ethofumesate was not mutagenic in the acceptable bacterial reverse mutation assay under the experimental test conditions.

*The study below (Thompson, 1992) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).*

## Reverse mutation assay (Thompson, 1992)

### Experimental design

The gene mutation test was performed on five different strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) in the presence or absence of an exogenous metabolising system (S9-mix). The concentrations of ethofumesate in the study were 8 - 5000 µg/plate.

### Results

No evidence of mutagenic activity was seen at any dose level. The positive controls (ENNG, 9AA, 4NOPD and 4NQO) gave a marked, positive result. The negative control (solvent) gave background levels.

### Comments

Under the experimental conditions in this study, ethofumesate is not mutagenic. The study follows the OECD guideline. There is a QA statement and a statement of compliance with GLP standards.

### ***B.6.4.1.2. Test for clastogenicity in mammalian cells***

<b>Reference:</b>	Technical Ethofumesate: Metaphase Chromosome analysis of human lymphocytes cultured in vitro
Author(s), year:	██ 1986
Report/Doc. number::	A83192 / M-155465-01-1
Guideline(s):	OECD 473 (1983)
GLP:	Yes
Deviations from OECD 473 (1997):	- no significant reduction in the mitotic index of the highest dose achieved
Acceptability:	Yes

## Material and Methods

### Test material:

Technical Ethofumesate

Batch No.: CR 4805/10

Purity: 96.2%

Appearance: off-white powder

Solvent/Vehicle: Dimethylsulphoxid (DMSO)

Positive Control:

In the absence of S-9 mix: Ethyl methanesulphonate

In the presence of S-9 mix: Cyclophosphamide

Cells:

Lymphocytes from aseptically collected human blood

Test Procedure:

First a preliminary toxicity test with the highest concentration of 110 µg/ml test compound (maximum concentration soluble in the test system) was conducted. Furthermore a metaphase analysis with concentrations of 11, 55 and 110 µg/ml test compound was performed. In both the preliminary toxicity test and the metaphase analysis cultured human lymphocytes, stimulated to divide by the addition of phytohaemagglutinin, were exposed to the test compound in both the presence and absence of a metabolic activation system (S-9 mix) derived from rat livers. Cell division was then arrested using colchicine and after hypotonic treatment, fixation and staining, mitotic index was calculated and metaphase spreads were examined for chromosomal damage in the metaphase analysis.

**Results**

In the preliminary toxicity test in the absence and presence of a metabolic activation system (S-9 mix) no substantial decreases in mitotic index were observed. In the metaphase analysis the results were negative at all dose levels with and without metabolic activation. At the highest tested dose no decrease in mitotic index was observed.

Both positive control compounds, ethyl methanesulphonate and cyclophosphamide, caused statistically highly significant increases in the proportion of metaphase spreads containing aberrations when compared with the relevant solvent controls.

**Conclusion**

Ethofumesate has no potential to induce chromosomal aberrations in human lymphocytes in culture under the conditions tested.

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<b>Reference:</b>	Chromosome analysis of Chinese hamster ovary cells treated in vitro with ethofumesate
Author(s), year:	1987
Report/Doc. number::	A87573 / M-161486-01-1
Guideline(s):	OECD 473 (1983)
GLP:	Yes
Deviations from OECD	No

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473 (1997):  
Acceptability: Yes

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## Material and Methods

### Test material:

Ethofumesate

Batch No.: not stated

Purity: 98-99%

Appearance: white powder

Solvent/Vehicle: Ethanol

### Positive Control:

In the absence of S-9 mix: Mitomycin C

In the presence of S-9 mix: Cyclophosphamide

### Cells:

CHO cells (CHO K-1 line) with the cell-cycle time of 12-14 hours

### Test Procedure:

#### Solubility test

The test substance was dissolved in ethanol obtaining concentrations of 11.11, 33.33 and 100.0 mg/ml. 25 µl of each test solution was added to 5 ml tissue culture medium.

#### Toxicity test

The test substance was dissolved in ethanol. The final concentrations of the test substance in the culture medium were 0.08, 0.24, 0.70, 2.15, 6.50, 19.45, 58.35, 175.0 µg/ml. In the absence and in the presence of an exogenous metabolizing system (S-9 mix), 50 µl of each of the test solutions were added to the culture medium. Ethanol was used as negative control. For each concentration of the test substance and for the controls one culture was used. In the absence of an exogenous metabolizing system the cultures were incubated for 21 hours at 37 °C in humidified air containing 5% CO<sub>2</sub>. In the presence of an exogenous metabolizing system (S-9 mix) the exposure period was reduced to 3 hours because of the toxicity of the S-9 mix for the cells. After the 3 hour incubation period the medium was removed, the cells were washed and supplied with 10 ml freshly prepared culture medium. The cells were incubated for an additional 9 or 18 hours (considering the different stages of the cell-cycle) at 37 °C in humidified air containing 5% CO<sub>2</sub>.

Two hours before the total end of the incubation period the cells were arrested in the metaphase stage of the mitosis by the addition of colcemid (0.1 µg/ml medium). Two microscope slides were prepared from each culture and stained in a 2% solution of Giemsa. At least 1000 nuclei per culture were examined to determine the mitotic index.

#### Chromosome aberration test

The test was carried out both in the absence and presence of an exogenous metabolizing system (S-9 mix) and with negative and positive controls. The test substance was dissolved in ethanol. The final concentrations of the test substance in the culture medium were 0, 1.30, 3.89, 11.67 and 35.0 µg/ml without S-9 mix and 0, 6.48, 19.45, 58.35 and 175.0 µg/ml with S-9 mix. For each concentration of the test substance and for the negative and positive controls duplicate cultures were used. Four microscopic slides were prepared from each culture. 200 well-spread metaphases (100 metaphases per culture) were analysed for a wide range of structural chromosome (gaps, breaks, fragments, dicentric, exchanges etc.) aberrations and other anomalies (endoreduplication, polyploidy). Otherwise the test was conducted according to the toxicity test.

### **Results**

#### Solubility test

The test substance appeared to segregate heavily in the culture medium at a final concentration of 500 µg/ml. Little segregation was seen at a final concentration of 166.65 µg/ml. 175.0 µg/ml was chosen as the highest concentration for the toxicity test.

#### Toxicity test

In the absence of the S-9 mix the mitotic index was reduced to about 56% of that of the vehicle control at a concentration of 19.45 µg/ml. In the presence of the S-9 mix and at a harvesting time of 12 hours the mitotic index was reduced to about 80% of that of the control at the highest concentration of 175.0 µg/ml. At a harvesting time of 21 hours no toxicity was observed at any of the concentrations. 35.0 µg/ml was chosen as the highest concentration for the chromosome aberration test in the absence of the S-9 mix and 175.0 µg/ml in the presence of the S-9 mix.

#### Chromosome aberration test

In the absence of the S-9 mix ethofumesate did not induce a statistically significant increase in the number of cells with structural chromosome aberrations at any of the concentrations. In the presence of the S-9 mix ethofumesate induced a statistically significant increase in the number of cells with structural chromosome aberrations at both the harvesting times of 12 and 21 hours, but only at the highest, highly cytotoxic, concentration (175.0 µg/ml). The positive control substances induced the expected increase in the incidence of structural chromosome aberrations. The negative control (solvent) gave background levels.

### **Conclusion**

Since ethofumesate gave positive response at highly cytotoxic dose levels only, the results can be considered as negative. Ethofumesate has no potential to induce chromosomal aberrations in CHO cells in culture under the conditions tested.



The study below (██████ 1992) was not listed in the reference list of the original DAR (1998) but was evaluated there. Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).

***In vitro* mammalian cytogenetic test** (██████ 1992)

*Experimental design*

Human lymphocytes, treated with ethofumesate were evaluated for chromosome aberrations at three dose levels together with negative and positive controls. Three treatment conditions were used: 4 hours exposure with the addition of S-9 mix with a cell harvest after 16 and 26 hour expression periods and a 20 hour continuous exposure in the absence of S-9 mix. The dose levels were 1250 to 5000 µg/ml with S-9 mix and 39 to 156 µg/ml without S-9 mix.

*Results*

The results were negative at all dose levels with and without metabolic activation. The positive control gave a clear, positive result. The negative control (solvent) gave background levels.

*Comments*

Under the experimental conditions in this study, ethofumesate has no clastogenic activity. The study follows the OECD guideline. There is a QA statement and a statement of compliance with GLP standards.

***B.6.4.1.3. Test for gene mutation in mammalian cells***

<b>Reference:</b>	Mouse lymphoma (6TG) fluctuation assay
Author(s), year:	Kennelly, J. C., 1986
Report/Doc. number::	A83191 / M-155464-01-1
Guideline(s):	OECD 476 (1984)
GLP:	Yes
Deviations from OECD 476 (1997):	No
Acceptability:	Yes

**Material and Methods**

Test material:

Technical Ethofumesate

Batch No.: CR 4805/10

Purity: 96.2%

Appearance: Whitish powder

Solvent/Vehicle:

Anhydrous, analytical grade dimethylsulphoxide (DMSO)

Positive Control:

In the absence of S-9 mix: 4-nitroquinoline-N-oxide (NQO)

In the presence of S-9 mix: Benzo(a)pyrene (BP)

Cells:

Wild type L5178Y mouse lymphoma cells

Test Procedure:

Two independent experiments were performed using duplicate cell cultures. In the first experiment, cultures were treated at 7.9, 25, 79 and 250 µg/ml. In Experiment 2, dose levels of 50, 100, 150, 200 and 250 µg/ml were used. A final concentration of 250 µg/ml was the maximum soluble concentration in the tissue culture medium. Each concentration was tested in the presence and absence of an exogenous metabolising system (S-9 mix). DMSO was used as the vehicle control in all the experiments. Incubation time of survival, viability and 6-thioguanin resistance test was 1-2 weeks (5% CO<sub>2</sub>).

**Results**

At the highest concentration of 250 µg/ml the survival values in the two experiments were 59 and 75 % of control values in the presence of S-9 mix and 67 and 82 % in its absence. Ethofumesate showed no evidence of mutagenic activity in the presence of S-9 mix in either experiment and was not mutagenic in the absence of S-9 mix in Experiment 2. In the absence of S-9 mix in Experiment 1, the slightly higher mutation frequencies seen at all dose levels of ethofumesate were well within the historical control range and there was no dose response relationship. Therefore they were considered to reflect the very low mutation frequency of the concurrent solvent control. The positive control substances all gave the expected response. The negative control (solvent) gave background levels.

**Conclusion**

In the in vitro mutagenicity study ethofumesate did not induce gene mutations in L5178Y mouse lymphoma cells.

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<b>Reference:</b>	An investigation into the possible induction of point mutations at the HGPRT locus of Chinese hamster ovary cells by ethofumesate
Author(s), year:	██████████ 1987
Report/Doc. number::	A87571 / M-161483-01-1
Guideline(s):	OECD 476 (1984)
GLP:	Yes
Deviations from OECD 476 (1997):	No
Acceptability:	Yes

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**Material and Methods**Test material:

Ethofumesate

Batch No.: not stated

Purity: 98-99%

Appearance: White powder

Solvent/Vehicle: Dimethylsulphoxide (DMSO)

Positive Control:

In the absence of S-9 mix: Ethyl methanesulphonate

In the presence of S-9 mix: Dimethyl nitrosamine

Cells:

Chinese hamster ovary cells (cell line CHO-K1, ATCC no. CCL 61)

Test Procedure:

Toxicity test

Cultures were treated at 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 µg/ml. Each concentration was tested in the presence and absence of an exogenous metabolising system (S-9 mix). DMSO was used as the vehicle control in all the experiments. Aliquots of 10 ml of the cell suspensions, containing at least  $1.75 \times 10^6$  cells, were dispensed into plastic tissue-culture flasks. The medium in the flasks was replaced by 9.9 ml fresh medium without calf serum (without metabolic activation) or 7.9 ml medium, 1.6 ml cofactor-mix and 0.4 ml S9 (with metabolic activation). 0.1 ml of the test solution and the positive controls were added to each flask as appropriate. After 5 hours of incubation medium was removed by aspiration and the cells were washed with phosphate buffered saline solution and resuspended in fresh growth medium. Incubation was continued for 18 hours. Aliquots of 10 ml of the cell suspension, containing  $2 \times 10^2$  cells, were plated to each of five plastic petri dishes to determine relative cell survival after incubation of 7 days.

Point mutation test

In the first experiment, cultures were treated at 0, 50, 150, 200, 225 and 250 µg/ml. In Experiment 2, dose levels of 0, 80, 160, 240, 320, 360 and 400 µg/ml were used without metabolic activation and 0, 100, 200, 250, 300, 350, 400, 450 and 500 µg/ml with metabolic activation. Aliquots of 20 ml of the cell suspensions, containing at least  $3.5 \times 10^6$  cells, were dispensed into plastic tissue-culture flasks. The medium in the flasks was replaced by 19.8 ml fresh medium without calf serum (without metabolic activation) or 15.8 ml medium, 3.2 ml cofactor-mix and 0.8 ml S9 (with metabolic activation). 0.2 ml of the test solution and the positive controls were added to each flask as appropriate. One flask was exposed to each concentration (with or without metabolic activation) of the test substance. After 5 hours of incubation medium was removed by aspiration and the cells were washed with phosphate buffered saline solution and resuspended in fresh growth medium. Incubation was continued for 18 hours. Aliquots of 10 ml of the cell suspensions, diluted to a density of  $2 \times 10^2$  cells, were plated to five plastic petri dishes per concentration and incubated for 7 days to determine initial relative cell survival (cytotoxicity). After incubation of overall 8 days, each cell suspension was diluted with medium lacking

hypoxanthine and thymidine and containing 6-thioguanine to a cell density of about  $2 \times 10^4$  cells/ml. Aliquots of 10 ml of the cell suspension were plated to ten plastic petri dishes per concentration and incubated for 7 days. After incubation the frequency of 6-thioguanine resistant mutants was measured. Furthermore, aliquots of 10 ml of the cell suspensions, incubated for 8 days and diluted to a density of  $2 \times 10^2$  cells, were plated to three plastic petri dishes per concentration and incubated for 7 days to determine final relative cell survival, which was necessary for calculation of the absolute mutant frequency.

## Results

A concentration dependent increase in the mutation frequency was not found in either of the duplicate tests. The initial survival of the cells exposed to the highest concentrations was significantly reduced. The positive control gave a clear, positive result. The negative control (solvent) gave background levels.

## Conclusion

Under the experimental conditions in this study, ethofumesate is not mutagenic in vitro at the HGPRT locus of CHO-cells.

### *B.6.4.1.4. Test for DNA damage and repair*

<b>Reference:</b>	Technical Ethofumesate: Assessment of Unscheduled DNA Synthesis using Rat Hepatocyte Cultures
Author(s), year:	██████████ 1988
Report/Doc. number::	A83194 / M-155467-01-1
Guideline(s):	OECD 482 (1986)
GLP:	Yes
Deviations:	No
Acceptability:	Yes

## Material and Methods

### Test material:

Technical Ethofumesate

Batch No.: CR 4805/10

Purity: 96.2%

Appearance: off-white powder

Solvent/Vehicle: Dimethylsulphoxide (DMSO)

### Animals:

Two young, adult, male Fischer 344 rats ██████████ One animal per experiment was used.

### Test Procedure

Two independent experiments were performed using cells from different animals. Primary cultures of rat hepatocytes (at least  $10^5$  cells) were prepared. Technical ethofumesate (1.56, 3.125, 6.25, 12, 25, 50, 100 and 200 µg/ml), vehicle control (10 µl), positive control (4, 8, and 16 µg/ml Mischler's Ketone) and 10µCi/ml tritiated thymidine ( $[6^3\text{H}]\text{-Tdr}$ ) were added as appropriate and the cultures were incubated at 37°C for 18-20 hours. Each concentration of the test substance, positive control and vehicle control was tested in quadruplicate wells. The fourth well, stained with trypan blue, was used to monitor the cell viability. The cells were examined microscopically. Unscheduled DNA synthesis was measured by autoradiography in 50 randomly-selected cells with normal appearing nuclei on each of the three prepared slides per concentration. For each cell, the number of nuclear grains was counted from which the mean number of cytoplasmic grains in 3 nuclear-sized areas adjacent to the nucleus was subtracted to give the net number of grains per nucleus. A negative value was obtained where the cytoplasmic grain count was higher than the nuclear count. The total net mean grain count was determined for each slide. For each treatment condition, the mean net nuclear grain count was then calculated from the total net mean grain counts of all 3 slides.

## Results

No evidence of unscheduled DNA synthesis was seen at any concentration of technical ethofumesate in either experiment. Precipitation and indications of toxicity were seen at the highest concentration (200 µg/ml) in both experiments and also at 100 µg/ml in Experiment 1. The concurrent positive controls demonstrated the sensitivity of the assay.

## Conclusion

Ethofumesate is not genotoxic in the DNA damage and repair/unscheduled DNA synthesis test.

### B.6.4.2. *In vivo* studies in somatic cells

<b>Reference:</b>	Technical Ethofumesate: Mouse Micronucleus Test
Author(s), year:	████████████████████ 1985
Report/Doc. number::	A83189 / M-155462-01-1
Guideline(s):	OECD 474 (1983)
GLP:	Yes
Deviations from OECD 476 (1997):	-The presence of micronuclei in 1000 polychromatic erythrocytes instead of at least 2000 was examined in one smear of each animal
Acceptability:	Yes

## Material and Methods

### Test material:

Technical Ethofumesate

Batch No.: CR 4805/10

Purity: 96.3 %

Appearance: brown crystalline powder

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Solvent/Vehicle: 1% w/v methylcellulose in deionised water

Positive Control: Mitomycin C prepared as a 0.2 mg solution in sterile 0.9% saline

Animals:

Specific pathogen free CD-1 outbred mice of Swiss origin ( ) weighing between 22 and 24 g on arrival

Test Procedure:

The animals were kept in a room with controlled environment consisting of 30 changes of air per hour, controlled temperature (22 °C) and light (12 hours light and 12 hours dark). Free access to drinking water and food (LAD 1 rodent diet, K and K Greef Limited, Croydon, Surrey) was allowed throughout the study. The test material was administered by gavage and the positive control mitomycin C was administered intraperitoneal. First a preliminary toxicity test was conducted. According to the preliminary toxicity test a dosage of 8100 mg technical ethofumesate per kg bodyweight was the maximum practicable dose that could be administered and was chosen for the micronucleus test. Thirty-five male and thirty-five female mice were used for the micronucleus test. The animals were observed regularly during the working day for a period of 72 hours for any mortalities or clinical signs.

Bone marrow smears from mice were obtained from the vehicle control and ethofumesate - treated groups at 24, 48 and 72 hours after dosing (five male and five female mice per time point) and from the positive control group 24 hours (five male and five female mice) after dosing. One smear from each animal was examined for the presence of micronuclei in 1000 polychromatic erythrocytes and the incidence of micronucleated normochromatic erythrocytes was assessed by examining at least 1000 erythrocytes from each animal.

**Results**

In females treated with 8100 mg ethofumesate /kg bw clinical signs like pilo-erection, hunched posture and ptosis were observed 1-3 hours after the administration.

The frequency of micronucleated polychromatic erythrocytes in the bone marrow smears from mice treated with technical ethofumesate was comparable to the frequency in the vehicle control mice at all sampling times.

The ratio of polychromatic to normochromatic erythrocytes in animals treated with technical ethofumesate was comparable to the ratio in the vehicle control animals.

The positive control compound, mitomycin C, produced highly significant increases in the frequency of micronucleated polychromatic erythrocytes and a significant decrease in the ratio of polychromatic to normochromatic erythrocytes.

Although no cytotoxicity was observed at 8100 mg/kg bw (the ratio of polychromatic to normochromatic erythrocytes in animals treated with ethofumesate was comparable to the ratio in the vehicle control animals) it is assumed that the ethofumesate administered at 8100 mg/kg bw reached the bone marrow. The assumption is based on the findings from the ADME studies where distribution of ethofumesate into tissues, after low and high dose administration, was very even and ethofumesate was detected in bones and blood. Additionally, in another mouse micronucleus study (■■■■■ 1993) and in the bone marrow cytogenetic test on chromosomal aberrations (■■■■■ 1994) the cytotoxicity was given at 4000 mg/kg bw.

## Conclusion

Under the experimental conditions in this study, ethofumesate is not clastogenic in mouse micronucleus test *in vivo*.

<b>Reference:</b>	Micronucleus assay in bone marrow cells by ethofumesate
Author(s), year:	■■■■■, 1992
Report/Doc. number::	A87572 / M-161484-01-1
Guideline(s):	OECD 474 (1983)
GLP:	Yes
Deviations from OECD 474 (1997):	-The presence of micronuclei in 1000 polychromatic erythrocytes instead of at least 2000 was examined in one smear of each animal
Acceptability:	Yes

## Material and Methods

### Test material:

Ethofumesate

Batch No.: 193

Purity: at least 97%

Appearance: solid white or slightly coloured crystals

Solvent/Vehicle: Polyethylene glycol 400

Positive Control: Cyclophosphamide

### Animals:

NMRI strain mice (■■■■■) weighing approximately 30g at start of treatment

### Test Procedure:

The animals were kept in a room with controlled environment consisting of controlled temperature ( $21 \pm 3$  °C), relative humidity (30-70 %) and light (12 hours light and 12 hours dark). Free access to drinking water and food (pelleted standard diet, Altromin 1324, Altromin Limited, Lage/Lippe, Germany) was allowed throughout the

study. The positive control and the test material were administered orally. According to a preliminary toxicity test a dosage of 5000 mg ethofumesate per kg bodyweight was the maximum attainable dose that could be administered. Six male and six female mice were used per dose (500, 1666, 5000 mg/kg bw), vehicle and positive control group and per time point of bone marrow smear taking.

Bone marrow smears from mice were obtained from the vehicle control and ethofumesate - treated groups at 24, 48 and 72 hours after dosing (five male and five female mice per time point) and from the positive control group 24 hours (five male and five female mice) after dosing. One smear from each animal was examined for the presence of micronuclei in 1000 polychromatic erythrocytes. To describe a cytotoxic effect due to the treatment with the test article the ratio between polychromatic and normochromatic erythrocytes (NCE) was determined in the same sample and reported as the number of NCEs per 1000 PCEs.

## Results

Two female and one male animal from the 1666 mg/kg bw dose group died after treatment. The reasons for these lethalties are unknown. The ratio of normochromatic to polychromatic erythrocytes was not affected by the treatment with ethofumesate, indicating that the test article had no cytotoxic properties at the dose levels tested. In comparison to the corresponding negative controls there was no enhancement in the frequency of the detected micronuclei at any preparation interval after treatment with ethofumesate. The positive control compound produced highly significant increases in the frequency of micronucleated polychromatic erythrocytes.

Although no cytotoxicity (the ratio of polychromatic to normochromatic erythrocytes in animals treated with ethofumesate was comparable to the ratio in the vehicle control animals) was observed it is assumed that the ethofumesate administered reached the bone marrow. The assumption is based on the findings from the ADME studies where distribution of ethofumesate into tissues, after low and high dose administration, was very even and ethofumesate was detected in bones and blood. Additionally, in another mouse micronucleus study (■■■■■, 1993) and in the bone marrow cytogenetic test on chromosomal aberrations (■■■■■, 1994) the cytotoxicity was given at 4000 mg/kg bw.

## Conclusion

Under the experimental conditions in this study, ethofumesate is not clastogenic in mouse micronucleus test *in vivo*.

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<b>Reference:</b>	Mutagenicity-Micronucleus Test in Swiss Albino Mice
Author(s), year:	■■■■■ 1993
Report/Doc. number::	OFC00004855 / M-351970-01-1
Guideline(s):	OECD 474 (1983)
GLP:	Yes
Deviations from OECD 474 (1997):	No
Acceptability:	Yes

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## Material and Methods



Test material:

Ethofumesate technical

Batch No.: 348/055

Purity: 98%

Appearance: white coloured odorless crystals

Solvent/Vehicle: Peanut oil

Positive Control: Cyclophosphamide

Animals:

Swiss Albino mice (Bred [REDACTED]  
[REDACTED] approximately 25-35g at start of study

Test Procedure:

The animals were kept in a room with controlled environment consisting of 12-15 air changes per hour, controlled temperature ( $22 \pm 3$  °C), relative humidity (40-70 %) and light (12 hours light and 12 hours dark). Free access to drinking water and food (Gold Mohur brand; manufactured by M/S Lipton India Ltd., Bangalore, a subsidiary of Unilever England) was allowed throughout the study. The positive control and the test material (40, 400 and 4000 mg/kg bw) were administered orally as gavage. The animals were treated twice, once daily for two consecutive days. The animals were observed twice a day for any mortalities or clinical signs. Body weight was determined on day one, two and at sacrifice.

Four to six slides of bone marrow smears from each mouse were obtained from the vehicle control and ethofumesate - treated groups (ten animals per sex from the vehicle control and five animals per sex from the ethofumesate-treated groups) and from the positive control group 24 hours (five animals per sex) after second treatment. From each animal a minimum of 2000 erythrocytes from four slides were examined. The following parameters were calculated: the number and percentage of polychromatic erythrocytes (PCE) with micronuclei, the number and percentage of normochromatic erythrocytes (NCE) with micronuclei, percentage RBCs with micronuclei and the ratio PCE : NCE.

**Results**

Some mice in the mid dose (400 mg/kg bw) and all mice in high dose (4000 mg/kg bw) lost body weight marginally due to treatment. No pharmacotoxic symptoms, mortality and gross lesions were seen in the treatment groups.

In comparison to the corresponding negative controls there was a statistically significant increase in the incidence of micronuclei at the high (male and female) and mid dose (female) level. Ethofumesate was also cytotoxic at the high dose level of 4000 mg/kg bw. No changes in the incidence of micronucleated cells were observed in the low dose groups compared to the control animals.

The positive control compound produced highly significant increases in the frequency of micronucleated erythrocytes.

### Conclusion

Under the experimental conditions of the mouse micronucleus test ethofumesate gave positive response at the dose levels of 400 and 4000 mg/kg bw but was cytotoxic at the highest dose.

<b>Reference:</b>	Genetic Toxicology – In vivo mammalian bone marrow cytogenetic test – Chromosomal analyses
Author(s), year:	██████████ 1994
Report/Doc. number::	OFC00004856 / M-351972-01-1
Guideline(s):	OECD 475(1984)
GLP:	Yes
Deviations from OECD 475 (1997):	<ul style="list-style-type: none"> <li>- Test material was administered twice within 2 days</li> <li>- Animals are sampled after 1.5 hours of metaphase arresting agent (instead of 3-5 hours)</li> <li>- Bone marrow was collected on only one occasion after dosing</li> <li>- 100 blast cells per slide were examined to determine the mitotic index (instead of 1000)</li> <li>- 50 metaphases per slide were analysed for structural chromosome aberrations (instead of 100)</li> <li>- only highest dose animals were evaluated for chromosome aberrations</li> </ul>
Acceptability:	Yes (but limited)

### Material and Methods

#### Test material:

Ethofumesate

Batch No.: 348/055

Purity: 98 %

Appearance: white coloured odourless crystals

Solvent/Vehicle: Postman brand refined groundnut (peanut) oil

Positive Control: Cyclophosphamide

Spindle Inhibitor: Colchicine

#### Animals:

Swiss Albino Mice (Bred at the ██████████  
██████████)

#### Test Procedure:

The animals were kept in a room with controlled environment consisting of controlled temperature ( $22 \pm 3$  °C), relative humidity (40-70%) and light (12 hours light and 12 hours dark). Free access to drinking water and food (Gold Mohur brand, manufactured by M/S Lipton India Ltd., Bangalore, a subsidiary of Unilever of England)

was allowed throughout the study. Male mice were weighing 32 to 38g and female mice were weighing 28 to 32g at the start of the study. First a dose range study was conducted. Two male and two female mice were used each at 4000 and 6000 mg/kg bw. According to the dose range study dosages of 0, 40, 400 and 4000 mg/kg bw were chosen for the final study. 5 male and 5 female mice were used for each dosage group. The test material was administered twice (one daily for two consecutive days) by gavage. The animals were observed twice a day for any mortalities or clinical signs. Dead animals were immediately necropsied. Body weight was noted daily on treatment day one, two and at sacrifice. 24 hours after the second dose each animal was injected intraperitoneally with 10 ml/kg bw of 0.04% solution of colchicine. The animals were sacrificed 90 minutes later to obtain cell suspension from the femur bone marrow. A microscope slides was prepared from cell suspensions of each animal and stained in a 2% solution of Giemsa. 100 blast cells per slide were examined to determine the mitotic index. 50 metaphases per slide were analysed for a wide range of structural chromosome aberrations (chromatid or chromosome gaps, breaks, acentric fragments, ring chromosomes, multiple chromatid break, pulverization, polyploidy and exchange figures). Only the aberrations in the high dose group were evaluated.

## Results

No treatment related pharmacotoxic symptoms and no findings at the necropsy were observed. Significant decrease in body weight was measured in low and high dose animals.

There was no evidence of an increase in the incidence of chromosome aberrations in animals treated with 4000 mg/kg bw when compared to the vehicle control groups (evaluated per sex). At the highest dose group mitotic index was significantly lower than vehicle control group indicating that the test compound was toxic at 4000 mg/kg bw. The positive control compound cyclophosphamide produced marked increases in the frequency of chromosome aberrations.

## Conclusion

Under the experimental conditions in this study ethofumesate is not clastogenic *in vivo*.

*The study below (██████ 1992) was not listed in the reference list of the original DAR (1998) but was evaluated there. Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998).*

### ***In vivo* mammalian bone marrow cytogenetic test - chromosomal analysis (██████ 1992)**

#### *Experimental design*

Groups of 10 rats (CD, 5 of each sex) were given a single i.p. dose of ethofumesate at the maximum tolerated dose (3000 mg/kg). Animals were killed 6, 24 or 48 hours later, the bone marrow extracted and slide preparations made and stained. Bone marrow cells were scored for the presence of chromosome aberrations. Appropriate positive controls were included.

#### *Results*

The positive control compound cyclophosphamide produced marked increases in the frequency of chromosome aberrations.

Under the experimental conditions in this study, ethofumesate is not clastogenic. The study follows the OECD guideline. There is a QA statement and a statement of compliance with GLP standards. The study seems to be of acceptable quality.

<b>Reference:</b>	Dominant Lethal Test in Wistar Rats
Author(s), year:	██████████ P.; 1992
Report/Doc. number::	OFC00004854 / M-351976-01-1
Guideline(s):	OECD 478 (1984)
GLP:	Yes
Deviations from Draft OECD 478 (September 2013):	<ul style="list-style-type: none"> <li>- age of animals was 20-24 weeks (instead of 8-12 weeks)</li> <li>- recommended limit dose (2000 mg/kg bw) exceeded (5000 mg/kg bw)</li> <li>- sacrifice of females on GD 16 (instead of GD 14-15)</li> <li>- 30 females/dose (instead of at least 40), however good statistical power achieved</li> </ul>
Acceptability:	Yes

Appearance: white coloured odourless crystals

Test Procedure:

The animals were kept in a room with controlled environment consisting of 10-15 air changes per hour, controlled temperature ( $22 \pm 3$  °C), relative humidity (52-70 %) and light (12 hours light and 12 hours dark). Free access to drinking water and food (pelleted rat feed; manufactured by M/S Lipton India Ltd., Bangalore, a subsidiary of Unilever England) was allowed throughout the study. The treated groups consisted of 30 and the control group of 10 males. The positive control was administered to 5 males at a dose of 100 mg/kg bw for 5 days and 500 mg/kg was administered to 5 males as a single dose. The test compound (0, 200, 1000 and 5000 mg/kg bw) was administered orally as gavage to male animals as a single dose. The animals were then allowed to mate with untreated adult virgin females (mating ratio 1:1) for 10 consecutive weeks. This resulted in 300 mated females pro treated group and negative control and 100 mated females pro positive control. The animals were observed twice a day during the first week and once a day during the remaining period for any mortalities or clinical signs. The body weight of males was determined on day zero, one, two, four and six post treatment and weekly thereafter. The body weight of females was determined initial and at sacrifice. The females were sacrificed on the 16<sup>th</sup> day of pairing. The uteri were examined for total pregnancies, implantations, resorptions, live implantations and pre- and post-implantation loss. After the sacrifice the changes of visceral organs in males were recorded and testes, epididymis, seminal vesicle and prostate collected.

## Results

No mortalities and no findings at necropsy were observed in males. The treatment tended to decrease the body weight slightly in mid and high dose males from week 4 until the termination. Ethofumesate did not induce dominant lethal effect in Wistar rats as the changes seen in fertility indices during 10 weeks post treatment period were minimal, inconsistent and not related to dose of the test compound and duration after treatment. The positive control compound ethyl methanesulphonate induced typical dominant lethal effect (a decrease in incidence of pregnancy to zero percent on week 3 and gradual recovery back to normal by 9 to 10 weeks and increased incidence of early resorptions during week one which gradually returned to normal level by week 9).

## Conclusion

Under the experimental conditions in this study ethofumesate does not produce dominant lethal effect in rats.

### B.6.4.4. Photomutagenicity

According to new data requirements for active substances (Regulation (EU) No 283/2013) testing of photomutagenicity may be required if the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is  $> 1\,000\text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ .

Ethofumesate absorbs electromagnetic radiation mostly below 290 nm. However, at 290 nm the extinction coefficient of ethofumesate is still  $> 1000\text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , which is above the trigger of  $1000\text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ . Therefore, testing of ethofumesate for its photomutagenicity might be required according to Regulation (EU) No 283/2013.

According to *Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) 283/2013 and Regulation (EU) No 284/2013* (SANCO/10181/2013– rev. 2, May 2013) it is stated that “*In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02*”.

No OECD Guideline is currently available for testing photomutagenicity and no study was provided by the notifiers. The waiving of the study is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.

### B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

One new long-term study (carcinogenicity study in Swiss albino mice; [REDACTED] 2001) was, by mistake, included in the dossier for purpose of re-newal of ethofumesate. Since the notifier did not claim adverse data for this study according to Article 56 (*Information on potentially harmful or unacceptable effects*) of Regulation (EC) No 1107/2009, this study was not evaluated in the DRAR (2014).

The evaluations of all studies presented below were already included in the original DAR (1998). Re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for chronic effects and carcinogenicity was proven. The RMS derived in the DRAR only one different NOAEL ([REDACTED] 1995) than originally derived in the DAR, however, exactly the NOAEL from this study was originally used for derivation of ADI. Based on the proposed change in the NOAEL, the RMS also proposed new ADI for re-newal of ethofumesate (for details please see corresponding data point in Volume 1).

#### B.6.5.1. Rat

<b>Reference:</b>	ETHOFUMESATE: 52 WEEK DIETARY TOXICITY STUDY IN RATS
Author(s), year:	[REDACTED] 1990
Report/Doc. number::	A89582 / M-165028-01-1
Guideline(s):	OECD 452 (1981)
GLP:	Yes
Deviations from OECD 452 (2009):	- Limit dose of 1000 mg/kg bw/d exceeded in the high dose when doses calculated with real body weight and real consumption data, however would be appropriate if the conversion factor of 0.05 for older rats is assumed - no chemical and biochemistry and haematology investigation after 3 months - no thyroid taken for organ weight estimation
Acceptability:	Yes

#### Material and Methods

Groups of 20 male and 20 female Sprague-Dawley rats, approximately 6 weeks old, were dosed daily for 52 weeks with ethofumesate (purity: at least 97%) via the diet at dose levels of 0, 2000, 7000 or 20000 ppm corresponding to 135, 470 and 1338 mg/kg bw/d in males and 164, 630 and 1849 mg/kg bw/d in females. Mortality and clinical signs were recorded twice a day. All animals were palpated at least once a week. Ophthalmoscopy observations were conducted in animals of control and high dose group once before treatment and during week 51 of treatment. Blood samples were taken from 10 males and 10 females from each group after 6 months and at the end of the study. Urine samples were collected from all animals in metabolism cages after 6 months and at the end of the study. Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus and uterus were weighed. All organs and tissues according to OECD 452 (2009) from control and high dose groups were passed for histopathological evaluation. Histological examination of kidney, liver and lung was performed also on all other animals from the low and intermediate dose groups.

## Results

There were 12 premature deaths showing no inter group differences including the control animals. There were no clinical signs attributable to administration of ethofumesate and no intergroup differences in the incidence of externally palpable masses in either sex.

There was a reduction in the overall body weight gain in the high dose males (-15%) when compared to the control, statistically significant at week 3, weeks 8-10 and from week 16 until the end of the study. In high dose males there was a slight decrease in total food consumption (-11%) compared to controls.

In the females, there was body weight gain reduction compared to controls in intermediate (-13%) and high dose animals (-24%), statistically significant at weeks 20-24 and from week 41 until the end of the study. No intergroup differences were observed in females regarding food consumption.

No ophthalmoscopy findings attributable to administration of ethofumesate were recorded after 6 months and at the end of the study.

No intergroup differences in haematology were measured in males at 6 months and at the end of the study. In females, platelets were increased in high dose females after 6 months (+ 33%) and at the end of the study (+31%). No other haematological changes in females were measured.

AST and ALT in serum were reduced in intermediate and high dose males and females (from about 30% to maximum 50% of the control), after 6 months and at the end of the study. The study author attributed these effects partially to ethofumesate and partially to unusually high variations of AST and ALT in control animals. The toxicological relevance of the decreased level of AST and ALT is questionable.

Total bilirubin in serum was reduced in the intermediate and high dose males after 6 months but not at the end of the study. Albumin was increased in the high dose males after 6 months (+5%) and at the end of the study (+9%) compared to control animals.

Regarding urine analysis no intergroup differences were observed.

In males, no intergroup differences regarding organ weights were recorded. In high dose females, absolute liver weight was statistically significantly increased in the high dose females. The relative liver weight in high dose females was 12% higher than in control females.

Histological evaluation revealed some treatment related liver changes in males and females of intermediate and high dose groups. There were increased focal cellular alterations with vascularisation of the liver in the intermediate and high dose males. Periportal cellular change of the liver was increased in high dose males. Periportal hepatocyte enlargement of the liver was increased in the intermediate and high dose females.

In other organs, no treatment related findings and differences between control and dosed groups were observed.

**Table B.6.5.1-1. Incidences of histopathological non-neoplastic findings in liver**

	Incidences of findings							
	Males				Females			
Findings	0 ppm	2000 ppm	7000 ppm	20000 ppm	0 ppm	2000 ppm	7000 ppm	20000 ppm
<b>Liver</b>								
Focal cellular alterations with vacuolar changes	0	1	5*	7**	0	0	0	0
Periportal cellular change	0	0	0	14***	0	0	0	0
Periportal hepatocellular enlargement	0	0	0	0	0	0	10***	13***

\* p < 0.05 (pairwise Fisher's test)

\*\* p < 0.01 (pairwise Fisher's test)

\*\*\* p < 0.001 (pairwise Fisher's test)

## Conclusion

The NOAEL of the study is 2000 ppm (135 mg/kg bw/d in males and 164 mg/kg bw/d in females) based on effects on liver histology of males (focal cellular alterations with vacuolar changes) and females (periportal hepatocellular enlargement) and reduced body weight gain in females (-13%) at 7000 ppm.

<b>Reference:</b>	ETHOFUMESATE: 104 WEEK DIETARY CARCINOGENICITY STUDY IN RATS
Author(s), year:	1991
Report/Doc. number::	A89583 / M-165030-01-1
Guideline(s):	OECD 451 (1981)
GLP:	Yes
Deviations from OECD 451 (2009):	No
Acceptability:	Yes

## Material and Methods

Groups of 50 male and 50 female Sprague-Dawley rats, approximately 6 weeks old, were dosed daily for 104 weeks with ethofumesate (purity: at least 97%) via the diet at dose levels of 0, 2000, 7000 or 20000 ppm corresponding to 115, 392 and 1161 mg/kg bw/d in males and 134, 529 and 1595 mg/kg bw/d in females.

Mortality and clinical signs were recorded twice a day. All animals were palpated at least once a week. Ophthalmoscopy observations were conducted in animals of control and high dose group once before treatment



and during week 104 of treatment. Blood and urine samples were taken from 10 males and 10 females from each group during weeks 77/78 and 103/104. For differential blood counts blood samples were taken after weeks 50/51, 76 and 103 from all surviving animals. Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes (and epididymes), thymus and uterus were weighed. All organs and tissues according to OECD 451 (2009) from control and high dose groups (and all premature decedents) were passed for histopathological evaluation. Histological examination of kidney, liver and lung was performed also on all other animals from the low and intermediate dose groups.

## Results

There were 174 premature deaths without notable inter group differences, the highest mortality being observed in females (58%) and males (44%) of the control group. There were no clinical signs that could be attributed to dosing with ethofumesate.

There was a reduction in the overall body weight gain in the high dose males and females (both -30%) when compared to the control. In males, a body weight gain reduction was also observed at low (- 15%) and intermediate dose (-12%) and was seen only towards the end of the study with variations within normal limits. In females the decrease in body weight gain was less significant (-3% in the low dose group and - 7% in the intermediate group).

No intergroup differences were observed in females regarding food consumption while only in high dose males a slight reduction in food consumption (8%) was measured.

No ophthalmoscopy findings attributable to administration of ethofumesate were recorded.

Considering haematological parameters, no intergroup differences were measured in males at week 78 and 104. In females of the high dose a slight decrease in the mean red blood cell volume was seen after 104 weeks. For differential blood counts, no intergroup differences in either males or females were measured at week 50/51, 76 and 103.

AST and ALT in serum were reduced in intermediate and high dose males (from about 30% to maximum 45% of the control), in the week 78 and 104. No comparable effects on AST and ALT were observed in females. The toxicological relevance of the decreased level of AST and ALT is questionable. Reductions in alkaline phosphatase, total bilirubin, calcium and total protein and an increase in glucose were not consistent between the weeks and sexes.

Terminal investigations revealed significantly increased liver weight (+ 26%) only in the high dose females.

### Non-neoplastic findings:

Periportal cell alteration of the liver was increased in the high dose males, no effects were observed in females. Centrilobular hepatocyte hypertrophy of the liver was increased in incidence and severity in males and females of intermediate and high dose groups. No other dose or treatment related non-neoplastic findings were recorded.

**Table B.6.5.1-2: Incidences of histopathological non-neoplastic findings in liver**

	Incidences of findings	
	Males	Females

Findings	0 ppm	2000 ppm	7000 ppm	20000 ppm	0 ppm	2000 ppm	7000 ppm	20000 ppm
<b>Liver</b>								
Centrilobular hepatocyte hypertrophy	5	5	11	21***	1	0	7	21***
Periportal cellular alteration	0	0	0	15***	0	0	0	0

\*\*\* p < 0.001 (pairwise Fisher's test)

#### Neoplastic findings:

There was a statistically significant increase in the combined incidence of testicular interstitial cell adenoma/focal hyperplasia in low, intermediate and high dose males. According to the study author this increase was comparable to the highest historical control values for the combined effect in their laboratory.

#### Conclusion

The target organ was the liver. The NOAEL of the study is 2000 ppm (corresponding to 115 mg/kg bw/d in males and 134 mg/kg bw/d in females), based on increased incidence of non-neoplastic findings (centrilobular hepatocyte hypertrophy) in liver of both males and females at 7000 ppm (corresponding to 392 mg/kg bw/d in males and 529 mg/kg bw/d in females). No treatment related carcinogenicity was observed in rats in the study.

<b>Reference:</b>	The effects of the dietary administration of NC 8438 to male and female rats for two years
Author(s), year:	██████████ 1976
Report/Doc. number::	A83155 / M-155430-01-1
Guideline(s):	The study predates the development of the OECD guideline
GLP:	No
Deviations from OECD 451 (2009):	- no ophthalmoscopy observations conducted - not all haematological and clinical biochemistry parameters measured at each sampling time points and partially from only 5 animals/sex - no histopathological examination of aorta, mammary gland and bone marrow
Acceptability:	Yes (limited since mortalities very high)

#### Material and Methods

Ethofumesate (purity : 92.5%) was administered in the diet to 6 groups (40-60 animals/sex/group) of Wistar rats, approximately 8 weeks old, for 2 years at concentrations of 0 (control), 8, 40, 200, 1000 or 5000 ppm. The values correspond to 0.3, 1.5, 7.4, 37.6 and 192.4 mg/kg bw/day for males and 0.4, 1.7, 8.7, 44.5 and 235.7 mg/kg bw/day for females. 5-10 animals/sex/group were sacrificed after 6, 12 and 24 months.

Mortality and clinical signs were recorded daily.

Blood samples were taken from all animals prior to the start of the treatment and from 10 males and 10 females from each dose group after 6 months treatment. After 12 months of treatment, 5 males and 5 females from the control and high dose groups were sampled and at 24 months, 10 males and 10 females again.

Urine samples were collected from 10 males and females from each group after 6 months treatment and from 5 males and 5 females of the control and high dose group after 12 months.

Adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus and uterus were weighed. All organs and tissues according to OECD 451 (2009) except aorta, mammary gland and bone marrow, from all surviving rats were passed for histopathological evaluation.

## Results

Survival was not affected during the first year of the study. At the end of the study mortality was statistically increased at the male high dose level (83 %) and was over 50% at all dose levels. The study author stated that three common syndromes (chronic nephritis, pituitary tumours and subcutaneous tumours) were the main reason for mortality and that there were randomly distributed amongst the treatment groups.

Body weight gain in females at the high dose level was reduced from week 4 to the end of the study and was 10 – 12 % lower than the control. Other groups were not affected. No intergroup differences were seen regarding food consumption.

Clinical observations, haematology, urinalysis and clinical chemistry did not yield any findings that were considered treatment-related.

The relative liver weight was increased for males (+13.7%) at the high dose level. No differences between groups were observed regarding other organs.

Autopsy and histopathological investigation revealed no evidence of treatment-related effects.

## Conclusion

The NOAEL was determined to 1000 ppm (corresponding to 37.6 mg/kg bw/d in males and 44.5 mg/kg bw/d for females) based on the high mortality, reduced body weight gain in females and increased liver weight in males at 5000 ppm. No treatment related carcinogenicity was recorded. Since the mortality in the study was very high the validity of the study is limited.

<b>Reference:</b>	Combined chronic toxicity and carcinogenicity study in wistar rats
Author(s), year:	██████ P., 1995
Report/Doc. number::	OFC00004858 / M-352005-01-1
Guideline(s):	OECD 453 (1981)
GLP:	Yes
Deviations from OECD 453 (2009):	- only control and high dose group taken for the chronic toxicity part of the study - no organ weight of epididymis, heart, spleen and thyroid recorded - no histopathological examination of aorta and epididymis
Acceptability:	Yes

## Material and Methods

Groups of 50 male and 50 female Wistar rats, approximately 6 weeks old, were dosed daily for 2 years with ethofumesate (purity : 98.0% ) via the diet at dose levels of 0, 100, 1000 or 10000 ppm. The values correspond to 6.9, 69 and 715 mg/kg bw/d in males and 9.8, 101 and 1169 mg/kg bw/d in females. Interim groups with 10 animals per sex (control) and 20 animals per sex (high dose of 10000 ppm, corresponding to 726 mg/kg bw/d for

both sexes) were included in the study. These animals were sacrificed after 12 months and non-neoplastic histological changes were investigated.

Mortality and clinical signs were recorded daily.

Blood samples were taken from first 20 surviving animals per sex and group at 3, 6, 12, 18 and 24 months.

Urine samples were collected from 10 males and females from each group one or two days before blood collection.

Adrenals, brain, kidneys, liver, ovaries and testes were weighed. All organs and tissues according to OECD 453 (2009), except aorta and epididymis, from all control and high dose animals and all dead and moribund sacrificed animals were passed for histopathological evaluation. Additionally, gross lesions from the low and mid dose groups were investigated.

## Results

No mortality was observed in 12 month interim sacrifice groups. There were 191 premature deaths, distributed through all the dose groups. There were no notable inter group differences except for the high dose males whose survival was slightly reduced. The survival at the end of the period was 54, 48, 56 and 51 % for the control, low, mid and high dose groups respectively.

The statistically significant differences in body weight gain were observed in males only at week 13 in the interim sacrifice group (10000 ppm), in the main test at week 13 in the low dose group (100 ppm) and in the main test at 13 weeks and 18 months in the mid dose group (1000 ppm). In the interim sacrifice group, males of the high dose group had reduced body weight gain of -15.7% after 13 weeks, while in the main treatment groups the reduced body weight gain was not dose dependent, always < 10% and ranged from -0.4% in the high dose group after 6 months to -9.8% in the low dose group after 13 weeks. In females, the reduced body weight gain > 10% was observed only in high dose females (10000 ppm) at the interim sacrifice at 12 months (-12.9%), in the main test in the mid dose females (1000 ppm) only transient after 12 months (-11.7%) and in the main test in the high dose females (10000 ppm) continuous at 12 months (-23.9%), 18 months (-22.5%) and 24 months (-24.1%).

**Table B.6.5.1-3: Body weight gain (g) in male rats**

Dose group (ppm)		Time				
		13 weeks	6 months	12 months	18 months	24 months
Interim sacrifice						
Control	Mean	210	258	281	-	-
	SD	17.1	18.7	18.0	-	-
10000	Mean	177*	243	272	-	-
	SD (% control)	19.2 (-15.7%)	23.8 (-5.8%)	31.5 (-3.2%)	-	-
Main treatment groups						
Control	Mean	203	254	277	299	294
	SD	26.5	30.3	38.9	41.7	36.9
100	Mean	183*	255	275	297	292
	SD (% control)	27.6 (-9.8%)	26.5 (+0.4%)	29.7 (-0.7%)	35.1 (-0.7%)	34.5 (-0.7%)

1000	Mean SD (% control)	184* 27.5 (-9.4%)	240 33.7 (-5.5%)	271 40.0 (-2.2%)	272* 38.8 (-9.0%)	274 42.3 (-6.8%)
10000	Mean SD (% control)	192 22.0 (-5.4%)	253 29.7 (-0.4%)	275 32.5 (-0.7%)	295 36.3 (-1.3%)	288 29.9 (-2.0%)

\*  $p < 0.05$  (ANOVA and Dunnett's pairwise comparison)

**Table B.6.5.1-4: Body weight gain (g) in female rats**

Dose group (ppm)		Time				
		13 weeks	6 months	12 months	18 months	24 months
Interim sacrifice						
Control	Mean	102	107	131	-	-
	SD	19.8	23.1	23.5		
10000	Mean	95	111	114	-	-
	SD (% control)	16.5 (-6.9%)	24.5 (+3.7%)	26.1 (-12.9)		
Main treatment groups						
Control	Mean	112	133	163	191	203
	SD	15.4	18.7	40.6	44.0	42.0
100	Mean	118	136	151	181	190
	SD (% control)	17.2 (+5.4%)	21.8 (+2.35)	31.5 (-7.4%)	31.2 (-5.2%)	39.0 (-6.4%)
1000	Mean	113	121*	144*	181	186
	SD (% control)	14.1 (+0.9%)	17.0 (-9.0%)	29.8 (-11.7%)	41.2 (-5.2%)	45.1 (-8.4%)
10000	Mean	109	123*	124*	148*	154*
	SD (% control)	18.2 (-2.7%)	19.8 (-7.5%)	23.4 (-23.9%)	29.8 (-22.5%)	34.4 (-24.1%)

\*  $p < 0.05$  (ANOVA and Dunnett's pairwise comparison)

In males, no intergroup differences in feed intake was measured, while in females there was a significantly higher feed intake in the high dose group.

No consistent treatment or dose related changes in haematological and clinical-chemical parameters and no changes in constituents of the urine were observed.

Terminal investigations revealed normal organ weights without intergroup statistical significance except for an increased relative ovarian weight in mid (+32%) and high dose (+50%) females. However, it is recognised that organ-to body-weight ratio has no prediction for ovary weight<sup>2</sup>. Ovary weight is optimally analysed using organ-to-brain weight ratio, but this analysis was not performed in the study. Additionally, since no dose-response was observed for absolute ovary weight from 100 to 1000 ppm and no statistical significance in absolute ovary weight was observed even for the 10000 ppm group, the increased relative ovary weight at 1000 and 10000 ppm

<sup>2</sup> Bailey at al., 2004: Relationships Between OrganWeight and Body/BrainWeight in the Rat: What Is the Best Analytical Endpoint. Toxicologic Pathology, 32:448–466, 2004

was rather considered as an isolated incidental finding. In other long-term rat studies (■■■■■ et al., 1991; ■■■■■, 1976) no similar effects were observed at comparable or higher doses.

**Table B.6.5.1-5: Ovary weights at terminal investigation**

	<b>0 ppm</b>	<b>100 ppm</b>	<b>1000 ppm</b>	<b>10000 ppm</b>
Absolute ovary weights (g)	0.072	0.092	0.089	0.098
(% of control)		(+28%)	(+24%)	(+36%)
Organ weight ratio (%)	0.028	0.035	0.037*	0.042*
(% of control)		(+25%)	(+32%)	(+50%)

\*  $p < 0.05$  (ANOVA and Dunnett's pairwise comparison)

At 12 and 24 months, no treatment related gross and histopathological lesions were observed. The statistical analysis showed that the incidence of tumours was significantly lower in high dose group females and in combined sex.

### Conclusion

In the original DAR (1998) the NOAEL was set at 100 ppm (corresponding to 6.9 mg/kg bw/d in males and 9.8 mg/kg bw/d in females), based on reduced body weight gain at 1000 ppm. For the re-newal of ethofumesate (2014) the RMS re-evaluated the study and concluded that at 1000 ppm no consistent effects on body weight gain, observed as the reduction above 10%, were measured. Also no other effects, except increased relative ovarian weight (+32%) without any histological findings in ovaries, were observed at 1000 ppm. Therefore, the RMS is of the opinion that NOAEL of this long-term study should be rather set at 1000 ppm (69 mg/kg bw/d in males and 101 mg/kg bw/d in females), based on reduced body weight gain in high dose females of approximately 24% measured from 12 to 24 months. The increased relative ovarian weight is considered to be of questionable relevance since not accompanied by any histopathological finding.

The NOAEL of 6.9 mg/kg bw/d from this chronic and carcinogenicity study (■■■■■, 1995) was basis for setting the ADI for the first approval of ethofumesate (DAR 1998). The new proposal for ADI is described under corresponding data point in Volume 1.

### B.6.5.2. Mouse

<b>Reference:</b>	ETHOFUMESATE: 80 WEEK ORAL (DIETARY) CARCINOGENICITY STUDY IN THE MOUSE
Author(s), year:	■■■■■ 1992
Report/Doc. number::	A89581 / M-165020-01-1
Guideline(s):	OECD 451 (1981)
GLP:	Yes
Deviations from OECD 451 (2009):	- only leucocyte differential count investigated - no clinical biochemistry measurements conducted
Acceptability:	Yes

## Material and Methods

Two hundred male and 200 female mice (CrI:CD-1(ICR) BR), approximately 6 weeks old, were divided into four groups each with 50 males and 50 females. Three groups received ethofumesate (purity: 97.0%) in the diet at concentrations of 0 (control) 1000, 3000 or 10000 ppm which corresponds to 161, 477 and 1601 mg/kg bw/d for males and 204, 644 and 2145 mg/kg bw/d for females. Treatment continued for 80 consecutive weeks.

Mortality and clinical signs were recorded daily.

Blood samples were taken from 10 animals (with lowest identification number) per sex and group during weeks 52 and 80.

Brain, kidneys, liver and testes (not ovaries) were weighed. All organs and tissues according to OECD 451 (2009) from all animals were passed for histopathological evaluation.

## Results

Forty one male and 47 female mice died or were killed *in extremis* during the study. Neither the incidence nor timing of these deaths was considered related to the administration of ethofumesate and there were no intergroup differences.

There were no clinical signs and no palpable masses related to ethofumesate. There were no intergroup differences regarding body weight gain and food consumption. Examination of blood smears did not indicate any effects on leucocyte differential counts related to ethofumesate treatment.

Relative liver weight was statistically significantly increased in mid dose (+ 17.3%) and high dose (+ 26.8%) females. No effects on organ weights in males were observed. The incidence of liver masses was slightly increased for males from low and high dose group but was lower in the intermediate group than in the control. No comparable findings were observed in females.

Macroscopic observations showed a slight increase in the incidence of yellow staining of the skin (12 – 15- 17- 26) and the loss of hair (10 – 13- 14- 15) was observed in males.

At 18 months, no treatment-related neoplastic or non-neoplastic changes were observed.

## Conclusion

At 3000 ppm only an increase in relative liver weight (+17.3%) was observed in females, not accompanied by any histopathological findings. Therefore, the NOAEL is set at 3000 ppm (corresponding to 477 mg/kg bw/d in males and 644 mg/kg bw/d in females), based on statistically significantly higher relative liver weight > 20% (+ 26.8%) in females at 10000 ppm, not accompanied by any histopathological findings.

### B.6.5.3. Other species

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<b>Reference:</b>	A CARCINOGENICITY STUDY OF TECHNICAL NC 8438 IN HAMSTERS (LIFETIME STUDY) CR 4805/4
Author(s), year:	1980
Report/Doc. number::	A83178 / M-155452-01-1
Guideline(s):	- The study predates the development of the OECD guideline 451
GLP:	No; QA statement available

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Deviations from OECD 451 (2009):	- the study includes conception, pregnancy, nursing of the offspring and entire life time of offspring - no detailed comparison with the OECD 451 has been done by the RMS
Acceptability:	Limited (hamster is not the species of choice and the study was conducted to include different life phases including exposure of the parents of the experimental animals)

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### Material and Methods

Ethofumesate (purity: 96.8%) was administered in the diet to 5 groups (50 animals/sex/group) of Syrian hamsters (BIO 15.16) for 19-22 months at concentrations of 0 (control), 0 (control), 80, 400 and 2000 ppm. The values correspond to 5.4, 26 and 130 mg/kg bw/day for males and 5.6, 28 and 143 mg/kg bw/day for females. Feeding with the test substance was started one week before conception by the mothers of the actual test animals and continued throughout pregnancy, during nursing of the offspring and throughout the entire life-time of the weaned offspring i.e. until mortality reached about 60%. Males were sacrificed at 98 weeks of age and females at 84 weeks. The animals were checked for mortality, body weight, clinical signs, food and water consumption, macroscopic examination, organ weights and histopathological examination.

### Results

Ethofumesate treatment did not affect survival, clinical signs, body weight or food consumption.

Absolute (+23%) and relative liver weights (+ 17%) of terminally sacrificed animals were significantly increased in females at the 2000 ppm dose level.

Gross and histopathologic investigation revealed no treatment-related neoplastic and non-neoplastic findings.

### Conclusion

NOAEL in this study was 400 ppm which corresponds to 26 and 28 mg/kg bw/day for males and females respectively, based on increased relative liver weight > 20% in the high dose group females, not accompanied by any histological findings at 2000 ppm.

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<b>Reference:</b>	TECHNICAL NC 8438 (CR 4805/3) TOXICITY STUDY IN BEAGLE DOGS (FINAL REPORT : DIETARY INTAKE FOR 104 WEEKS)
Author(s), year:	██████████ 1980
Report/Doc. number::	A83176 / M-155450-01-1
Guideline(s):	- The study predates the development of the OECD guideline 451
GLP:	No; QA statement available
Deviations from OECD 451 (2009):	No
Acceptability:	Yes

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### Material and Methods

Ethofumesate (purity: 96.6% – 98.0%) was administered in the diet to 4 groups (8 animals/sex/group) of Beagle dogs for 24 months at concentrations of 0 (control), 800, 4000 and 20000 ppm. This corresponds to ca 25, 118 and 632 mg/kg bw/d for males and 0, 24, 109 and 619 mg/kg bw/d for females. One animal/sex/group was sacrificed after 12 months.



Mortality and clinical signs were recorded daily. Ophthalmoscopic observations and laboratory investigations were performed on all animals once before dosing and after 3, 6, 12, 18 and 23 months.

Brain, liver, thymus, thyroid, pituitary, spleen, prostate, uterus, adrenals, heart, pancreas, kidneys, gonads, lungs and spinal cord were weighed and processed for histopathological investigations. Additional organs according to OECD 451 were investigated for histopathological findings.

## Results

One male dog of high dose group was sacrificed *in extremes* at week 69.

There were no intergroup differences regarding body weight, food consumption and clinical signs. No findings in ophthalmoscopic investigation were related to ethofumesate treatment.

The findings in the haematology parameters were all within normal limits.

Serum alkaline phosphatase (AP) levels were higher at the high dose level at each time point but were within normal limits. Serum alanine aminotransferase (ALT) was increased in the high dose level animals at week 78 (+27%) and week 104 (+48%).

Relative liver weight was increased at the high dose level (males and females liver weight combined) being 22% higher than in the control animals. No intergroup differences were observed in weights of other organs. A histopathologic examination showed no findings attributable to treatment with ethofumesate.

## Conclusion

The NOAEL was set to 4000 ppm which corresponds to 118 and 109 mg/kg bw/day for males and females, based on increased relative liver weight and increased liver enzyme values at 20000 ppm.

### B.6.6. REPRODUCTIVE TOXICITY

#### B.6.6.1. Generational studies

The evaluations of all studies presented below were already included in the original DAR (1998). No additional studies were submitted for the purpose of renewal. Re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for multigeneration studies was proven.

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<b>Reference:</b>	ETHOFUMESATE: DIETARY RAT GENERAL REPRODUCTIVE PERFORMANCE DOSE RANGING STUDY
Author(s), year:	██████████ 1989
Report/Doc. number::	A87578 / M-161494-01-1
Guideline(s):	OECD 416 (1983)
GLP:	No; QA statement available
Deviations from OECD 416 (2001):	Dose range finding study, with low number of animals and limited parameters assessed
Acceptability:	Yes; limited information

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## Material and Methods

Groups of 6 male and 6 female Sprague-Dawley rats (F0 generation) were dosed via the diet with ethofumesate (purity : not stated) at levels of 0, 3750, 7500, 15000 and 30000/50000 ppm. The high dose group was dosed at 30000 ppm for the first two weeks of the pre-mating period and at 50000 ppm for the remaining time of the study since no conclusive evidence of toxicity was observed at 30000 ppm.

The males were exposed to the test article continuously for three weeks before mating, throughout the mating period and for a further two weeks. The females were exposed continuously for three weeks prior to mating, throughout the mating period, pregnancy and parturition and until necropsy on day 21 post partum. The clinical condition, body weights and food consumption were recorded. For mating, each female was paired with a male from the same dose group for up to seven days. Pregnant females were allowed to litter and rear their offspring (F1 generation) to day 21 post partum. Litter size, body weights and clinical condition of the pups were recorded during lactation.

## Results

### F0 generation:

For the males, there were no mortalities. For the females, four animals of 50000 ppm dose group were either sacrificed because of poor clinical condition (hunched posture, emaciation, lethargy, piloerection, fur staining) or found dead, all during lactation period.

For males, a slightly lower body weight was observed over the 7 weeks in all treated groups, but without statistical and biological significance (maximum – 4% in the highest dose group). Statistically significantly lower body weight gain was observed in females of 50000 ppm group during premating (weeks 2 and 4) and during whole pregnancy and lactation and in females dosed with 7500, 15000 and 50000 ppm on the first day post partum. Clinical observations in females included hunched posture, emaciation, lethargy and fur staining at the high dose level. At necropsy, abnormalities which induced thoracic haemorrhages and gastrointestinal lesions were observed in females at the 50000 ppm level.

Regarding fertility and mating performance, no differences were observed except for the 50000 ppm group females, where only 4 out of 6 females mated.

### F1 generation:

There were no differences in the duration of gestation, litter size and sex ratios that could be conclusively related to treatment. At 50000 ppm there was an adverse effect on pup survival and the clinical condition (small pups, lethargic and hypothermic), and most pups died between days 10 and 14 pp. At other treatment doses there were no differences in the pup survival indices or clinical condition that could be conclusively related to treatment. Pup body weights at 50000 ppm were slightly lower than the controls but not statistically significant. One pup with short body and cleft palate was found in 3750 ppm group but this was not considered treatment related.

## Conclusion

No NOAEL was specified in the study report (since the study was conducted only as a range finding study) or in the original DAR. For purpose of renewal the parental NOAEL of 50000 ppm is proposed for males since no effects on males were observed at highest dose tested and a parental NOAEL of 15000 ppm for females, based on mortality, lower body weight gain and clinical signs at 30000/50000 ppm. The foetal NOAEL is proposed also at 15000 ppm based on adverse effect on pup survival and bad clinical condition at 30000/50000 ppm. The reproductive NOAEL is proposed at 15000 ppm based on reduced mating performance at 30000/50000 ppm.

<b>Reference:</b>	ETHOFUMESATE: DIETARY RAT TWO-GENERATION REPRODUCTION TOXICITY STUDY VOL I-II
Author(s), year:	██████████, 1990
Report/Doc. number::	A87579 / M-161496-01-1
Guideline(s):	OECD 416 (1983)
GLP:	Yes
Deviations from OECD 416 (2001):	No sperm parameters like sperm motility, sperm morphology, sperm count were assessed Only ovaries, testes and pituitary gland weighed
Acceptability:	Yes

## Material and Methods

Groups of 25 male and 25 female Sprague-Dawley rats (F<sub>0</sub> generation), 5 to 6 weeks old, were dosed with ethofumesate (purity: 97%) via the diet at levels of 0, 3000, 10000 and 30000 ppm. The achieved dosages were calculated by RMS and were approximately 200, 700 and 2200 mg/kg bw/d for F<sub>0</sub> males (pre- and post-mating values combined) and were for F<sub>0</sub> females 220, 800 and 2700 mg/kg bw/d during pre-mating, 200, 700 and 2000 mg/kg bw/d during gestation and 370, 1300 and 3800 mg/kg bw/d during lactation. For F<sub>1</sub> males the mean achieved dosage was 290, 970 and 3000 mg/kg bw/d (pre- and post-mating values combined) and for F<sub>1</sub> females 350, 1200 and 3900 mg/kg bw/d during pre-mating, 200, 640 and 1890 mg/kg bw/d during gestation and 350, 1200 and 3500 during lactation.

The males were exposed to the test article for ten weeks prior to mating, throughout the mating period and until necropsy at weaning of the F<sub>1</sub> generation. The females were exposed for two weeks prior to mating, throughout the mating period and until necropsy at weaning of the F<sub>1</sub> generation.

Pregnant females were allowed to litter and rear their offspring to weaning on day 21 post partum. Each male which failed to mate was paired with an untreated female; the pregnancy status of the female was assessed 10 days after mating.

After weaning, 25 male and 25 female F<sub>1</sub> pups were selected from each group for the second generation. The selected F<sub>1</sub> generation animals were dosed via the diet with ethofumesate at the same levels as the F<sub>0</sub> generation; vaginal smears were examined during this period. Pregnant females were allowed to litter and rear their offspring to weaning on day 21 post partum.

The following organs and tissues were subject to histopathological evaluation: vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland, lesions. Only ovaries, testes and pituitary gland were weighed.

## Results

F<sub>0</sub> and F<sub>1</sub> generation parent animals:

There were no mortalities or treatment-related clinical observations.

Body weight and gain:

In males of F<sub>0</sub> generation statistically significantly ( $p < 0.05$ ) lower body weight was observed from week 12 until the sacrifice only in the 30000 ppm dosed group. In males of F<sub>1</sub> generation dosed with 30000 ppm significantly lower body weight was observed sporadically (week 15 and 19). No effects on body weight were observed in males dosed with 3000 and 10000 ppm.

In females of F<sub>0</sub> generation statistically significantly ( $p < 0.001$ ) lower body weight was observed during the whole study duration until the sacrifice in the 30000 ppm dosed group. This was also measured in the first and second week of pregnancy and in the first week post partum in females dosed with 10000 ppm. In females of F<sub>1</sub> generation statistically significantly lower body weight was observed in animals dosed with 10000 ppm and 30000 ppm from the age of 6 or 7 weeks through the pregnancy and in the post partum period until the sacrifice. No effects on body weight were observed in females of F<sub>0</sub> and F<sub>1</sub> generation dosed with 3000 ppm.

Mating performance and fertility:

Mating performance, fertility, vaginal smear patterns were not affected by ethofumesate treatment.

Necropsy findings and organ weights:

No macroscopic or microscopic necropsy findings were observed which could be considered treatment-related.

Absolute weight of testes was not statistically significantly different than in the control neither in F<sub>0</sub> nor in F<sub>1</sub> males at any dose. At 30000 ppm, relative testes weight was statistically significantly higher than in control males in both generations (+ 12%). However, the absolute weight of testes is considered to be the more reliable value since testes weight is less dependent of reduction in body weight gain. The toxicological significance of this finding is questionable in absence of any histopathological effects.

It is also recognised that testes are not modelled well by either organ-to-body weight ratio or organ-to-brain weight ratio, but no alternative analysis such as analysis of covariance was performed (Bailey et al, 2004<sup>3</sup>).

No effects on weight of pituitary were observed in males.

**Table B.6.6.1-1. Organ weights (males)**

Organ	F <sub>0</sub> generation				F <sub>1</sub> generation			
	Control	3000 ppm	10000 ppm	30000 ppm	Control	3000 ppm	10000 ppm	30000 ppm
Testes	4.15 ±	4.05 ±	4.17 ±	4.28 ±	4.02 ±	4.11 ±	4.28 ±	4.21 ±

<sup>3,4</sup> Bailey et al., 2004: Relationships Between OrganWeight and Body/BrainWeight in the Rat: What Is the Best Analytical Endpoint. Toxicologic Pathology, 32:448–466, 2004

(absolute weight) g	0.37	0.3	0.28	0.39	0.51	0.29	0.27	0.51
Testes (relative weight) g/kg bw	6.52 ± 0.6	6.67 ± 0.55	6.85 ± 0.77	<b>7.35 ± 0.91***</b> (+12.7%)	7.10 ± 0.93	7.27 ± 0.75	7.69 ± 0.80	<b>7.97 ± 1.01***</b> (+12.3%)

\*\*\* significantly different from control,  $p < 0.001$ , Student's t test

Absolute and relative weight of pituitary was statistically significantly different than in the control animals in both  $F_0$  and  $F_1$  females at 30000 ppm. No differences in relative pituitary weight were measured. In  $F_0$  females statistically significantly lower absolute and relative ovaries weight was recorded at all dose levels. In  $F_1$  females statistically significantly lower absolute and relative ovaries weight was recorded in animals dosed with 10000 and 30000 ppm, but not in animals of 3000 ppm dose group. It is recognised that organ-to body-weight ratio is less predictive for ovary weight than organ-to-brain weight ratio, however, this analysis was not performed in the study. It is also recognised that pituitary gland is not modelled well by either organ-to-body weight ratio or organ-to-brain weight ratio, but no alternative analysis such as analysis of covariance was performed<sup>4</sup>.

The toxicological significance of these findings is questionable in absence of any histopathological effects.

**Table B.6.6.1-2. Organ weights (females)**

Organ	F <sub>0</sub> generation				F <sub>1</sub> generation			
	Control	3000 ppm	10000 ppm	30000 ppm	Control	3000 ppm	10000 ppm	30000 ppm
Pituitary (absolute weight) g	0.017 ± 0.003	0.017 ± 0.002	0.016 ± 0.002	<b>0.015 ± 0.002*</b>	0.016 ± 0.002	0.017 ± 0.003	0.015 ± 0.002	<b>0.0147 ± 0.002*</b>
Pituitary (relative weight) g/kg bw	0.05 ± 0.008	0.051 ± 0.005	0.051 ± 0.007	0.051 ± 0.006	0.051 ± 0.007	0.051 ± 0.006	0.048 ± 0.006	0.05 ± 0.007
Ovaries (absolute weight) g	0.158 ± 0.015	<b>0.138 ± 0.021***</b>	<b>0.121 ± 0.022***</b>	<b>0.093 ± 0.014***</b>	0.147 ± 0.025	0.144 ± 0.027	<b>0.130 ± 0.028*</b>	<b>0.110 ± 0.029***</b>
Ovaries (relative weight) g/kg bw	0.473 ± 0.05	<b>0.413 ± 0.06***</b> (-12.7%)	<b>0.37 ± 0.064***</b> (-21.8%)	<b>0.315 ± 0.045***</b> (-33.4%)	0.475 ± 0.120	0.449 ± 0.117	<b>0.415 ± 0.088*</b> (-12.6%)	<b>0.380 ± 0.095**</b> (-20.0%)

\* significantly different from control,  $p < 0.05$ , Student's t test

\*\* significantly different from control,  $p < 0.01$ , Student's t test

\*\*\* significantly different from control,  $p < 0.001$ , Student's t test

$F_1$  and  $F_2$  generation litters:

Ethofumesate treatment did not affect gestation index, duration of gestation, pup survival or pup sex ratio.

In  $F_2$  generation at 30000 ppm litter size at birth was statistically significantly lower than the control values (13.5 pups in control versus 10.4 pups at 30000 ppm). This effect was not observed in  $F_1$  generation litters.

At 30000 ppm pup body weights were significantly lower than the control values for both F<sub>1</sub> and F<sub>2</sub> generation from day 7 to 21 post partum. Similar effects were also observed at 10000 ppm for the F<sub>2</sub> generation only. At 3000 ppm no effects on pup weights were observed.

Clinical condition and necropsy findings were not affected by ethofumesate treatment. No malformations related to treatment were recorded. Slight retardation of pups development, expressed as number of pups with eyes opened on day 15 post partum, was recorded in F<sub>1</sub> generation litters at 30000 ppm and in F<sub>2</sub> generation litters at 10000 and 30000 ppm. This is consistent with lower pups body weight in both generations at same dose levels.

### Conclusion

The parental NOAEL is proposed at 3000 ppm (approximately 200 mg/kg bw/d) based on statistically significantly lower body weight of females and statistically significantly lower absolute and relative (-21.8%) ovaries weight at 10000 ppm in both generations. The lower weight of ovaries was not accompanied by any histopathological finding. The relative (-12.7%) and absolute ovary weight at lowest dose (3000 ppm) was not considered as LOAEL since the reduction of 12.7% in relative ovary weight was not accompanied by any histopathological finding.

The reproductive NOAEL is proposed at 10000 ppm (approximately 640 mg/kg bw/d) based on statistically significantly lower litter size in F<sub>2</sub> generation at 30000 ppm.

The offspring NOAEL is proposed at 3000 ppm (approximately 200 mg/kg bw/d) based on significantly lower pup body weight and retardation of development (expressed as number of pups with eyes open at day 15 post partum) at 10000 ppm in F<sub>2</sub> generation litters.

<b>Reference:</b>	TECHNICAL NC 8438: MULTIGENERATION STUDY IN THE RAT - FINAL REPORT - VOLUMES I AND II
Author(s), year:	██ 1980
Report/Doc. number::	A83174 / M-155448-01-1
Guideline(s):	the study predates OECD Guideline 416 (1983)
GLP:	Report completed after the effective date of FDA Good Laboratory Practice Regulation (1979); some deviations from GLP, e.g that the protocol was not signed by the Study Director
Deviations from OECD 416 (2001):	No sperm parameters like sperm motility, sperm morphology, sperm count were assessed Number of animals per group lower than 20 since the females were divided in sub-groups for different observations Low number of animals (five from control and high dose each) taken for histopathological observation
Acceptability:	Yes; limited information

### Material and Methods

Groups of 30 male and 30 female rats ██████████ were dosed via the diet at levels of 0, 200, 1000 and 5000 ppm ethofumesate (purity: 97.8%). The concentrations corresponded to 16, 78 and 397 mg/kg bw/day for males and 18, 88 and 454 mg/kg bw/day for females (no differentiation upon different stages was made in the

report). The animals were dosed for 90 days before pairing and throughout the mating, gestation and lactation periods of two successive litters, F<sub>1</sub>A and F<sub>1</sub>B. Using random selected animals from the second litter for continuation of the study, the process was repeated on two further occasions, providing information on F<sub>2</sub>A, F<sub>2</sub>B, F<sub>3</sub>A and F<sub>3</sub>B litters. After the second mating the females from each group were divided into two approximately equal-sized sub-groups. One sub-group was sacrificed on day 21 of gestation and the other was allowed to deliver and rear the young until weaning. The following observations were done on females sacrificed on Day 21 of gestation: maternal toxicity, number of corpora lutea in each ovary, number of foetuses in each uterine horn, assessment of foetal viability, individual foetal and placental weight, uterine position and sex of foetuses, external and internal examination of all foetuses, calculation of pre- and post-implantation loss. In females allowed to litter following observations were made: gestation length, parturition, and litter size at birth, birth-weight of offspring and viability of offspring.

Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroid and uterus were weighed. A series of organs and tissues were prepared for microscopic evaluation which was confined to five of the control and high dose animals.

## Results

General condition and mortalities:

No signs of reaction to treatment and no treatment related mortalities were observed in any generation.

Bodyweight gain and food consumption:

Body weight gain in males was similar in all groups and all generations. Body weight gain was from slightly to significantly reduced at the 5000 ppm level in females in different generations. Food consumption and water intake were unaffected by treatment.

Reproductive performance:

The duration and regularity of oestrus cycles, pre-coital interval, mating performance and conception rates were unaffected by the treatment. Gestation length was comparable for all groups in all generations; no dystocia was observed. Litter size at birth was similar in all groups and not dose dependent.

Foetal effects :

F<sub>0</sub> to F<sub>1</sub>A, F<sub>0</sub>to F<sub>1</sub>B (dams allowed to give birth), F<sub>1</sub>to F<sub>2</sub>A, F<sub>1</sub>to F<sub>2</sub>B (dams allowed to give birth), F<sub>2</sub>to F<sub>3</sub>A and F<sub>2</sub>to F<sub>3</sub>B (dams allowed to give birth): The general condition of offspring was unaffected by treatment. Live births and viability indices, birth weight and body weight gain of offspring during the lactation period, sex ratio at birth and at weaning were similar in all groups and not dose dependent.

F<sub>0</sub>to F<sub>1</sub>B (dams sacrificed on day 21 of gestation): There was a slight increase in pre-implantation loss and a corresponding decrease in the number of viable foetuses at 5000 ppm but this was attributed to two females

having one implantation only. At 5000 ppm there was increased number of foetuses with incomplete ossification of cranial bones.

F<sub>1</sub> to F<sub>2</sub>B (dams sacrificed on day 21 of gestation): No treatment related changes were recorded in the numbers of corpora lutea, implantations or viable foetuses, or in the extent of pre- and post-implantation losses. No treatment related effects were observed in the post mortem examination of foetuses.

F<sub>2</sub>to F<sub>3</sub>B (dams sacrificed on day 21 of gestation): No treatment related changes were recorded in the numbers of corpora lutea, implantations or viable foetuses, or in the extent of pre- and post-implantation losses. No treatment related effects were observed in the post mortem examination of foetuses.

#### Macroscopic observations:

No macroscopic abnormalities were observed in any generation that could be attributed to treatment with ethofumesate.

#### Organ weight analysis:

No consistent effects on organ weights were observed in animals of F<sub>0</sub> and F<sub>1</sub> generation. Except for the highest dose of 5000 ppm, no treatment related or dose dependents effects on organ weights were measured. In F<sub>0</sub> generation dosed at 5000 ppm only statistically significantly lower absolute and relative thyroid weight in females was measured. No effects on organ weights in males of F<sub>0</sub> generation were measured. In F<sub>1</sub> generation at 5000 ppm there was measured a statistically significantly higher absolute weight of lungs, liver and kidneys and statistically significantly lower relative weight of thyroid and testes in males. In females of F<sub>1</sub> generation only the relative and absolute uterus weight were significantly higher. In F<sub>2</sub> generation no effects on organ weights related to treatment were measured.

**Table B.6.6.1-3. Organ weights**

Organ	Females				Males			
	Control	200 ppm	1000 ppm	5000 ppm	Control	200 ppm	1000 ppm	5000 ppm
<b>F<sub>0</sub></b>								
Thyroid (absolute weight) g	0.023	0.021	0.021	<b>0.019**</b>	0.027	0.024	0.025	0.026
Thyroid (relative weight) % bw	0.0063	0.0055	0.0058	<b>0.0051* (-19%)</b>	0.004	0.0036	0.0038	0.004
<b>F<sub>1</sub></b>								
Lungs (absolute weight) g	1.9	1.7	1.8	1.8	2.3	2.4	2.4	<b>2.6*</b>
Liver (absolute weight) g	12.9	14.5	12.5	12.1	19.7	<b>22.3*</b>	20.2	<b>23.5**</b>
Kidneys (absolute	2.5	2.8	2.6	2.6	4.1	4.4	4.0	<b>4.6*</b>



weight) g								
Uterus (absolute weight) g	0.57	<b>0.72*</b>	0.64	<b>0.72*</b>	-	-	-	-
Uterus (relative weight) % bw	0.17	0.18	0.19	<b>0.22*</b> (+29.4%)	-	-	-	-
Thyroid (relative weight) % bw	0.0048	0.0052	0.0056	0.0047	0.0038	0.0036	0.0036	<b>0.0031**</b> (-18.4%)
Testes (relative weight) % bw	-	-	-	-	0.59	0.54	0.57	<b>0.53*</b> (-10.2%)

\* significantly different from control,  $p < 0.05$ , analysis of variance

\*\* significantly different from control,  $p < 0.01$ , analysis of variance

#### Histopathology:

Histopathological assessment of selected males and females of control and high dose revealed no changes that could be associated with the treatment.

#### Conclusion

In general, ethofumesate caused only minor signs of toxicity in this 3-generation reproduction study. The parental NOAEL is proposed at 1000 ppm (78 mg/kg bw/d in males and 88 mg/kg bw/d in females), based on decrease in body weight gain in females and unspecific organ weight changes of questionable relevance (without any histopathological findings in the organs) at 5000 ppm. The foetal NOAEL is also proposed at 1000 ppm, based on increased number of foetuses with incomplete ossification of cranial bones in F<sub>1</sub>B generation only dosed with 5000 ppm. The reproduction NOAEL is proposed > 5000 ppm (> 397 mg/kg bw/d in males and 454 mg/kg bw/d in females) since no treatment related effects on reproductive performance were observed up to the highest dose tested.

In the DAR (1998) only NOAEL of 1000 ppm (for parental animals) was derived, no specification for offspring and reproduction was made. The current proposal for the NOAELs (parental and foetal) follows the set NOAEL of 1000 ppm from the DAR, although the RMS recognises that the observed, inconsistent effects of questionable toxicological relevance in parental animals and offspring might also justify the higher NOAEL of 5000 ppm (highest tested dose). However, the NOAEL of 1000 ppm is in line with the NOAELs of other multigeneration studies.

<b>Reference:</b>	Ethofumesate technical - Two generation reproduction study in Wistar rats
Author(s), year:	██████████. 1993
Report/Doc. number::	OFC00004862 / M-352016-01-1
Guideline(s):	OECD Guideline 416 (1983)
GLP:	Yes

Deviations from OECD 416 (2001):	No sperm parameters like sperm motility, sperm morphology, sperm count were assessed
Acceptability:	No organs weights were assessed
	Yes

### Material and Methods

Groups of 30 male and 30 female Wistar rats were dosed via the diet at levels of 0, 100, 1000 and 10000 ppm ethofumesate (purity : 98%). The calculation of actual daily doses (table below) were done by the applicant in 2013 and submitted in the supplementary dossier for renewal of ethofumesate. The animals were dosed for 10 weeks before pairing. The first mating produced the F<sub>1</sub> litter from which the second parental generation (P<sub>1</sub>) was selected to continue the study. This procedure was repeated in the P<sub>1</sub> generation to produce the F<sub>2</sub> litter which was sacrificed at weaning. No organs were weighed. Histopathological examination was done on ovaries, uterus, vagina, testes, epididymis, seminal vesicles, prostate, coagulating glands, pituitary, adrenals, liver and kidneys.

**Table B.6.6.1-4. Actual daily doses [mg/kg bw/d]**

Concentration in feed	100 ppm	1000 ppm	10000 ppm
<b>P<sub>0</sub> generation</b>			
Males – pre mating and mating	5.9	60.9	654.0
Females - pre mating	8.3	90.7	939.7
Females - gestation	7.4	79.6	840.4
Females - lactation	14.3	154.2	1514.0
<b>P<sub>1</sub> generation</b>			
Males – pre mating and mating	6.9	76.7	880.9
Females - pre mating	10.9	119.8	1322.6
Females - gestation	9.9	109.7	1099.8
Females - lactation	18.4	196.1	2028.2

### Results

General condition and mortalities :

No dose related clinical signs, mortality or incidence of cannibalism were observed in the study.

Bodyweight gain and food consumption:

In males, statistically significant and consistent reduction in body weight gain was observed in animals dosed with 10000 ppm in both generations. In P<sub>0</sub> generation males, the decreased body weight gain was maximum 12 % of control, mainly at the begin of the study, while from week 7 to week 16 the decrease in body weight gain was below 10% comparing to control animals. In P<sub>1</sub> generation males, the decreased body weight gain was maximum 15 % of control, mainly at the begin of the study, while from week 9 to week 16 the decrease in body weight gain was far below 10% comparing to control animals. No consistent or dose related changes in body weight gain were observed in males dosed at 100 and 1000 ppm.

In females, statistically significant reduction in body weight gain was observed in animals dosed with 1000 and 10000 ppm in P<sub>0</sub> generation and in animals dosed with 10000 ppm in P<sub>1</sub> generation. Although the decrease in body weight gain in P<sub>0</sub> females dosed with 1000 ppm was statistically significant it was always below 10% comparing to control. In P<sub>0</sub> generation females dosed with 10000 ppm, the decrease in body weight gain was between 12 and 13% comparing to control animals during the course of the study. In P<sub>1</sub> generation females dosed with 10000 ppm, statistically significant decrease in body weight gain higher than 10% was observed only from week 4 to week 5.

No dose or treatment related effect on food consumption was observed during the study.

#### Reproductive performance:

The duration and regularity of oestrus cycles, pre-coital interval, mating performance and conception rates were unaffected by the treatment. Gestation length was comparable for all groups in all generations; no dystocia was observed.

In P<sub>0</sub> generation, the percentage of pre-implantation loss and mean number of implantations in females dosed with 10000 ppm was statistically significantly higher than in control animals. The mean litter size was statistically significantly lower at 10000 ppm than in the control group.

In P<sub>1</sub> generation no negative effects on fertility indices were observed, to the contrary, the reproductive performance was better in the treated groups than in the control animals.

#### Foetal effects:

In the P<sub>0</sub> generation the live birth index, the 21 day survival index and the number of male pups were statistically significantly lower at 10000 ppm than in the control group. No effects on pups body weight was measured during the lactation period.

In the P<sub>1</sub> generation, the number of live litters, mean viable litter size, number of live pups on day 1 and survival index for days 4, 7, 14 and 21 were (significantly) higher in treated groups than in the control. No effects on pups body weight was measured during the lactation period.

#### Macroscopic observations:

No treatment or dose related effects on gross pathological lesions were observed.

#### Histopathology:

No treatment or dose related histopathological effects on reproductive organs were observed.

### Conclusion

In the DAR (1998) only NOAEL of 1000 ppm was derived without further specification if for parental, foetal or reproduction effects. The current proposal for the NOAELs (parental, foetal and reproductive) follows the set NOAEL of 1000 ppm from the DAR.

In general, most of the effects observed in parental and offspring animals in the P<sub>0</sub> generation were either not confirmed in the P<sub>1</sub> generation or were observed to much lesser extent.

The parental NOAEL is proposed at 1000 ppm (60.9 mg/kg bw/d in males and 90.7 mg/kg bw/d in females) based on decrease in body weight gain observed in males and females of both parental generations dosed with 10000 ppm. The reproductive NOAEL is proposed at 1000 ppm, based on higher incidences of pre-implantation loss and lower mean litter size in P<sub>0</sub> generation dosed at 10000 ppm (not confirmed in P<sub>1</sub>). The foetal NOAEL is proposed also at 1000 ppm, based on the lower live birth index, the 21 day survival index and the number of male pups at 10000 ppm in P<sub>0</sub> generation (not confirmed in P<sub>1</sub>).

### B.6.6.2. Developmental toxicity studies

The evaluations of all studies presented below were already included in the original DAR (1998). No additional studies were submitted for the purpose of renewal. Re-wording was conducted and additional information was included in DRAR where considered necessary for better overview. Finally, the validity of studies in view of updated OECD guidelines for developmental toxicity studies was proven.

#### B.6.6.2.1. Rat

<b>Reference:</b>	ETHOFUMESATE: ORAL (GAVAGE) RANGE FINDING STUDY IN THE PREGNANT RAT
Author(s), year:	1991
Report/Doc. number:	A87574 / M-161487-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Limited number (seven) of animals per group Shorter administration period (day 6 to 15) Limited parameters retrieved (no skeletal or soft tissue examination of foetuses done)
Acceptability:	Yes (limited information); range finding study

### Material and Methods

Four groups of seven Sprague-Dawley female rats, 10 to 12 weeks old, were dosed with ethofumesate (purity: > 97%) orally, by gavage, at dose levels of 0, 300, 1000 or 2000 mg/kg bw/day from day 6 to 15 of gestation. The females were sacrificed at day 20 of gestation, necropsied and their uterine contents assessed.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 3, 6, 9, 12, 15, 18 and 20. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea and number and intrauterine position of implantations recorded. Foetuses were sacrificed and weighed, examined only externally and sexed.

### Results

All animals survived until the scheduled sacrifice. There were no adverse clinical or necropsy findings. Pregnancy incidence, body weight gain, food intake, pre- or post- implantation loss, litter size, sex ratio or foetal weight were not affected by the treatment at any dose levels.

## Conclusion

According to the results of this range finding study with limited number of animals and parameters assessed, the maternal and the foetal NOAELs are both proposed at 2000 mg/kg bw/d, based on absence of any treatment related findings at highest tested dose.

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<b>Reference:</b>	ETHOFUMESATE: ORAL (GAVAGE) TERATOLOGY STUDY IN THE RAT
Author(s), year:	██████ 1991
Report/Doc. number:	A87575 / M-161489-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 6 to 15 of gestation)
Acceptability:	Yes

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## Material and Methods

Four groups of 24 mated female Sprague-Dawley rats, 10 to 12 weeks old, were administered ethofumesate (purity: > 97%) orally, by gavage (in 1% methyl cellulose), at dose levels of 0, 1000, 2000 and 4000 mg/kg bw/d from day 6 to day 15 of gestation. All the animals were kept until day 20 of gestation when they were sacrificed and their uterine contents examined.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, 6, 9, 12, 15, 18 and 20. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea and number and intrauterine position of implantations recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

## Results

All the animals survived till the scheduled sacrifice. Pale faeces (coloured as the test article) were observed in all treated animals, but the effect was transient and was considered not to be a toxic effect. Body weight gain, food intake, pregnancy rate, corpora lutea and implantations were within normal limits in all the treated groups. There was no effect of treatment on post-implantation loss, litter size or sex ratio, litter weight or foetal weight. There were no treatment related malformations and no differences in incidence of visceral and skeletal variations.

## Conclusion

According to the results of the study, the maternal and the foetal NOAELs are both proposed at 4000 mg/kg bw/d, based on absence of any treatment related findings at this highest tested dose. Although the administration period was shorter (from day 6 to 15 of gestation) than the one from the current OECD Guideline (administration until the end of gestation) it is assumed that the effects on dams and offspring, which were not observed after administration of ethofumesate during first two trimester of gestation, will also not occur in the last trimester. Therefore, the study is considered valid and acceptable.

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<b>Reference:</b>	TECHNICAL ETHOFUMESATE: ORAL TERATOLOGY (DEVELOPMENTAL TOXICITY) STUDY IN THE RAT
Author(s), year:	██████ 1991
Report/Doc. number:	A83205 / M-155477-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 6 to 15 of gestation) 10fold interval between intermediate and high dose
Acceptability:	Yes

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### Material and Methods

Four groups of 24 mated female Sprague-Dawley rats, approximately 12 weeks old, were administered ethofumesate (purity: 97%) orally, by gavage (in 1% methyl cellulose), at dose levels of 0, 10, 100 and 1000 mg/kg bw/d from day 6 to day 15 of gestation. All the animals were kept until day 20 of gestation when they were sacrificed and their uterine contents examined.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, 3, 6, 7, 8, 9, 12, 15, 18 and 20. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea and number and intrauterine position of implantations recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

### Results

There were no deaths in dams at any dose level and the treatment-related clinical signs were limited to transient post-dosing salivation at 1000 mg/kg. At this dose, treatment-related increases in the mean body weight gain and mean water intake and a reduction in the mean food intake were also observed following the initiation of dosing, but these effects were both slight and transient. There were no treatment-related maternal necropsy findings at any dose level.

Body weight gain, food intake, pregnancy rate, corpora lutea and implantations were within normal limits in all treated groups. There was no effect of treatment on post-implantation loss, litter size or sex ratio, litter weight or foetal weight. There were no treatment related malformations and no differences in incidence of visceral and skeletal variations.

### Conclusion

According to the results of the study, the maternal and the foetal NOAELs are both proposed at 1000 mg/kg bw/d, based on absence of any adverse treatment related findings at this highest tested dose. Although the administration period was shorter (from day 6 to 15 of gestation) than the one from the current OECD Guideline (administration until the end of gestation) it is assumed that the effects on dams and offspring, which were not observed after administration of ethofumesate during first two trimester of gestation, will also not occur in the last trimester. Therefore, the study is considered valid and acceptable.

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<b>Reference:</b>	Teratogenicity study in Wistar rats (Limit dose test)
Author(s), year:	██████████ 1991
Report/Doc. number:	OFC00004865 / M-352018-01-1 and Amendment OFC00004864 / M-352142-01-1 (1999)
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 6 to 15 of gestation)
Acceptability:	Yes

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### Material and Methods

One group of 20 mated female Wistar rats, 18 to 20 weeks old, was administered ethofumesate (purity: 98%) orally, by gavage (in 1% peanut oil), at 1000 mg/kg bw/d from day 6 to day 15 of gestation. 30 animals served as control group and received only vehicle. All animals were kept until day 20 of gestation when they were sacrificed and their uterine contents examined.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, daily from day 6 to 15 and on day 20. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea, number of implantations, embryonic resorptions and fetal resorptions recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

### Results

There were no toxicologically treatment related deaths in dams (one treated dam dies because of the incorrect gavage) and no treatment-related clinical signs at 1000 mg/kg. No inter group differences were recorded in body weight and body weight gain.

There were no intergroup differences in number of corpora lutea, implantations, embryonic and foetal resorptions, foetal weight and sex ratio. The number of dams with any resorption was even lower in the treatment group.

There were no treatment related external variations and malformations.

Regarding visceral findings in foetuses, the incidence of displaced umbilical artery was significantly higher in 1000 mg/kg bw group. Regarding skeletal findings, incidence of incomplete/partial ossification of intraparietal bones was statistically significantly higher in the treated group.

In the original DAR (1998) the significantly higher incidence of malpositioned umbilical artery in foetuses of 1000 mg/kg bw group was not mentioned, whereas for incomplete intraparietal ossification it was stated that this is a malformation. According to the new nomenclature of findings (DevTox<sup>5</sup>) neither malpositioned umbilical artery nor the incomplete intraparietal ossification are considered to be malformations, but variations.

Ethofumesate was already discussed at ECB (10 – 21 May 1999, ECBI/43/99 Rev. 2) regarding developmental effects. It was concluded that ethofumesate does not fulfil the criteria for classification for developmental effects.

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<sup>5</sup> <http://www.devtox.org/nomenclature/organ.php>

In the final Evaluation table (Doc 6487/VI/99 rev 6 (05.02.2001)) for inclusion of ethofumesate in Annex I of Directive 91/414/EC it was concluded that ethofumesate does not pose a risk for teratogenic effects.

However, since no detailed argumentations/discussions from ECB are available, the RMS concluded to add more information in the DRAR to support the original decision on non-teratogenicity of ethofumesate.

The RMS requested from the TaskForce to provide historical control data for these two findings, covering the requirements for historical control data set in Commission Regulation (EU) No 283/2013. The TaskForce provided raw data and evaluation of 14 studies conducted between 1990 and 1995 in the same laboratory and so covering a five-year period, centred as closely as possible on the date of the index study. The RMS considered the HCD appropriate for comparison since following parameters were common: species and strain; name of the supplier; specific colony identification; general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed; approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death; description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations; name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study.

Based on the evaluation of historical control data the RMS concluded that both findings are within historical control data. For incomplete intraparietal ossification additionally it could be shown that in the HCD up to 100% of fetuses in one litter and up to 60% of control dams can be frequently affected while for the current study 83% of examined fetuses per litter and 30% of dams were recorded. For displaced umbilical artery additionally it could be shown that in the HCD up to 100% of fetuses in one litter and up to 55% of control dams can be frequently affected while in the current study 75% of examined affected fetuses per litter and 25% affected dams were recorded.

**Table B.6.6.2.1-1. Incidences of findings and comparison to historical control data**

Findings	Dose [mg/kg bw/d]		Laboratory historical control data (14 studies 1990 – 1995)
	Control	1000 mg/kg bw/d	
Visceral findings			
% fetuses with displaced (malpositioned) umbilical artery [%]	0.8	9.5*	0 – 13%
Skeletal findings			
% fetuses with incomplete/partial ossification of intraparietal bones	2.1	11.6*	0 – 24.2%

\* significantly different from control,  $p < 0.05$ , contingency test

## Conclusion

According to the results of the study, the maternal and the foetal NOAEL are both proposed at 1000 mg/kg bw/d, based on absence of any adverse treatment related findings at this highest tested dose.



**B.6.6.2.2. Rabbit**

<b>Reference:</b>	ETHOFUMESATE: ORAL (GAVAGE) RANGE FINDING STUDY IN THE PREGNANT RABBIT
Author(s), year:	██████ 1991
Report/Doc. number:	A87576 / M-161491-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 7 to 19 of gestation) Low number of animals (5) per group since range finding study
Acceptability:	Yes (limited information); range finding study

**Material and Methods**

Four groups of 5 mated female New Zealand white rabbits were given ethofumesate (purity: not stated) by gavage, at dose levels of 0, 300, 1500 or 2000 mg/kg bw/day from day 7 to day 19 of gestation. Control animals were given the vehicle (1% w/v aqueous methyl cellulose). All the animals were kept until day 29 of gestation, sacrificed and their uterine contents assessed.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, 7, 12, 19, 23 and 29. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea, number of implantations, embryonic resorptions and foetal resorptions recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

**Results**

Three animals at the high dose level were sacrificed during the treatment period having shown excessive reduction in body weight gain from the start of treatment and poor condition. Similar signs were apparent in two animals at the intermediate dose level which were sacrificed during the post-dosing period (days 22 and 23). One animal in the control group died on day 10 of gestation.

There was a dosage-related reduction in the body weight gain at the intermediate and high dose levels. In these two groups periods of anorexia (not eating) were observed. In the low dose group the body weight gain was comparable to control animals.

All animals were pregnant. Pre-implantation loss was lower in all treated groups comparing to control (however this was prior to dosing). Post-implantation loss was higher in the high dose group (15.8%) than in the control (4.9%), but no differences to control were observed in the low (5.8%) and intermediate (0%) dose group.

Litters size was slightly reduced in the high dose group. Sex ratio was within normal limits in all groups. There was no conclusive effect of treatment on foetal weight in any dose group. There was no indication of an adverse effect of treatment on the incidence of malformations or variations.

**Conclusion**

Administration of ethofumesate at 2000 mg/kg bw/d elicited marked maternal toxicity characterised by mortality, reduction in body weight gain and reduced food intake. A higher incidence of intrauterine deaths (post-implantation loss) was also noted at 2000 mg/kg bw/d, but there was no effect on foetal growth or

development. Similar but less marked maternal effects were observed at 1500 mg/kg bw/d. At this dose level no effects on foetuses were observed.

The maternal NOAEL is proposed at 300 mg/kg bw/d, based on mortality, reduced body weight gain and reduced food intake at 1500 mg/kg bw/d. The foetal NOAEL is proposed at 1500 mg/kg bw/d, based on increased intrauterine deaths at 2000 mg/kg bw/d.

<b>Reference:</b>	ETHOFUMESATE: ORAL (GAVAGE) TERATOLOGY STUDY IN THE RABBIT
Author(s), year:	██████████, 1991
Report/Doc. number:	A87577 / M-161492-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 7 to 19 of gestation) The number of pregnant animals (15 and 14) in intermediate and high dose group slightly lower than the lowest number of animals (16) to be achieved according to OECD 414
Acceptability:	Yes

## Material and Methods

Four groups of 16 mated female New Zealand white rabbits were given ethofumesate (purity: > 97%), by gavage, at dose levels of 0, 300, 600 or 1200 mg/kg bw/day from day 7 to day 19 of gestation. Control animals were given the vehicle (1% w/v aqueous methyl cellulose). All the animals were kept until day 29 of gestation, sacrificed and their uterine contents assessed.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, 7, 12, 19, 23 and 29. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea, number of implantations, embryonic resorptions and foetal resorptions recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

## Results

One animal at the high dose level was sacrificed on day 21 of gestation after showing excessive weight loss (19%) and abortion.

A significant reduction ( $p < 0.001$ , analysis of variance, Dunnett's test) in the weight gain and food intake was observed in the high dose group at the start of treatment (days 7 to 12) and the mean weight gain and food intake of the animals in the high dose group was lower than controls over the dosing period. The body weight gain in the low and the intermediate group were either slightly lower or comparable to controls.

Pregnancy incidence was within normal limits in all groups. The mean number of corpora lutea and implantations was comparable to controls in all treated groups. No intergroup differences in post implantation loss or litter size were observed. Sex ratio was within normal limits in all groups.

There was no treatment related effect on foetal weight in any dose group. There was no indication of an adverse effect of treatment on the incidence of malformations or variations.

## Conclusion

Administration of ethofumesate at 1200 mg/kg bw/d elicited maternal toxicity characterised by reduction in body weight gain and reduced food intake and a single occurrence of abortion. No effects on offspring were observed at any dose level.

The maternal NOAEL is proposed at 600 mg/kg bw/d, based on reduced body weight gain and reduced food intake at 1200 mg/kg bw/d. The foetal NOAEL is proposed at 1200 mg/kg bw/d, the highest dose tested.

Although the administration period was shorter (from day 7 to 19 of gestation) than the one from the current OECD Guideline (administration until the end of gestation) it is assumed that the effects on dams and offspring, which were not observed after administration of ethofumesate during first two trimester of gestation, will also not occur in the last trimester. Therefore, the study is considered valid and acceptable.

<b>Reference:</b>	Teratogenicity study in rabbits (Limit test)
Author(s), year:	██████████, 1993
Report/Doc. number:	OFC00004866 / M-352093-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 6 to 18 of gestation) The number of surviving pregnant animals at 1000 mg/kg bw/d (limit test) was lower (12) than the lowest number of animals (16) to be achieved according to OECD 414
Acceptability:	Yes; limited information based on reduced number of treated dams

## Material and Methods

26 and 19 mated female New Zealand white rabbits were treated by gavage with vehicle (0.5% aqueous methyl cellulose) and 1000 mg/kg bw/d ethofumesate (purity : 98%) respectively, from day 6 to day 18 of gestation (Limit test). All animals were kept until day 28 of gestation, sacrificed and their uterine contents assessed.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation day 0, daily from day 6 through day 18 and on day 28 of gestation. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea, number of implantations, embryonic resorptions and foetal resorptions recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

## Results

At the end of the study, 20 animals from control and 12 from the treatment group were pregnant and survived the treatment. In the control group two dams died because of wrong gavaging, which was also the case for five dams from the dosed group.

The animals exposed to 1000 mg/kg bw/d ethofumesate showed clinical signs such as weakness and a nasal discharge.

Body weights, body weight gain and food intake during different periods of treatment did not statistically differ between the control and the treated animals.

No differences in number of corpora lutea, number of implantations, pre- and post-implantation loss were observed between the controls and treated animals. The mean litter size, sex ratio and litter/foetal weight were comparable between control and treated animals.

No intergroup differences were observed regarding external variations and malformations in foetuses.

Statistically significant intergroup differences in delayed or incomplete ossification was observed in various bones, however, in most cases the incidences of incomplete ossification were higher in the control than in the treated group.

Regarding visceral findings, a statistically significant ( $p < 0.05$ ) increase in incidence of a dilated right heart ventricle was observed in 9 fetuses in 3 litters (10.6% of all examined fetuses from 1000 mg/kg bw/d group) while no incidence was observed in the control group.

In the original DAR (1998) the higher incidence of dilated right heart ventricle was mentioned but not further evaluated. According to the nomenclature of findings (DevTox<sup>6</sup>) dilated heart ventricle is not considered to be a malformation.

Ethofumesate was already discussed at ECB (10 – 21 May 1999, ECBI/43/99 Rev. 2) regarding developmental effects. It was concluded that ethofumesate does not fulfil the criteria for classification for developmental effects. In the final Evaluation table (Doc 6487/VI/99 rev 6 (05.02.2001)) for inclusion of ethofumesate in Annex I of Directive 91/414/EC it was concluded that ethofumesate does not pose a risk for teratogenic effects.

However, since no detailed argumentations/discussions from ECB are available, the RMS concluded to add more information in the DRAR to support the original decision on non-teratogenicity of ethofumesate.

The RMS requested from the TaskForce to provide historical control data for this finding, covering the requirements for historical control data set in Commission Regulation (EU) No 283/2013. The TaskForce provided raw data and evaluation of 12 studies conducted between 1993 and 1998 in the same laboratory and so covering a five-year period, centred as closely as possible on the date of the index study. The RMS considered the HCD appropriate for comparison since following parameters were common: species and strain; name of the supplier; specific colony identification; general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed; approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death; description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations; name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study.

Based on the evaluation of historical control data the RMS concluded that dilated right heart ventricle is within historical control data. Additionally it could be shown that also in the HCD up to 100% of fetuses in one litter can be affected as it was observed in the current study.

**Table B.6.6.2.2-1. Incidences of findings and comparison to historical control data**

Findings	Dose [mg/kg bw/d]		Laboratory historical control data (12 studies 1993 – 1998)
	Control	1000 mg/kg bw/d	
Visceral findings			
% fetuses with	0%	10.6% *	0 – 15%

<sup>6</sup> <http://www.devtox.org/nomenclature/organ.php>

dilated right ventricle of the heart [%]			
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\* significantly different from control,  $p < 0.05$ , contingency test

## Conclusion

Ethofumesate administered at 1000 mg/kg bw/d caused clinical signs in dams, but no effects on mortality or body weight gain and food consumption were observed. Regarding foetuses, no treatment related effects were observed on body weight or incidences of variations or malformations. Both maternal and foetal NOAEL are proposed at 1000 mg/kg bw/d based on absence of any adverse treatment related findings at the limit dose.

<b>Reference:</b>	SN 49.913 (ETHOFUMESATE) - EMBRYOTOXICITY INCLUDING TERATOGENICITY STUDY IN THE RABBIT AFTER DAILY INTRAGASTRIC ADMINISTRATION FROM DAY 6 TO DAY 18 OF GESTATION
Author(s), year:	1986
Report/Doc. number:	A83190 / M-155463-01-1
Guideline(s):	OECD Guideline 414 (1981)
GLP:	Yes
Deviations from OECD 414 (2001):	Shorter administration period (day 6 to 18 of gestation) The number of surviving pregnant animals at 3000 mg/kg bw/d was very low (9)
Acceptability:	Yes; limited information

## Material and Methods

Four groups of 25 mated female New Zealand white rabbits were given ethofumesate (purity: not stated) orally, by gavage, at dose levels of 0, 30, 300, or 3000 mg/kg bw/day from day 6 to day 18 of gestation. All the animals were kept until day 28 of gestation, sacrificed and assessed for effects of the compound.

All females were examined daily to detect mortality and any clinical signs. Body weight was recorded on gestation days 0, 6, 13, 18, 23 and 28. After sacrifice the ovaries and uteri were removed and pregnancy status, number of corpora lutea, number of implantations, embryonic resorptions and foetal resorptions recorded. Foetuses were sacrificed and weighed, examined externally and sexed. The necropsy was conducted in order to reveal any abnormalities or malformations.

## Results

During the last days and after termination of treatment (between days 16 and 24) eleven females of the high dose group died and one additional female was sacrificed moribundly on day 17. In low and intermediate dose groups the little mortality was not considered to be treatment related. There were severe discrepancies comparing these effects with effects of the preliminary study (not evaluated) where 3000 mg/kg bw/d were well tolerated by the dams.

A significant reduction ( $p < 0.05$ , analysis of variance, Scheffe test) in the body weight gain was observed in the high dose group at the start of treatment (days 6 to 13) and there was no compensation during the rest of the treatment period.

At 3000 mg/kg bw/d increased abortions (three comparing to one in the control group) were observed. At 300 and 3000 mg/kg bw/d an increased incidence of post-implantation loss was observed, this was statistically significant only at 3000 mg/kg bw/d.

At 3000 mg/kg bw/d the mean foetal weight was slightly decreased and there was a slight retardation of foetal development observed as incomplete ossification of different bones.

No anomalies or malformations were observed which could be considered treatment related.

## Conclusion

Administration of ethofumesate at 3000 mg/kg bw/d elicited maternal toxicity characterised by mortality, abortions and reduction in body weight gain and foetal toxicity characterised by significantly increased post-implantation loss, decreased mean foetal weight and delayed ossifications.

The maternal NOAEL is proposed at 300 mg/kg bw/d, based on mortality, abortions and reduction in body weight gain at 3000 mg/kg bw/d. The foetal NOAEL is also proposed at 300 mg/kg bw/d, based on significantly increased resorptions, decreased mean foetal weight and delayed ossifications.at 3000 mg/kg bw/d.

In the original DAR (1996) the RMS Sweden concluded that the study does not seem to be of acceptable quality.

## B.6.7. NEUROTOXICTY

No studies were evaluated in the original DAR (1998) and no additional studies were submitted for the purpose of renewal.

### B.6.7.1. Neurotoxicity studies in rodents

There are no indications from the existing database that ethofumesate has effects on the nervous system. Therefore, no specific neurotoxicity studies are required.

### B.6.7.2. Delayed polyneuropathy studies

Studies on delayed neuropathy are not required since ethofumesate is not an organophosphate and based on available information does not have the potential to cause delayed neuropathy.

## B.6.8. OTHER TOXICOLOGICAL STUDIES

### B.6.8.1. Toxicity studies on metabolites and relevant impurities

No studies on metabolites were evaluated in the original DAR (1998) and this was also not considered necessary. For purpose of renewal the notifier TaskForce provided a package of genotoxicity studies *in vitro* with the assumed metabolite called “BCS - CU88901” (the sodium salt of metabolite NC 20645 – carboxylic acid). The notifier TaskForce argued that the sodium salt of metabolite NC 20645 – carboxylic acid is a groundwater metabolite and that therefore the data package for this metabolite is necessary. Based on the FOCUS calculations

there are no groundwater metabolites of ethofumesate exceeding the trigger of 0.1 µg/l. Therefore, the studies on genotoxicity of sodium salt of metabolite NC 20645 are not considered necessary. Studies are evaluated below (in case they are needed in the future) although not considered “relied upon” in the current assessment.

Additionally, the RMS concluded in the ADME studies that carboxylic acid is the main metabolite in rat urine, constituting up to 97% of urine radioactivity. Thus, the toxicity of the ethofumesate-carboxylic acid is considered covered by the studies conducted with ethofumesate and no studies with this metabolite are considered necessary. It is subsequently followed that the sodium-salt of ethofumesate carboxylic acid would not have any other toxicological properties than those observed in the toxicity studies with the active substance.

***B.6.8.1.1. BCS - CU88901 (the sodium salt of metabolite NC 20645 – carboxylic acid)***

<b>Reference:</b>	Salmonella typhimurium reverse mutation assay with BCS-CU88901 (Na salt of NC 20645)
Author(s), year:	Sokolowski, A., 2012
Report/Doc. number::	1457201/ M-427430-01-1
Guideline(s):	OECD 471 (1997)
GLP:	Yes
Deviations:	No
Acceptability:	Yes

**Material and Methods**

<b>1. Test material:</b>	BCS-CU88901 (NA salt of NC 20645)
Description:	brown powder
Lot/Batch no:	SES 11754-3-8
Purity:	69.2% (dose adjusted to content)
Stability of test compound:	guaranteed for study duration; expiry date: 2013-10-05
<b>2. Vehicle and/or positive control:</b>	Vehicle: deionised water used for solvent control, test substance and positive controls except for 4-nitro-o-phenylene-diamine and 2-aminoanthracene. DMSO used for 4-nitro-o-phenylene-diamine and 2-aminoanthracene
	Positive controls: Sodium azide (Na-azide), 4-nitro-o-phenylene-diamine (4-NOPD) methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)
<b>3. Test system:</b>	Salmonella typhimurium strains TA1535, TA1537, TA100, TA98, TA102
metabolic activation:	S9 mix from phenobarbital/β-naphthoflavone induced rat livers

## Study design and methods

### 1. Treatment:

Dose: 0-3-10-33-100-333-1000-2500- 5000 µg/plate +/-S9 mix  
(3 and 10 µg/plate tested in the first assay only)

positive controls:

NaN<sub>3</sub> 10 µg/plate (TA1535 and TA100) -S9 mix

4-NOPD 10 µg/plate (TA 98) -S9 mix

4-NOPD 50 µg/plate (TA 1537) -S9 mix

MMS 3 µl/plate (TA 102) -S9 mix

2-AA 2.5 µg/plate (TA1535, TA1537, TA98, TA100) +S9 mix

2-AA 10 µg/plate (TA102) +S9 mix

Application volume: 0.1 mL/plate

Incubation time / temperature: 48 h, 37°C

## Results

No substantial increase in revertant colony numbers of any of the five test strains was observed following treatment with BCS-CU88901 at any dose level, neither in the presence or absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies confirming the sensitivity of the assay.



Table 6.8.1.1-1: Summary of mean values without S9 mix

	Strain				
Concentration [µg/plate]	TA1535	TA1537	TA98	TA100	TA102
Deionised water	12	12	28	99	322
Untreated	12	10	30	104	305
3	14	13	31	84	304
10	14	12	30	101	291
33	11	13	29	97	314
100	13	11	36	101	341
333	14	13	32	87	299
1000	14	15	35	92	314
2500	13	12	28	101	328
5000	16	8	28	93	318
NaN3 10	1645			1967	
4-NOPD 10			319		
4-NOPD 50		80			
MMS 3					4180
Concentration [µg/plate]	TA1535	TA1537	TA98	TA100	TA102
Deionised water	21	25	28	96	390
Untreated	16	23	33	100	345
33	16	29	32	115	367
100	18	34	28	101	358
333	20	22	27	108	405
1000	17	29	30	101	348
2500	14	24	31	95	363
5000	23	25	28	111	394
NaN3 10	1794			1914	
4-NOPD 10			306		
4-NOPD 50		124			
MMS 3					2269

Table 6.8.1.1-2: Summary of mean values with S9 mix

Concentration [µg/plate]	Strain				
	TA1535	TA1537	TA98	TA100	TA102
Deionised water	25	15	47	106	344
Untreated	23	17	47	100	351
3	22	17	53	110	317
10	22	14	42	110	324
33	26	18	49	104	359
100	23	17	41	107	366
333	23	18	47	97	319
1000	22	16	47	107	333
2500	23	17	50	104	387
5000	21	15	51	100	409
2-AA 2.5	282	191	1488	2024	
2-AA 10					2602
Concentration [µg/plate]	TA1535	TA1537	TA98	TA100	TA102
Deionised water	23	27	43	128	556
Untreated	21	26	44	137	504
33	26	30	49	118	555
100	20	31	37	128	582
333	23	31	43	129	603
1000	19	30	47	121	491
2500	24	34	40	131	534
5000	25	32	48	122	543
2-AA 2.5	349	277	2103	2403	
2-AA 10					3014

### Conclusion

Under the conditions of the study BCS-CU88901 (Na salt of NC 20645) was non-mutagenic with and without S9 mix in the Salmonella typhimurium reverse mutation assay.

<b>Reference:</b>	In vitro chromosome aberration test in Chinese hamster V79 cells with BCS-CU88901 (Na Salt of NC 20645)
Author(s), year:	Bohnenberger, S., 2012
Report/Doc. number::	1457202 / M-429561-01-1
Guideline(s):	OECD 473 (1997)
GLP:	Yes
Deviations:	No
Acceptability:	Yes

### Material and Methods

<b>1. Test material:</b>	BCS-CU88901 (NA salt of NC 20645)
Description:	brown powder
Lot/Batch no:	SES 11754-3-8
Purity:	69.2% (dose adjusted to content)
Stability of test compound:	guaranteed for study duration; expiry date: 2013-10-05

- 
- |  |  |
|--|--|
| <b>2. Vehicle and/or positive control:</b> | Negative: Nutrient medium<br>Solvent: De-ionised water<br><br>Positive controls:<br>Ethylmethane sulfonate (EMS) Lot No. A0297962 in nutrient medium without S9<br>Cyclophosphamide (CPA) Lot No. 120M1253V<br>In normal saline with S9.                         |
| <b>3. Test system:</b>                     | Chinese hamster V79 lung cells obtained from Labor fur Mutagenitatssprufungen Technical University Darmstadt, Germany. The karyotype was checked before freezing. They have a modal chromosome number of 22 and a rapid population doubling time of c. 13 hours. |
| <b>4. Test compound concentrations</b>     | BCS-CU88901 was used up to the top concentration of 4281 µg/ml   |

Three independent experiments were performed. In the first experiment the exposure period was 4 hours with and without S9 mix. In the second experiment the exposure period was 4 hours with S9 mix. In the third Experiment the exposure period was 18 hours without S9 mix. The chromosomes were prepared 18 hours after start of treatment with the test item.

In each experimental group two parallel cultures were set up. At least 100 metaphases per culture were evaluated for structural chromosome aberrations.

The highest treatment concentration in the study, 4281.0 µg/mL (approximately 10 mM) was chosen with regard to the molecular weight and the purity (69.2 %) of the test item and with respect to the OECD Guideline for in vitro mammalian cytogenetic tests.

## Results

No precipitation of the test item in the culture medium was observed. No relevant influence on osmolality or pH value was observed.

In the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration.

In the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.5 - 4.0 % aberrant cells, excluding gaps) were slightly above the range of the solvent control values (0.0 - 2.0 % aberrant cells, excluding gaps), but within the range of the laboratory historical solvent control data. However, one single statistically significant increase in chromosomal aberrations was observed in the third experiment in the presence of S9 mix after treatment with 4281.0 µg/mL (2.5 % aberrant cells, excluding gaps). The value was well within the range of the historical solvent control data (0.0 - 4.0 % aberrant cells, excluding gaps) and therefore was regarded as biologically irrelevant.

No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (1.3 - 2.9 %) as compared to the rates of the solvent controls (1.4 - 3.0 %).

No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (0.0 - 0.1 %) as compared to the rates of the solvent controls (0.0 - 0.7 %).

Either EMS (600.0 or 1000.0 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

## Conclusion

Under the conditions of the study BCS-CU88901 did not induce chromosome aberrations in Chinese hamster V79 cells and was therefore considered to be non-clastogenic in this chromosome aberration test.

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<b>Reference:</b>	Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) - BCS-CU88901 (Na salt of NC 20645)
Author(s), year:	Wollny, H. E., 2012
Report/Doc. number::	1457203 / M-436262-01-1
Guideline(s):	OECD 476 (1997)
GLP:	Yes
Deviations:	No
Acceptability:	Yes

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## Material and Methods

- 1. Test material:** BCS-CU88901 (NA salt of NC 20645)
- Description: brown powder
- Lot/Batch no: SES 11754-3-8
- Purity: 69.2% (dose adjusted to content)
- Stability of test compound: guaranteed for study duration; expiry date: 2013-10-05
- 2. Vehicle and/or positive control:** Negative: Nutrient medium
- Solvent: Deionised water
- Positive controls:  
 Ethylmethane sulfonate (EMS) Lot No. A0297962 in nutrient medium without S9  
 7,12-dimethylbenz(a)anthracene Lot No. 040M1231 in dimethyl sulfoxide with S9.
- 3. Test system:** Chinese hamster V79 lung cells obtained from Labor für Mutagenitätssprüfungen Technical University Darmstadt, Germany. The karyotype was checked before freezing. They have a modal chromosome number of 22 and a rapid population doubling time of 12-16 hours.

## Study design and methods

### Pre-Test on Toxicity

A pre-test was performed in order to determine the toxicity of the test item.

The highest concentration used in the pre-test was 4300 µg/mL equal to approximately 10 mM. Test item concentrations between 33.6 µg/mL and 4300 µg/mL were used to evaluate toxicity in the presence (4 hours treatment) and absence (4 hours and 24 hours treatment) of metabolic activation. No relevant cytotoxic effect indicated by a reduction of the colony counts was noted up to the maximum concentration of 4300 µg/mL with and without metabolic activation following 4 and 24 hours treatment.

No precipitation was noted up to the maximum concentration with and without metabolic activation. There was no relevant shift of the osmolarity or the pH of the medium even at the maximum concentration of the test item measured in the pre-experiment. Based on the results of the pre-experiment, the individual concentrations of the main experiments were selected. The individual concentrations were spaced by a factor of 2. Doses applied in the gene mutation assay with BCS-CU88901 (concentrations given in bold letters were chosen for mutation rate analysis).

**Table 6.8.1.1-3: Concentrations of BCS-CU88901 Tested**

Exposure Period	S9 Mix	Concentrations µg/ml					
		Experiment I					
4 hours	-	134.4	268.8	537.5	1075	2150	4300
4 hours	+	134.4	268.8	537.5	1075	2150	4300
		Experiment II					
24 hours	-	134.4	268.8	537.5	1075	2150	4300
4 hours	+	134.4	268.8	537.5	1075	2150	4300

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The first experiment was terminated prior to the generation of any data on mutagenicity due to microbial contamination. The first experiment was repeated as experiment IA. The data generated in experiment IA were reported under experiment I. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation

### Results

No precipitation of the test item occurred up to the maximum concentration with and without metabolic activation.

No relevant cytotoxic effects indicated by a relative cloning efficiency or a relative cell density below 50% in both parallel cultures occurred in any of the experimental parts.

No relevant and reproducible increase in mutant colony numbers/10<sup>6</sup> cells was observed in the main experiment up to the maximum concentration. The threshold was reached or exceeded at 268.8 µg/mL in the first culture of the second experiment without metabolic activation. This increase however, was neither reproduced in the parallel culture under identical conditions nor dose dependent as indicated by the lacking statistical significance.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental parts.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

### Conclusion

Under the conditions of the study BCS-CU88901 did not induce gene mutations in Chinese hamster V79 cells and was therefore considered to be non-mutagenic in this test.

### B.6.8.2. Supplementary studies on the active substance

Two acute intraperitoneal studies were evaluated in the original DAR (1998). One of the studies (■■■■, 1993) was originally submitted by Barclay Chemicals R&D Limited. Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study summary was copied from the DAR (1998) in the DRAR (2014).

<b>Reference:</b>	Acute intraperitoneal toxicity test of ethofumesate technical in rats
Author(s), year:	■■■■, 1993
Report/Doc. number::	M-436188-01-1
Guideline(s):	No OECD Guideline for acute intraperitoneal toxicity
GLP:	Yes
Deviations:	-
Acceptability:	Yes

### Material and Methods

Five male and five female Wistar rats were given ethofumesate (purity: not stated in the study report) by a single intraperitoneal dose. The test material was administered using syringe and needle as a 10% suspension in Na-carboxy-methyl cellulose (0.5%) in volume of 20 ml/kg and the dose level was 2000 mg/kg bw. The observation period was 14 days.

### Results

No mortalities were observed. LD<sub>50</sub> for rats was greater than 2000 mg/kg bw for males and females.

Severe clinical signs were observed up to 6 hours after the application, including reduced activity, abnormal gait, decreased body and abdominal tone, squattered position, decreased respiratory rate and sunken flank.

No treatment related pathological changes were noted at necropsy.

### Conclusion

Under the conditions of the study and based on the information given in the study report, intraperitoneal LD<sub>50</sub> in male and female rats was above 2000 mg/kg bw.

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*The study below (████, 1993) was originally submitted by Barclay Chemicals R&D Limited and was evaluated in the DAR (1998). Since the new RMS does not have the complete original dossier from 1990-ies this study was not subject for re-wording or inclusion of additional information. The study was copied from the DAR (1998).*

#### **Acute intraperitoneal toxicity in rats (████, 1993)**

##### *Experimental design*

Five male and five female albino rats of the Sprague-Dawley strain were given ethofumesate by a single intraperitoneal dose. The test material was administered as a suspension in peanut oil and the dose level was 2000 mg/kg bw. The observation period was 14 days.

##### *Results*

LD<sub>50</sub> for rats was greater than 2000 mg/kg bw for males and females.

Clinical signs: none

Necropsy findings: no abnormalities were noted.

##### *Comments*

This is a limit test. There is no OECD guideline for this type of study. There is a QA statement and a statement of compliance with GLP standards.

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### **B.6.8.3. Studies on endocrine disruption**

There are no indications from the existing database that ethofumesate has effects on the endocrine system. No recognised endocrine disrupting effects were observed *in vivo* and it is considered unlikely that any mechanistic study would add any relevant information. However, the existing database does not completely comply with the current guidelines for detecting endocrine disruption and thus the most sensitive end points have not been investigated.

At present no specific studies on endocrine disruption for ethofumesate are required. Ethofumesate also does not fulfil the criteria for endocrine disruptors stated in the Regulation (EC) 1107/2009.

## **B.6.9. MEDICAL DATA AND INFORMATION**

### **B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies**

#### **TaskForce:**

A literature search on possible specific information on poisoning incidents, medical case reports or epidemiological investigations was conducted. The search in a large number of scientific bibliographical



databases did not return any meaningful reference addressing either poisoning incidents or other human data with ethofumesate, or any information on potential neurotoxic effects of this substance.

**UPL:**

No new literature search was conducted. UPL referred to the information given in the Review report for the first approval of the ethofumesate.

In a current occupational health statement it is confirmed that during the last 10 years there have been no incidences of reported occupational diseases upon medical examinations of workers engaged in the production and handling of ethofumesate.

**B.6.9.2. Data collected on humans****TaskForce:**

No data were collected on humans.

**UPL:**

No data were collected on humans.

**B.6.9.3. Direct observations****TaskForce:**

Signs and Symptoms of Poisoning:

In humans headaches and a single case of drowsiness, most likely due to the formulation solvent, have been reported.

In acute high dose animal experiments tremor, lethargy, dyspnoea and ataxia have been observed.

**UPL:**

In the HSDB Databank (Human Health Effects) a few cases of accidental exposure to ethofumesate are reported: In one case an employee developed a headache when he worked in the smell of the technical material. There were two cases of contamination with the spray dilution of an emulsifiable concentrate formulation of ethofumesate plus another product; in one of these cases no toxic effects were seen, in the other there was transient weakness and light-headedness, possibly caused by the solvent. In the final case, eye contamination with an emulsifiable concentrate formulation of ethofumesate may have produced a corneal ulcer; again this may have been a solvent effect.

**B.6.9.4. Epidemiological studies**

There have been no epidemiological studies conducted in groups exposed to ethofumesate.

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**B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test**

Please see 6.9.3.

**B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment****TaskForce:**

Remove patient from exposure/terminate exposure.

Thorough skin decontamination with copious amounts water and soap, if available with polyethylene glycol 300 followed by water.

Note: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polyethyleneglykol 300 is not required.

Flushing of the eyes with lukewarm water for 15 minutes

Induction of vomiting does not seem to be required in regard of the low toxicity

It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious.

Induced vomiting can remove maximum 50% of the ingested substance.

Note: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!

**UPL:**

No specific medical treatment is proposed in the relevant literature.

General rules and medical treatment upon exposure to possibly toxic chemicals apply.

## B.6.10. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.1.1	[REDACTED]	1974	FURTHER MAMMALIAN METABOLISM STUDIES ON NC 8438 [REDACTED] Bayer CropScience, Report No.: A82951, Edition Number: <u>M-155228-01-1</u> EPA MRID No.: acc.36360 Date: 1974-03-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.1	[REDACTED]	1977	THE METABOLISM OF 14C-ETHOFUMESATE IN THE HAMSTER [REDACTED] Bayer CropScience, Report No.: A82961, Edition Number: <u>M-155238-01-1</u> Date: 1977-03-24 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.1	[REDACTED]	1977	THE PHARMACOKINETICS AND METABOLISM OF 14C-ETHOFUMESATE IN THE DOG [REDACTED] Bayer CropScience, Report No.: A82960, Edition Number: <u>M-155237-01-1</u> Date: 1977-03-24 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.1	[REDACTED] J.	1991	THE METABOLISM OF 14C-ETHOFUMESATE IN THE RAT [REDACTED] Bayer CropScience, Report No.: A87552, Report includes Trial Nos.:150624 Edition Number: <u>M-161454-01-1</u> Date: 1991-08-27 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.1	[REDACTED]	1994	Ethofumesate - ADME-Study in Rats [REDACTED] Feinchemie Schwebda , Report No.: OFC00004935, Edition Number: <u>M-351968-01-1</u> Date: 1994-07-18 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.1.1	[REDACTED]	1992	THE METABOLISM OF 14C-ETHOFUMESATE IN RATS [REDACTED] Bayer CropScience, Report No.: A82967, Report includes Trial Nos.:SMS 298/920303 TOX 90540 Edition Number: <u>M-155244-01-1</u> EPA MRID No.: 42364503, 42689903 Date: 1992-06-04 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.1	[REDACTED]	1993	Metabolism study of 14C-labelled ethofumesate after single oral and intravenous administration to sprague-dawley rats [REDACTED] Feinchemie Schwebda, Report No.: <u>M-468464-01-1</u> , Edition Number: <u>M-468464-01-1</u> Date: 1993-12-01	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebra te study Y/N	Data protection claimed Y/N	Justificat ion if data protectio n is claimed	Owner	Previous evaluatio n
KCA 5.1.1	[REDACTED]	1977	GLP/GEP: yes, unpublished INVESTIGATION OF RESIDUE ACCUMULATION IN THE RAT FOLLOWING REPEATED ADMINISTRATION OF 14C ETHOFUMESATE [REDACTED] Bayer CropScience, Report No.: A82962, Edition Number: <u>M-155239-01-1</u> EPA MRID No.: acc.93280 Date: 1977-07-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.1.3	Koester, J.	2013	[Phenyl-UL-14C]ethofumesate: Isolation and identification of metabolite(s) from an in-vitro study with rat and human liver microsomes Bayer CropScience, Report No.: EnSa-13-0841, Edition Number: M-469296-01-1 Date: 2013-11-11 GLP/GEP: yes, unpublished	N	Y	-	Bayer CropScience	Submitte d for the purpose of renewal (2014)  (consider ed by RMS as additiona l informati on only)
KCA 5.1.3	[REDACTED]	2013	[Phenyl-UL-14C]ethofumesate: Metabolic stability and profiling in liver microsomes from rats and humans for inter-species comparison [REDACTED] Bayer CropScience, Report No.: S45316, Edition Number: M-471058-01-1 Date: 2013-11-29 GLP/GEP: yes, unpublished	N	Y	-	Bayer CropScience	Submitte d for the purpose of renewal (2014)  (consider ed by RMS as additiona l informati on only)
IIA, 5.2.1	[REDACTED]	1988	Acute oral toxicity study in the rat: thofumesate technical. [REDACTED] Report No.: BY-881109	Y	N	-	Barclay Chemicals R&D Limited	In DAR (1998)
IIA, 5.2.1	[REDACTED]	1988	Acute oral toxicity study in the mouse: Ethofumesate technical. [REDACTED] Report No.: BY-881117A	Y	N	-	Barclay Chemicals R&D Limited	In DAR (1998)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.2.1	██████	1988	Ethofumesate technical powder - Acute oral toxicity (limit test) in the rat ██████ Bayer CropScience, Report No.: A83223, Report includes Trial Nos.: TOX/92286 Edition Number: <u>M-155494-01-1</u> Date: 1988-08-31 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.1	██████	1991	Acute oral toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats ████████████████████ Feinchemie Schwebda, Report No.: OFC00004836, Edition Number: <u>M-351974-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.2.1	██████	1991	Acute oral toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Swiss albino mice ████████████████████ Feinchemie Schwebda, Report No.: OFC00004837, Edition Number: <u>M-351978-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.2.1	██████	1992	ETHOFUMESATE: ACUTE ORAL TOXICITY (LIMIT TEST) IN THE RAT ██████ Bayer CropScience, Report No.: A87559, Report includes Trial Nos.: 245/60 Edition Number: <u>M-161466-01-1</u> Date: 1992-04-13 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.1	██████ D. J.	1992	ETHOFUMESATE: ACUTE ORAL TOXICITY (LIMIT TEST) IN THE MOUSE ██████ Bayer CropScience, Report No.: A87560, Report includes Trial Nos.: 245/64 Edition Number: <u>M-161468-01-1</u> Date: 1992-06-08 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.2	██████	1979	Ethofumesate technical CR 4805/4 acute dermal toxicity study in rabbits ████████████████████ Bayer CropScience, Report No.: A83173, Report includes Trial Nos.: 411795 Edition Number: <u>M-155447-01-1</u> EPA MRID No.: acc.30419 Date: 1979-03-27 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.2	██████	1988	TECHNICAL ETHOFUMESATE	Y	N	-	Bayer	In DAR

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			POWDER: ACUTE DERMAL TOXICITY (Limit Test) IN THE RAT [REDACTED] Bayer CropScience, Report No.: A83224, Report includes Trial Nos.: TOX/92287 Edition Number: <u>M-155495-01-1</u> Date: 1988-08-31 GLP/GEP: yes, unpublished				CropScience	(1998)
KCA 5.2.2	[REDACTED]	1991	Acute dermal toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats [REDACTED] Feinchemie Schwabach, Report No.: OFC00004838, Edition Number: <u>M-351980-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabach)	In DAR (1998)
KCA 5.2.2	[REDACTED]	1992	ETHOFUMESATE: ACUTE DERMAL TOXICITY (LIMIT TEST) IN THE RAT [REDACTED] Bayer CropScience, Report No.: A87561, Report includes Trial Nos.: 245/61 Edition Number: <u>M-161469-01-1</u> Date: 1992-04-07 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.3	[REDACTED]	1986	Acute toxicological study of ethofumesate-Stefes after inhalation by the rat. [REDACTED] Report No.: ??	Y	N	-	Barclay Chemicals R&D Limited	In DAR (1998)
KCA 5.2.3	[REDACTED]	1989	ETHOFUMESATE: ACUTE INHALATION TOXICITY STUDY IN RATS 4 HOUR EXPOSURE [REDACTED] Bayer CropScience, Report No.: A87562, Edition Number: <u>M-161471-01-1</u> Date: 1989-11-21 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.3	[REDACTED]	1991	Acute inhalation toxicity study with ethofumesate technical (FSG 03189 H/27 Feb. 90) in Wistar rats [REDACTED] Feinchemie Schwabach, Report No.: OFC00004840, Edition Number: <u>M-351989-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabach)	In DAR (1998)
KCA 5.2.3	[REDACTED]	1988	ETHOFUMESATE TECHNICAL: ACUTE INHALATION TOXICITY STUDY FOUR-HOUR EXPOSURE IN THE RAT [REDACTED] Bayer CropScience, Report No.: A83217, Edition Number: <u>M-155489-01-1</u> Date: 1988-08-02 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebra te study Y/N	Data protection claimed Y/N	Justificat ion if data protectio n is claimed	Owner	Previous evaluatio n
KCA 5.2.4	████████	1991	Primary skin irritation study with ethofumesate technical (FSG 03189 H/27 Feb.90) in new zealand white rabbits ████████ Feinchemie Schwebda , Report No.: OFC00004841, Edition Number: <u>M-351993-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.2.4	████████	1992	Ethofumesate: Acute dermal irritation test in the rabbit ████████ ████ ██████████ Bayer CropScience, Report No.: A87563, Report includes Trial Nos.: 245/65 Edition Number: <u>M-161472-01-1</u> Date: 1992-06-23 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.4	████████	1991	TECHNICAL ETHOFUMESATE: RABBIT SKIN IRRITANCY STUDY ████████ Bayer CropScience, Report No.: A83207, Report includes Trial Nos.: TOX 90535 Edition Number: <u>M-155479-01-1</u> EPA MRID No.: 41949205 Date: 1991-03-13 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.5	████████	1991	Primary eye irritation study with ethofumesate technical (FSG 03189 H/27 Feb.90) in New Zealand white rabbits ████████ Feinchemie Schwebda , Report No.: OFC00004842, Edition Number: <u>M-351996-01-1</u> Date: 1991-03-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.2.5	████████	1992	Ethofumesate: Acute eye irritation test in the rabbit ████████ ████ ██████████ Bayer CropScience, Report No.: A87564, Report includes Trial Nos.: 245/66 Edition Number: <u>M-161473-01-1</u> Date: 1992-06-29 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.5	████████	1991	TECHNICAL ETHOFUMESATE: RABBIT EYE IRRITANCY STUDY ████████ Bayer CropScience, Report No.: A83208, Report includes Trial Nos.: TOX 90536 Edition Number: <u>M-155480-01-1</u> EPA MRID No.: 41949204 Date: 1991-03-13 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.6	████████	1984	Technical ethofumesate CR 4805. Delayed contact hypersensitivity in the guinea-pig –	Y	N	-	Bayer CropScience	In DAR (1998)

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			and Amendment 1 [REDACTED] Bayer CropScience, Report No.: <u>M-155461-02-1</u> , Report includes Trial Nos.: TOX 83103 Edition Number: <u>M-155461-02-1</u> Date: 1984-01-16 ...Amended: 2005-05-20 GLP/GEP: yes, unpublished					
KCA 5.2.6	[REDACTED]	1991	Skin sensitization study with ethofumesate technical (FSG 03089 H/27 Feb. 90) in Guinea Pigs (Buehler Test) [REDACTED] Feinchemie Schwabach, Report No.: OFC00004843, Edition Number: <u>M-351999-01-1</u> Date: 1991-11-18 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabach)	In DAR (1998)
KCA 5.2.6	[REDACTED]	1989	ETHOFUMESATE: SENSITISATION TEST IN THE GUINEA PIG (MAGNUSSON AND KLIGMAN MAXIMISATION METHOD) [REDACTED] Bayer CropScience, Report No.: A87565, Report includes Trial Nos.: A/M/10804 Edition Number: <u>M-161474-01-1</u> Date: 1989-01-30 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.2.7	Hepperheimer, A.	2012	Cytotoxicity assay In vitro with BALB/c 3T3 cells: Neutral red (NR) test with Ethofumesate during simultaneous irradiation with artificial sunlight United Phosphorus Ltd., 1474000 Harlan cytotox Cell Research GmbH (Harlan CCR) GLP/GEP: yes, unpublished	N	Y	New study necessary according to new data requirements	UPL	Submitted for the purpose of renewal (2014)



Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.3.1	[REDACTED]	1994	ETHOFUMESATE: ORAL (CAPSULE/GAVAGE) MAXIMUM TOLERATED DOSE (MTD) AND 28 DAY REPEAT DOSE RANGE FINDING STUDY IN THE DOG [REDACTED] Bayer CropScience, Report No.: A87567, Edition Number: <u>M-161477-01-1</u> Date: 1994-04-25 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.1	[REDACTED]	1989	Desmedipham / ethofumesate; dietary dose range finding study in rats [REDACTED] Bayer CropScience, Report No.: A63715, Edition Number: <u>M-147174-01-1</u> Date: 1989-01-13 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.1	[REDACTED]	1988	ETHOFUMESATE: PRELIMINARY DOSE RANGE FINDING STUDY IN RATS BY DIETARY ADMINISTRATION FOR 4 WEEKS [REDACTED] Bayer CropScience, Report No.: A87566, Edition Number: <u>M-161475-01-1</u> Date: 1988-06-23 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.1	[REDACTED]	1991	28 day dietary study in Wistar rats - Ethofumesate technical (FSG 03189 H/27 FEB.90) [REDACTED] Feinchemie Schwebda , Report No.: OFC00004844, Edition Number: <u>M-352006-01-1</u> Date: 1991-07-22 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.3.2	[REDACTED]	1990	Ethofumesate technical: 90 day oral toxicity study in the rat. [REDACTED] Report No.: BY-900117	Y	N	-	Barclay Chemicals R&D Limited	In DAR (1998)
KCA 5.3.2	[REDACTED] J.	1994	ETHOFUMESATE: 13 WEEK ORAL (GAVAGE) TOXICITY STUDY IN THE DOG [REDACTED] Bayer CropScience, Report No.: A87568, Edition Number: <u>M-161478-01-1</u> Date: 1994-04-25 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.2	[REDACTED]	1989	ETHOFUMESATE TECHNICAL: NINETY-DAY (Dietary Administration) TOXICITY STUDY IN THE RAT [REDACTED] Bayer CropScience, Report No.: A83225, Report includes Trial Nos.: 43/30 Edition Number: <u>M-155496-01-1</u> Date: 1989-06-07	Y	N	-	Bayer CropScience	In DAR (1998)

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KCA 5.3.2	[REDACTED]	1990	GLP/GEP: yes, unpublished ETHOFUMESATE 13 WEEK ORAL (DIETARY) DOSE RANGE FINDING STUDY IN THE MOUSE. [REDACTED] Bayer CropScience, Report No.: A89579, Edition Number: <u>M-165016-01-1</u> EPA MRID No.: 44156201 Date: 1990-07-01 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.2	[REDACTED]	1989	ETHOFUMESATE: TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS [REDACTED] Bayer CropScience, Report No.: A89580, Edition Number: <u>M-165018-01-1</u> EPA MRID No.: 44093601 Date: 1989-06-18 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.3.2	[REDACTED]	1992	90 day oral toxicity study in Wistar rats - Ethofumesate technical (FSG H/27 FEB.90) [REDACTED] Feinchemie Schwabda, Report No.: OFC00004845, Edition Number: <u>M-352012-01-1</u> Date: 1992-04-10 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabda)	In DAR (1998)
KCA 5.3.3	[REDACTED]	1991	TECHNICAL ETHOFUMESATE: RABBIT TWENTY ONE DAY DERMAL TOXICITY STUDY [REDACTED] Bayer CropScience, Report No.: A83209, Report includes Trial Nos.: TOX 90537 Edition Number: <u>M-155481-01-1</u> Date: 1991-05-20 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.1	Allen, J. A., Brooker, P. C., Birt, D. M., Godfrey, S.	1986	TECHNICAL ETHOFUMESATE: METAPHASE CHROMOSOME ANALYSIS OF HUMAN LYMPHOCYTES CULTURED IN VITRO Schering AG, Berlin, Germany Bayer CropScience, Report No.: A83192, Report includes Trial Nos.: TOX 86086 Edition Number: <u>M-155465-01-1</u> EPA MRID No.: 41214203 Date: 1986-12-11 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.1	Davis, P. B.	1987	AN INVESTIGATION INTO THE POSSIBLE INDUCTION OF POINT MUTATIONS AT THE HGPRT LOCUS OF CHINESE HAMSTER OVARY CELLS BY ETHOFUMESATE TNO; Bayer CropScience, Report No.: A87571, Report includes Trial Nos.: 17818	N	N	-	Bayer CropScience	In DAR (1998)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Edition Number: <u>M-161483-01-1</u> Date: 1987-06-25 GLP/GEP: yes, unpublished					
KCA 5.4.1	Gant, R. A.	1994	ETHOFUMESATE BACTERIAL MUTATION ASSAY Huntingdon Research Centre Ltd., Huntingdon, United Kingdom Bayer CropScience, Report No.: A83222, Report includes Trial Nos.: TOX 94329 Edition Number: <u>M-155493-01-1</u> EPA MRID No.: 43529501 Date: 1994-12-07 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.1	Kennelly, J. C.	1986	TECHNICAL ETHOFUMESATE: MOUSE LYMPHOMA (6TG) FLUCTUATION ASSAY Microtest; Bayer CropScience, Report No.: A83191, Report includes Trial Nos.: BFC 2/ML TOX 86085 Edition Number: <u>M-155464-01-1</u> EPA MRID No.: 41710501 Date: 1986-11-11 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.1	Sokolowski, A.	2013	Salmonella typhimurium reverse mutation assay with ethofumesate, technical Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1523800, Edition Number: <u>M-449020-01-1</u> Date: 2013-03-14 GLP/GEP: yes, unpublished	N	Y	Required to test current production material	Bayer CropScience	Submitted for the purpose of renewal (2014)
KCA 5.4.1	Suresh, T. P.	1993	Mutagenicity - Salmonella typhimurium reverse mutation assay (Ames test) - Ethofumesate technical Rallis India Ltd., Research Centre, Bangalore, India Feinchemie Schwebda, Report No.: 904-MUT:AMES, Edition Number: <u>M-359339-01-1</u> Date: 1993-04-30 GLP/GEP: yes, unpublished	N	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.4.1	Thompson, P.W.	1992	Ethofumesate technical: Reverse mutation "Ames test" using Salmonella typhimurium. SafePharm Laboratories Ltd. Report No: 134/35	N	N	-	Barclay Chemicals R&D Limited	In DAR (1998)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.4.1	[REDACTED]	1987	CHROMOSOME ANALYSIS OF CHINESE HAMSTER OVARY CELLS TREATED IN VITRO WITH ETHOFUMESATE [REDACTED] Bayer CropScience, Report No.: A87573, Report includes Trial Nos.: B87-0471 Edition Number: <u>M-161486-01-1</u> Date: 1987-05-08 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.1	[REDACTED]	1987	EXAMINATION OF ETHOFUMESATE FOR MUTAGENIC ACTIVITY IN THE AMES TEST [REDACTED] Bayer CropScience, Report No.: A87570, Report includes Trial Nos.: B87-0064/05 Edition Number: <u>M-161481-01-1</u> Date: 1987-04-15 GLP/GEP: yes, unpublished	N	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.2	[REDACTED]	1985	TECHNICAL ETHOFUMESATE: MOUSE MICRONUCLEUS TEST [REDACTED] Bayer CropScience, Report No.: A83189, Report includes Trial Nos.: TOX 85060 Edition Number: <u>M-155462-01-1</u> EPA MRID No.: 41214217 Date: 1985-12-11 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.2	[REDACTED]	1988	TECHNICAL ETHOFUMESATE: ASSESSMENT OF UNSCHEDULED DNA SYNTHESIS USING RAT HEPATOCYTE CULTURES [REDACTED] Bayer CropScience, Report No.: A83194, Report includes Trial Nos.: TOX 87229 Edition Number: <u>M-155467-01-1</u> EPA MRID No.: 41214204 Date: 1988-04-28 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.2	[REDACTED]	1993	Mutagenicity-micronucleus test in swiss albino mice [REDACTED] Feinchemie Schwebda , Report No.: OFC00004855, Edition Number: <u>M-351970-01-1</u> Date: 1993-05-06 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.4.2	[REDACTED]	1994	Genetic toxicity - In vivo mammalian bone marrow cytogenetic test - Chromosomal analysis [REDACTED] Feinchemie Schwebda , Report No.: OFC00004856, Edition Number: <u>M-351972-01-1</u> Date: 1994-04-19	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)

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			GLP/GEP: yes, unpublished					
KCA 5.4.2	[REDACTED]	1992	ETHOFUMESATE: MICRONUCLEUS ASSAY IN BONE MARROW CELLS OF THE MOUSE [REDACTED] Bayer CropScience, Report No.: A87572, Edition Number: <u>M-161484-01-1</u> Date: 1992-11-30 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.4.3	[REDACTED]	1992	Dominant lethal test in wistar rats [REDACTED] Feinchemie Schwabach, Report No.: OFC00004854, Edition Number: <u>M-351976-01-1</u> Date: 1992-11-04 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabach)	In DAR (1998)
KCA 5.5	[REDACTED]	1992	Ethofumesate - 104 week dietary carcinogenicity study in rats (Addendum 1) [REDACTED] Bayer CropScience, Report No.: A89584, Edition Number: M-165033-01-1 EPA MRID No.: 44093604 Date: 1992-06-02 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1980	A CARCINOGENICITY STUDY OF TECHNICAL NC 8438 IN HAMSTERS (LIFETIME STUDY) CR 4805/4 [REDACTED] Report No.: A83178, Report includes Trial Nos.: TOX 74004 Edition Number: M-155452-01-1 EPA MRID No.: acc.243881 Date: 1980-08-04 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1980	TECHNICAL NC 8438 (CR 4805/3) TOXICITY STUDY IN BEAGLE DOGS (FINAL REPORT : DIETARY INTAKE FOR 104 WEEKS) [REDACTED] Bayer CropScience, Report No.: A83176, Report includes Trial Nos.: TOX 77020 Edition Number: M-155450-01-1 EPA MRID No.: 000243884, 00062822, acc.243884 Date: 1980-02-20 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1990	ETHOFUMESATE: 52 WEEK DIETARY TOXICITY STUDY IN RATS [REDACTED] Bayer CropScience, Report No.: A89582, Report includes Trial Nos.: 437609 Edition Number: M-165028-01-1 EPA MRID No.: 44093602 Date: 1991-08-21	Y	N	-	Bayer CropScience	In DAR (1998)

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KCA 5.5	[REDACTED]	1991	GLP/GEP: yes, unpublished ETHOFUMESATE: 104 WEEK DIETARY CARCINOGENICITY STUDY IN RATS [REDACTED] Bayer CropScience, Report No.: A89583, Report includes Trial Nos.: 437609 Edition Number: M-165030-01-1 EPA MRID No.: 44093603 Date: 1992-06-02 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1992	ETHOFUMESATE: 80 WEEK ORAL (DIETARY) CARCINOGENICITY STUDY IN THE MOUSE [REDACTED] Bayer CropScience, Report No.: A89581, Edition Number: M-165020-01-1 EPA MRID No.: 44156202 Date: 1992-06-26 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1976	The effects of the dietary administration of NC 8438 to male and female rats for two years [REDACTED] Bayer CropScience, Report No.: A83155, Edition Number: M-155430-01-1 EPA MRID No.: acc.41853 Date: 1976-01-01 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1976	The Effects of Dietary Administration of NC 8438 to male and female rats for two years - SUMMARY [REDACTED] United Kingdom Bayer CropScience, Report No.: A89487, Edition Number: M-164863-01-1 EPA MRID No.: 00041853 GLP/GEP: n.a., unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.5	[REDACTED]	1995	Combined chronic toxicity and carcinogenicity study in wistar rats [REDACTED] Feinchemie Schwebda , Report No.: OFC00004858, Edition Number: M-352005-01-1 Date: 1995-06-26 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)



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KCA 5.6.1	[REDACTED]	1989	ETHOFUMESATE: DIETARY RAT GENERAL REPRODUCTIVE PERFORMANCE DOSE RANGING STUDY [REDACTED] Bayer CropScience, Report No.: A87578, Edition Number: M-161494-01-1 Date: 1989-03-09 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.1	[REDACTED]	1990	ETHOFUMESATE: DIETARY RAT TWO-GENERATION REPRODUCTION TOXICITY STUDY VOL I-II [REDACTED] Bayer CropScience, Report No.: A87579, Edition Number: M-161496-01-1 Date: 1990-08-22 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.1	[REDACTED]	1993	Ethofumesate technical - Two generation reproduction study in Wistar rats [REDACTED] Feinchemie Schwabda , Report No.: OFC00004862, Edition Number: M-352016-01-1 Date: 1993-11-08 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabda)	In DAR (1998)
KCA 5.6.1	[REDACTED]	1980	TECHNICAL NC 8438: MULTIGENERATION STUDY IN THE RAT - FINAL REPORT - VOLUMES I AND II [REDACTED] Bayer CropScience, Report No.: A83174, Report includes Trial Nos.: TOX 77003 Edition Number: M-155448-01-1 EPA MRID No.: 00062823, acc.62823 Date: 1980-09-26 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.2	[REDACTED]	1991	ETHOFUMESATE: ORAL (GAVAGE) RANGE FINDING STUDY IN THE PREGNANT RAT [REDACTED] Bayer CropScience, Report No.: A87574, Report includes Trial Nos.: 520-/23 Edition Number: M-161487-01-1 Date: 1991-07-11 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.2	[REDACTED]	1991	ETHOFUMESATE: ORAL (GAVAGE) TERATOLOGY STUDY IN THE RAT [REDACTED] Bayer CropScience, Report No.: A87575, Edition Number: M-161489-01-1 Date: 1991-07-08 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.2	[REDACTED]	1991	ETHOFUMESATE: ORAL (GAVAGE) RANGE FINDING STUDY IN THE PREGNANT RABBIT [REDACTED]	Y	N	-	Bayer CropScience	In DAR (1998)

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			Bayer CropScience, Report No.: A87576, Edition Number: <u>M-161491-01-1</u> Date: 1991-07-05 GLP/GEP: yes, unpublished					
KCA 5.6.2	██████	1991	ETHOFUMESATE: ORAL (GAVAGE) TERATOLOGY STUDY IN THE RABBIT ██████; Bayer CropScience, Report No.: A87577, Edition Number: <u>M-161492-01-1</u> Date: 1991-07-08 GLP/GEP: no, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.2	██████	1991	TECHNICAL ETHOFUMESATE: ORAL TERATOLOGY (DEVELOPMENTAL TOXICITY) STUDY IN THE RAT ██████████████ Report No.: A83205, Report includes Trial Nos.: 194/37, 194/38 TOX 90207 Edition Number: <u>M-155477-01-1</u> EPA MRID No.: 42067701 Date: 1991-05-10 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.6.2	██████	1991	Teratogenicity study in wistar rats ████████████████████ Feinchemie Schwabda , Report No.: OFC00004865, Edition Number: <u>M-352018-01-1</u> Date: 1991-12-03 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabda)	In DAR (1998)
KCA 5.6.2	██████	1993	Teratogenicity study in rabbits ████████████████████ Feinchemie Schwabda , Report No.: OFC00004866, Edition Number: <u>M-352093-01-1</u> Date: 1993-04-16 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwabda)	In DAR (1998)
KCA 5.6.2	██████	1999	Amendment - Teratogenicity study in wistar rats ████████████████████ Feinchemie Schwabda , Report No.: OFC00004864, Edition Number: <u>M-352142-01-1</u> Date: 1999-05-26 GLP/GEP: no, unpublished	Y	N	-	Adama (former Feinchemie Schwabda)	Submitted for the purpose of renewal (2014)
KCA 5.6.2	██████	1986	SN 49.913 (ETHOFUMESATE) - EMBRYOTOXICITY INCLUDING TERATOGENICITY STUDY IN THE RABBIT AFTER DAILY INTRAGASTRIC ADMINISTRATION FROM DAY 6 TO DAY 18 OF GESTATION ████████████████████ Bayer CropScience, Report No.: A83190, Edition Number: <u>M-155463-01-1</u> Date: 1986-01-28 GLP/GEP: yes, unpublished	Y	N	-	Bayer CropScience	In DAR (1998)
KCA 5.8.1	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with BCS-CU88901 (Na salt of NC 20645)	Y	Y	Assumed by the notifier to	Bayer CropScience	Submitted for the purpose



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			Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1457201, Edition Number: <u>M-427430-01-1</u> Date: 2012-02-21 GLP/GEP: yes, unpublished			be triggered by groundwa ter guidance document (SANCO/ 221/2000)		of renewal (2014), however, not considere d necessary
KCA 5.8.1		2012	In vitro chromosome aberration test in Chinese hamster V79 cells with BCS- CU88901 (Na Salt of NC 20645) [REDACTED] Bayer CropScience, Report No.: 1457202, Edition Number: <u>M-429561-01-1</u> Date: 2012-04-11 GLP/GEP: yes, unpublished	Y	Y	Assumed by the notifier to be triggered by groundwa ter guidance document (SANCO/ 221/2000)	Bayer CropScience	Submitt ed for the purpose of renewal (2014), not considere d necessary
KCA 5.8.1		2012	Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) - BCS- CU88901 (Na salt of NC 20645) [REDACTED] Bayer CropScience, Report No.: 1457203, Edition Number: <u>M-436262-01-1</u> Date: 2012-08-07 GLP/GEP: yes, unpublished	Y	Y	Assumed by the notifier to be triggered by groundwa ter guidance document (SANCO/ 221/2000)	Bayer CropScience	Submitt ed for the purpose of renewal (2014), not considere d necessary
KCA 5.8.2		1993	Ethofumesate technical L4047: Acute intrapertitoneal toxicity (limit test) in the rat. [REDACTED]. Report No: 134/60	Y	N	-	Barclay Chemicals R&D Limited	In DAR (1998)
KCA 5.8.2		1993	Acute intraperitoneal toxicity test of ethofumesate technical in rats [REDACTED] Feinchemie Schwabda, Report No.: <u>M-436188-01-1</u> , Edition Number: <u>M-436188-01-1</u> Date: 1993-01-31 GLP/GEP: yes, unpublished	Y	N	-	Adama (former Feinchemie Schwebda)	In DAR (1998)
KCA 5.9	Klipsch, K.; Reddig, H.	2008	Ethofumesate (CAS: 26225-79-6) - Report on a literature search for the following topics: Human clinical data / case reports / epidemiology and additionally neurotoxicity EBRC Consulting GmbH, Hannover, Germany Feinchemie Schwabda , Report No.: <u>M-352095-01-1</u> , Edition Number: <u>M-352095-01-1</u> Date: 2008-09-30 GLP/GEP: yes, unpublished	N	N	-	Adama (former Feinchemie Schwebda)	Submitt ed for the purpose of renewal (2014)
KCA 5.9	Shanker, B.	2014	Occupational Health Statement United Phosphorus Ltd., not applicable GLP/GEP: no Published: no	N	N	-	UPL	Submitt ed for the purpose of renewal (2014)

