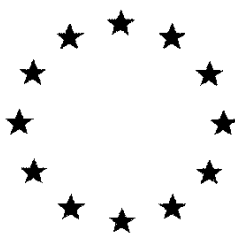


# ***European Commission***



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

**FLUFENACET**  
**Volume 3 – B.6 (AS)**  
**Toxicology and metabolism data**

Rapporteur Member State: Poland  
Co-Rapporteur Member State: France

**Version History**

<b>When</b>	<b>What</b>
August 1997	Initial assessment. <b>Draft Assessment Report</b> for first inclusion to Annex I. RMS: FR
April 2016	<b>Draft Renewal Assessment Report</b> prepared according to the Commission; Regulation (EU) N° 1107/2009; RMS: PL; Co-RMS: FR
May 2017	Revision after Co-RMS comments

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## B.6. TOXICOLOGY AND METABOLISM DATA

For the renewal of approval of flufenacet, notifier (Bayer CropScience), submitted complete dossier by the deadline of April 2015. The dossiers for the renewal contain 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 flufenacet (*COMMISSION DIRECTIVE 2003/84/EC of 25 September 2003 amending Council Directive 91/414/EEC to include flurtamone, flufenacet, iodosulfuron, dimethenamid-p, picoxystrobin, fosthiazate and silthiofam as active substances*).

Regarding the old studies, originally evaluated in the DAR (RMS: France), 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. The RMS also paid attention to new criteria for classification and labelling according to Regulation (EC) 1272/2008.

**Search of the scientific peer reviewed open literature** was conducted by RMS: Poland, covering a period from 2005 to 2015. It was stated that 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.]). The query was conducted in resources: ELSEVIER ScienceDirect Webpage; Springer International Publishing Webpage; NIH-TOXNet Webpage; Europe PMC Webpage [developed by the European Bioinformatics Institute, JISC, The University of Manchester and the British Library].

In the opinion of RMS, the information contained in open source literature does not contain any additional information that could materially affect the determination of endpoints for flufenacet. The published animal studies have characterized toxic effect and outcomes using endpoints not required by guideline studies.

The document published by the US EPA [Chemicals Evaluated for Potential carcinogenic Office of Pesticide Programs, U.S. Environmental Protection Agency, Annual Cancer Report 2015] confirms the lack of carcinogenic potential for flufenacet -Not Likely to Be carcinogenic to Humans. (see page 13 cited Report)

## INTRODUCTION

Flufenacet was included in Annex I of Directive 91/414/EEC on 01/01/2004, as notified in Directive 2003/84/EC dated 25 September 2003 where in there is no specific provision under Part B which needs to be considered related to toxicological data. The Monograph prepared by the Rapporteur Member State France in the context of the inclusion of flufenacet in Annex 1 of the Council Directive 91/414/EEC, the Review Report for flufenacet (7469/VI/98-Final – 3<sup>rd</sup> July 2003) as well as the Evaluation table of flufenacet (7468/VI/98-rev. 10(27.12.2001) are considered to provide the relevant scientific information for the review of the active substance. Studies, which were already submitted by Bayer for the first EU review, are contained in the Monograph/Review Report (July 2003)/ Evaluation table (December 2001) and in the baseline dossier provided by Bayer CropScience. A synonymous name for flufenacet used at several locations in this supplemental dossier is FOE 5043 or the abbreviation FFA.

The following table provides an overview on the batches of flufenacet used in all toxicological studies on this compound. Studies not evaluated during the first EU review are written in bold letters.

**Table 6-1: Overview of flufenacet batches used for toxicity studies**

Batch Number	Purity (%)*	Study type	Reference
17001/90	94.8	Acute inhalation toxicity, rat	██████████, 1990 <a href="#">M-004844-01-1</a>
17001/90	92.6-93.8	Skin irritation, rabbit	██████████, 1992 <a href="#">M-004846-01-1</a>
17001/90	92.6-93.8	Eye irritation, rabbit	██████████, 1992 <a href="#">M-004847-01-1</a>
17001/90	92.6-93.8	Skin sensitization, Guinea pig	██████████, 1992 <a href="#">M-004845-01-1</a>
17001/90	93.8-94.8	Dog, 13-week oral (diet) toxicity	██████████, 1995 <a href="#">M-004977-02-1</a>
17001/90	92.6-94.8	Mouse, 13-week oral (diet) (range-finding)	██████████, 1995 <a href="#">M-004985-01-1</a>
17001/90	92.6-94.8	Rat, 13-week oral (diet)	██████████, 1995 <a href="#">M-004999-01-1</a>
17001/90	92.6	<i>In vitro</i> Unscheduled DNA synthesis (UDS), rat hepatocytes	██████████, 1992 <a href="#">M-004577-01-1</a>
17001/90 FL 0036	92.6-94.8 95.2-99.0	Mechanistic study on thyroid hormone effects, 21-day, rat, diet	██████████, 1995 <a href="#">M-004982-03-1</a>
FL 036	95.0-99	Dog, 12-month chronic oral (diet) toxicity	██████████, 1995 <a href="#">M-005001-02-2</a>
FL 036	97.0-98.5	Rat, 21-day dermal toxicity	██████████, 1995, <a href="#">M-004981-01-1</a>
FL 036	97.1-97.5	<i>In vitro</i> mammalian cell gene mutation test (HGPRT), Chinese hamster cells V79	Brendler-Schwaab, 1994 <a href="#">M-004634-01-1</a>
FL 036	97.5	<i>In vitro</i> mammalian chromosome aberration test, Chinese hamster cells	Gahlmann, 1995 <a href="#">M-004692-01-1</a>
FL 036	97.5	<i>In vivo</i> Micronucleus test, mouse	██████████, 1993 <a href="#">M-004588-01-1</a>
FL 036	95.2-98.5	Mouse, 18-months feeding (carcinogenicity)	██████████, 1995 <a href="#">M-005060-02-1</a>
FL 036	95.2-99.0	Rat, 24-month feeding (carcinogenicity and chronic toxicity)	██████████, 1995 <a href="#">M-005062-02-1</a>
FL 036	95.2-99.0	Rat dietary two-generation reproduction study	██████████, 1995 <a href="#">M-004984-03-1</a>
FL 036	97.2	Rat, oral developmental	██████████, 1995, <a href="#">M-004976-02-1</a>
FL 036	98.5	Rabbit, oral developmental	██████████, <a href="#">M-004979-01-1</a>
FL 036	97.4-97.8	Rat, acute oral neurotoxicity	██████████, 1995 <a href="#">M-004986-02-1</a>
FL 036	98.0-98.2	Rat, 90-day neurotoxicity study (diet) (screening study)	██████████, 1995, <a href="#">M-005014-01-2</a>



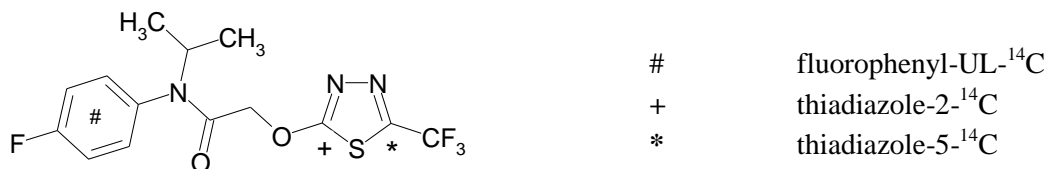
Batch Number	Purity (%)*	Study type	Reference
FL 036 NLL 3643-5	99.0 98.0	Acute oral toxicity, rat	██████████, 1993 <a href="#">M-004865-02-1</a>
NLL 3643-5	98.1	Acute oral toxicity, rat	██████████, 1991 <a href="#">M-004850-01-1</a>
NLL 3643-5	98.0-98.1	Acute oral toxicity, rat	██████████, 1992 <a href="#">M-004864-01-1</a>
NLL 3643-5	98.0-98.1	Acute dermal toxicity, rat	██████████, 1992 <a href="#">M-004843-01-1</a>
<b>603-0013</b>	<b>96.0-96.9</b>	<b>Rat, oral developmental neurotoxicity</b>	██████████, 2000 <a href="#">M-026105-01-1</a>
898313105	96.8	Skin sensitization, Guinea pig	██████████, 1994 <a href="#">M-004637-01-1</a>
898313105	96.8	Bacterial reverse mutation assay	Herbold, 1995 <a href="#">M-004696-01-1</a>
<b>920902ELB01</b>	<b>99.5</b>	<b>Skin sensitization, Guinea pig</b>	██████████, 1995 <a href="#">M-004677-01-1</a>
<b>EDHB001715</b>	<b>97.5</b>	<b>Skin sensitization, LLNA (mouse)</b>	██████████, 2004 <a href="#">M-090513-01-1</a>
<b>EDHB001715</b>	<b>97.0</b>	<b>Rat, 1-week inhalation toxicity</b>	██████████, 2008 <a href="#">M-300005-01-1</a>
<b>EDHB001715</b>	<b>97.0</b>	<b>Rat, 4-week inhalation toxicity</b>	██████████, 2008 <a href="#">M-302961-01-2</a>
<b>NK61AX0177</b>	<b>96.8</b>	<b>Bacterial reverse mutation assay</b>	Sokolowski, 2010 <a href="#">M-395211-01-1</a>
<b>NK61AX0177</b>	<b>96.8</b>	<b>Rat, oral (diet) developmental toxicity (range-finding)</b>	██████████, 2012 <a href="#">M-434509-01-1</a>
<b>NK61AX0177</b>	<b>96.8</b>	<b>Rat, comparative thyroid sensitivity assay (gestational exposure phase)</b>	██████████, 2012 <a href="#">M-435619-01-1</a>
<b>NK61AX0177</b>	<b>96.8</b>	<b>Rat, comparative thyroid sensitivity assay (gestational &amp; lactational exposure phase)</b>	██████████, 2012 <a href="#">M-435313-01-1</a>
<b>NK61AX0177</b>	<b>96.8</b>	<b>Rat, comparative thyroid sensitivity assay (gavage exposure of pups)</b>	██████████, 2012 <a href="#">M-435126-01-1</a>
<b>NK61CK0650</b>	<b>98.2</b>	<b>Phototoxicity test</b>	Heppenheimer, 2013 <a href="#">M-464615-01-1</a>

\* Purity as stated in study reports

### B.6.1 Absorption, distribution, metabolism and excretion (toxicokinetics)

#### B.6.1.1 Toxicokinetics: single dose and repeated dose, oral route in rats

Flufenacet (FOE 5043) was  $^{14}\text{C}$ -labelled at three different positions of the molecule for investigation of metabolism studies in plants and animals:



A study on absorption, distribution, metabolism and excretion (ADME) of  $^{14}\text{C}$ -labelled flufenacet with rats was conducted with all three label positions [REDACTED] (1995): The metabolism of FOE 5043 in rats, unpublished report of [REDACTED], Comp. No. [M-002247-01-1](#)).

This study was submitted with the dossier for Annex I listing of flufenacet according to EU directive 91/414/EEC and summarized in the Tier 2 summary for the active substance, under Annex IIA, Point 5.1.1.1 (2000). As a consequence, it has already been evaluated by the corresponding registration authorities in the EU.

This study is briefly summarized in the following sections of the Monograph of flufenacet (FOE 5043, fluthiamide) and its addenda published 1997.

Level 2 “Overall Conclusions; Section 2.4.1 “Effects having relevance to human and animal health arising from exposure to the active substance or to their transformation products”  
and  
Annex B5 “Toxicological and metabolism studies”, Section B.5.1.1 “Biokinetics and metabolism in rats”

**A short summary of the ADME study from this Monograph is repeated in the following:**

#### Toxicokinetics and metabolism

##### Dosing regimen

Low dose	1.0 mg/kg body weight, single oral dose
Multiple dosing	14 * 1.0 mg/kg body weight, oral dose once per day (non-radioactive compound) and 24 hours after the last dose 1.0 mg/kg body weight, single oral dose (radioactive compound)
High dose	150.0 mg/kg body weight, single oral dose

Sex: male and female rats

“The biokinetic and metabolism study on rats showed a high degree of absorption of radioactivity followed by fast elimination from the body. After oral administration of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 more than 87% of the recovered radioactivity was excreted via urine and faeces within 72 hours in all dose groups tested. The plasma curve analysis after dosing of [fluorophenyl-UL-<sup>14</sup>C]- and [thiadiazole-2-<sup>14</sup>C]-labelled FOE 5043 revealed that only the fluorophenyl part of the molecule underwent enterohepatic circulation. Absorption commenced immediately after administration. The concentration in the different organs and tissues were relatively low and showed only slight differences with respect to dose and sex.

The identification rate ranged from 60 to 75% of the recovered radioactivity in the experiments with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 and was 92% on average in the experiments with [thiadiazole-2-<sup>14</sup>C]FOE 5043. After application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 all metabolites identified contained only the fluorophenyl moiety of the active ingredient, because the thiadiazole ring was cleaved off prior to further metabolisation. This was confirmed by the results obtained after application of [thiadiazole-2-<sup>14</sup>C]FOE 5043. The major metabolites were the glucuronic acid of thiadone (M24), the oxalylacetic acid conjugate of thiadone (M26) and free thiadone (M9).

Glutathione conjugation appeared to be the major, and possibly the exclusive, metabolic pathway for [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in rats. Although the glutathione itself was not detected, the presence of a variety of glutathione-derived metabolites provided sufficient evidence for the glutathione pathway. Almost all metabolites identified were glutathione related compounds. The major metabolite in all dose groups was the N-acetylcysteine conjugate of fluorophenylacetanilide (M10).

For a better understanding of the biokinetic behaviour and metabolism of some FOE 5043 plant metabolites, the bioavailability of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate as well as [thiadiazole-2-<sup>14</sup>C]-N-glucoside was investigated after oral administration to rats. Both compounds were excreted unchanged with urine and faeces. Due to the extremely low residues in tissues and carcass, there should be no detectable residues in animal tissues neither from the fluorophenyl acetamide moiety nor from the thiadiazole moiety of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues.”

<b>Toxicokinetics critical end points</b>	
Rate and extent of absorption	high degree of absorption: 75 – 80% of oral dose (fluorophenyl-label) 93 – 97% of oral dose (thiadiazole-2- and thiadiazole-5-label) based on urine excretion, tissue distribution and exhaled $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$
Distribution	quick redistribution from blood
Potential for accumulation	none
Rate and extent of excretion	[fluorophenyl-UL- $^{14}\text{C}$ ]FOE5043: 59 - 79% with urine, 8 - 30% with faeces (72h) [thiadiazole-2- $^{14}\text{C}$ ]FOE5043: 41 - 59% with urine, 2 - 6 with faeces (72h), 22 - 32% $\text{CO}_2$ and 12 - 23% $\text{CH}_4$ in the expired air [thiadiazole-5- $^{14}\text{C}$ ]FOE5043: 82 – 89% with urine, 6 – 7 % with faeces (72h)
Main animal metabolites	<i>N</i> -acetylcysteine conjugate of <i>N</i> -isopropyl-fluorophenyl-acetanilide (M10), glucuronic acid of thiadone (M24), oxalylacetic acid conjugate of thiadone (M26), thiadone (M9)

<b>Previous evaluation</b>	<b>In DAR for original approval (1997)</b>  <b>The metabolism of FOE 5043 in rats studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>		
Title:		
Document No:		
Report No:		
Guidelines and data requirements:		
GLP		

#### Material and methods:

[fluorophenyl-UL- $^{14}\text{C}$ ]FOE 5043 with a specific radioactivity of 65.6 mCi/mMole (radiochemical purity 98.9%) was administered in 2% Cremophor/water solution at different dose levels to groups of 5 male and female rats as follows:

- Low dose 1.0 mg/kg body weight, single oral dose
- Multiple dosing 14 \* 1.0 mg/kg body weight, oral dose once per day (non-radioactive compound) and 24 hours after the last dose 1.0 mg/kg body weight, single oral dose (radioactive compound)
- High dose 150.0 mg/kg body weight, single oral dose

[Thiadiazole-2- $^{14}\text{C}$ ] FOE 5043 with a specific radioactivity of 18.47 mCi/mMole (radiochemical purity 98 %) as well as [thiadiazole-5- $^{14}\text{C}$ ] FOE 5043 with a specific radioactivity of 15.1 mCi/mMole (radiochemical purity

99.8 %) was administered to a group of 5 male and female rats at a low dose of 1.0 mg/kg body weight (single oral dose) and to 5 male rats at a high dose of 150 mg/kg body weight (single oral dose).

### Findings:

#### Absorption

Since in the low dose experiments after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 the renal excretion was above 70% of the administered radioactivity, a bile-fistulation experiment was not rendered as necessary. The radioactivity excreted with urine was significantly higher than with faeces. The single low dose demonstrated that male rats absorbed at least 75% of the given dose, while with female rats this figure amounted to 80%. After administration of the high dose (150 mg/kg) the renal excretion rate decreased to 59% of the given dose for male rats and did not change significantly for female rats with 76% (Table 6.1.1a). The lower value for male rats was probably due to an increased amount of unabsorbed radioactivity.

**Table 6.1.1 a: Excretion of total radioactivity and radioactive residues in the rat after oral application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 (values are given in % of administered radioactivity)**

Excretion of radioact. and radioact. residues	1 mg/kg male	pretreatm. 1 mg/kg male	150 mg/kg male	1 mg/kg female	pretreatm. 1 mg/kg female	150 mg/kg female
Urine	72	76	59	79	79	76
Faeces	20	17	30	11	8	14
Total excreted	92	93	89	90	87	90
Ratio Urine/Faeces	3.6	4.5	2.0	7	9.9	5.4
Tissues	3	3	3	1	2	2
Cage Rinse	3	4	3	5	7	4
Recovery	98	100	95	96	96	96

A comparable excretion behavior was observed in the studies with [thiadiazole-2-<sup>14</sup>C]FOE 5043 and [thiadiazole-5-<sup>14</sup>C]FOE 5043 demonstrating high renal excretion as well as high amounts of <sup>14</sup>CO<sub>2</sub> in the breathe out air (approx. 30%) in the experiment with [thiadiazole-2-<sup>14</sup>C] FOE 5043 (Tables 6.1.1b and 6.1.1c).

**Table 6.1.1b: Excretion of total radioactivity and radioactive residues in the rat after oral application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 (values are given in % of administered radioactivity)**

Excretion of radioactivity and radioactivity residues	1 mg/kg male	150 mg/kg male	1 mg/kg female
Urine	51	59	41
Faeces	6	4	2
<sup>14</sup> CO <sub>2</sub>	27	22	32
Total excreted*	84(99)	85(97)	75(98)
Ratio Urine/Faeces	8.5	14.8	20.5
Tissues	1	1	1
Cage Rinse	<1	2	<1
Recovery	85	88	76

Lost**	15	12	23
Total	100	100	99 <sup>#</sup>

\* values in brackets include the amount of "lost"

\*\* <sup>14</sup>CH<sub>4</sub> was assumed to constitute of all the unrecovered radioactivity

# total does not equal to 100 % due to rounding

The analysis of the plasma curves using the [fluorophenyl-UL-<sup>14</sup>C] label demonstrated that absorption commenced immediately after administration. Each plasma curve exhibited two peaks of concentration. The first one was reached after 1 hour. The plasma concentration then decreased to reach a second maximum 6 to 24 hours later (high dose). The appearance of a second peak indicates an enterohepatic circulation in which radioactivity enters systemic circulation after elimination with bile and reabsorption from the intestinal tract.

The plasma curves of the rats treated with fthiadiay.ole-2-<sup>14</sup>C]FOE 5043 were different from those of the rats treated with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043. There was only a single plasma concentration maximum in the thiadiazole-label experiment compared with the two maxima in the fluorophenyl-label experiment. This indicates that the thiadiazole ring and the fluorophenyl ring of FOE 5043 were being separated during the first pass prior to entering systemic circulation. The thiadiazole moiety appeared to be absorbed quickly and reached a maximum concentration about 2 hours after dosing. The elimination of thiadiazole-derived residues from the blood followed first order kinetics.

The plasma curve analysis after dosing of [fluorophenyl-UL-<sup>14</sup>C]- and [thiadiazole-2-<sup>14</sup>C]-labelled FOE5043 revealed that only the fluorophenyl part of the molecule underwent enterohepatic circulation. Hence plasma curve analysis was rendered unnecessary after [thiadiazole-5-<sup>14</sup>C] application.

**Table 6.1.1c: Excretion of total radioactivity and radioactive residues in the rat after oral application of [thiadiazole-5-<sup>14</sup>C]FOE 5043 (values are given in % of recovered radioactivity)**

Excretion of radioactivity and radioactivity residues	1 mg/kg male	150 mg/kg male	1 mg/kg female
Urine	89	82	87
Faeces	6	7	4
Total excreted*	95	89	91
Ratio Urine/Faeces	14.8	11.7	21.8
Tissues	2	7	7
Cage Rinse	2	4	2
Recovery	99*	100	100

\* total does not equal to 100 % due to rounding

### Distribution

The comparison of the plasma curves of the different dose groups showed that the dosage of 150 mg/kg influenced the shape of the plasma curve inasmuch that the first maximum was reduced and the second one

increased. This possibly indicates that after high dosage a bigger portion of lipophilic metabolites are formed which are more susceptible to enterohepatic circulation.

The total amount of radioactivity in the tissues did not exceed 3% of the administered radioactivity. The recovery ranged from 95 to 100% (Tab. 6.2.a).

A summary of the distribution of the radioactivity concentration amongst the different organs and tissues after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 is given in Table 5.1.1d. The concentrations in general were relatively low. The relative concentration in all organs except liver did not exceed the concentration in blood. This demonstrates that the radioactivity is being quickly redistributed from the organs to the blood. The significantly higher concentration in GIT as compared to that in the organs is further support to the existence of a pronounced enterohepatic circulation. The relative concentrations in the organs showed only slight differences with respect to dosage and sex.

**Table 6.1.1d: Relative concentration of radioactivity (P) in individual parts of the body of rats after oral application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 (all values are multiplied with the factor 100)**

Biological Material	1 mg/kg male	pretreatm. 1 mg/kg male	150 mg/kg male	1 mg/kg female	pretreaun. 1 mg/kg female	150 mg/kg female
Blood	2.5	2.3	3.0	2.7	2.0	2.3
Bone	0.7	0.5	0.9	0.6	0.5	0.6
Brain	1.1	0.8	1.6	0.9	0.9	1.3
Fat	0.8	0.6	0.9	0.8	0.6	0.9
Gonad/Ovaries	0.7	0.7	1.3	1.3	1.1	1.7
Heart	1.7	1.4	2.1	1.3	1.2	1.7
Kidney	1.7	1.6	2.6	1.7	2.0	2.2
Liver	3.1	3.2	5.3	3.0	1.2	3.5
Lung	1.6	1.3	2.3	1.8	0.8	1.8
Muscle	1.0	0.9	1.4	0.9	1.1	1.2
Spleen	1.3	1.2	2.0	1.3	1.6	1.5
Skin	1.8	1.7	2.6	3.0	1.2	2.4
Thyroid	2.3	1.2	2.7	1.3	1.2	1.5
Uters	---	---	---	1.0	0.8	1.2
GIT	11.6	12.6	6.6	3.4	2.6	2.2
Carcass	0.4	0.4	0.6	0.5	0.4	0.5

p=  $\frac{\text{measured activity} / \text{g tissue or plasma}}{\text{administered activity} / \text{g body weight}}$

The relative concentrations of radioactivity in tissues after application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 are summarized in Table 6.1.1e. The recoveries ranged from 76 to 88 % of the applied dose. The remaining 12 to 23 % of the dose was determined to be methane based on analysis of respired gases in a special experiment. The radioactivity remaining in the tissues accounted for only 1% of the total radioactive residue (TRR).

The recovery of the total radioactivity after application of [thiadiazole-5-<sup>14</sup>C]FOE 5043 ranged from 93 to 102 % (Table 6.1.le). The residues remaining in the tissues were very low and accounted for <7 % of the total radioactive residue (Table 6.1.le).

**Table 6.1.1e: Relative concentration of radioactivity (P) in individual parts of the body of rats after oral application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 and [thiadiazole-5-<sup>14</sup>C]FOE 5043 (all values are multiplied with the factor 100)**

Biological Material	[thiadiazole-2- <sup>14</sup> C]FOE 5043			[thiadiazole-5- <sup>14</sup> C]FOE 5043		
	1 mg/kg male	1 mg/kg female	150 mg/kg male	1 mg/kg male	1 mg/kg female	150 mg/kg male*
Blood	6.0	1.0	1.7	25.3	34.4	16.9
Bone	1.2	0.9	1.0	2.7	3.5	2.4
Brain	0.3	0.2	0.3	3.0	4.2	2.5
Fat	0.4	0.4	0.5	2.3	2.7	1.6
Gonad/Ovaries	0.3	1.1	0.4	3.0	6.0	2.5
Heart	0.5	0.6	0.6	7.8	11.6	6.5
Kidney	1.2	1.5	1.7	6.6	11.0	5.6
Liver	4.1	4.0	3.2	8.2	10.7	5.3
Lung	0.6	0.8	0.9	7.8	11.8	6.1
Muscle	0.3	0.3	0.3	3.3	4.5	2.7
Spleen	0.8	0.8	0.6	6.2	10.3	4.2
Skin	1.8	1.4	1.8	5.8	7.7	4.7
Thyroid	0.7	1.2	0.7	4.5	5.8	3.1
Uters	---	0.9	---	---	6.9	---
GIT	0.9	1.1	1.3	4.1	6.4	4.2
Carcass	0.2	0.2	0.3	1.0	1.2	1.2

$p = \frac{\text{measured activity / g tissue or plasma}}{\text{administered activity / g body weight}}$

\* values based on the results of 4 animals, one rat was injured on dosing (punctured esophagus); a portion of the dose entered the lung and no feed uptake/excretion occurred. The rat was sacrificed early.

### Excretion

The experiment with [thiadiazole-2-<sup>14</sup>C]FOE 5043 revealed that significant amounts (up to 32%) of radioactivity were expired as I<sub>4</sub>CO<sub>2</sub>. In addition, up to 23% was attributed to CH<sub>4</sub>. Although no CO<sub>2</sub> measurements were made in the studies using [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 and [thiadiazole-5-<sup>14</sup>C]FOE, it can be assumed that only small quantities may have been formed, because most of the radioactivity was recovered in the excreta and tissues (Tables 6.1.1a-c).

Concerning total excretion of radioactivity, there was no difference in sex with regard to the recovered radioactivity (96-100 %). Within each dose group, a greater portion of radioactivity was excreted with urine by females, indicating that female rats were able to absorb and metabolize FOE 5043 more efficiently than males. After oral administration of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 more than 87% of the recovered radioactivity was excreted via urine and faeces within 72 hours in all dose groups tested (Table 6.1.1a). The majority of the radioactivity was excreted renally [average ratio: 4.4:1 (urine:faeces)]. A minimum of 40% of the total



radioactivity was excreted within the first 24 hours posttreatment, 75% within 48 hours posttreatment, and 86% within 72 hours posttreatment. Two groups, high and low dose females, were not sacrificed until 96 hours posttreatment, when >90% of the dose had been excreted. Excretion appeared to be slightly reduced at early time points in the high dose compared to the low dose for both sexes. The highest level of urinary excretion for both sexes was observed in the multiple dose group, possibly indicating that chronic treatment induced metabolism.

After oral administration of [thiadiazole-2-<sup>14</sup>C]FOE 5043 more than 75% of the recovered radioactivity was excreted via urine, faeces and the respired air within 72 hours in all dose groups tested (Table 6.1.1b). The majority of the radioactivity was excreted renally [average ratio: 12.6:1 (urine:faeces)]. A minimum of 59% of the total radioactivity was excreted within the first 24 hours posttreatment and 75% within 48 hours posttreatment. Excretion appeared to be slightly less at early time points in the high dose than in the low dose. Between 12 and 23% of the applied dose were determined to be CH<sub>4</sub>.

After oral administration of [thiadiazole-5-<sup>14</sup>C]FOE 5043 more than 89 % of the recovered radioactivity was excreted via urine and faeces within 72 hours in all dose groups tested (Table 6.1.1c). The majority of the radioactivity was excreted renally [average ratio: 15.2:1 (urine:faeces)]. A minimum of 70% of the total radioactivity was excreted within the first 24 hours posttreatment and 86 % within 48 hours posttreatment. Excretion appeared to be slightly reduced at early time points in the high dose as compared to the low dose.

### Metabolism

Table 5.1.1f gives the quantitative distribution of the identified metabolites in the sum of urine and faeces 72 hours after administration as % of the recovered radioactivity.

All metabolites identified contained only the fluorophenyl-moiety of the active ingredient, the thiadiazole ring was cleaved off prior to further metabolism. The compilation shows that the main metabolite in all dose groups was the *N*-acetylcysteine conjugate of fluorophenylacetamide (M10) with up to 55%. Other significant metabolites were dcs-isopropyl-fluorophenylacetanilide methylsulfone (M15) which accounted for 16% of the total radioactive residue, the sulfoxide of M10 (M12). 2-amino-5-fluoro phenol (M21) with 8% and the hydroxyd *N*-phenyl-(2-methylsulfonyl)acetamide (M17) with 7%. This metabolite was identified to consist of two position isomers (regioisomers) which were chromatographically separated, but the percent distribution reflects the sum of both isomers. Each of the other metabolites accounted for less than 4% of the total radioactive residue. Unchanged parent compound was detected in faeces of male rats of the high dose group and in urine of male rats after multiple dosing in a small amount of 2%. The only metabolites that were found in faeces but not in urine were identified as bis(*N*-(4-fluorophenyl)-*N*-(1-methylethyl)acetamide (M19) and hydroxy-isopropylfluorophenylacetanilide methylsulfone (M29). Metabolites detected in both urine and faeces were M17, M6, M7, M10, M15 and parent compound. A proposed metabolic pathway in animals is included in Figure 6.2. Glutathione conjugation appeared to be the major, and possibly the exclusive, metabolic pathway for [fluorophenyl-UL-1-<sup>14</sup>C]FOE 5043 in rats. Although the glutathione conjugate itself was not detected, the presence of a variety of glutathione-derived metabolites provided sufficient evidence for the glutathione pathway. Almost all metabolites identified were glutathione-related compounds. The major metabolite (M10)

can be formed by glutamyltranspeptidase-mediated hydrolysis of a glutathione conjugate to produce a cysteinyl conjugate which is acetylated to result in des-isopropyl-fluorophenylacetanilide methylsulfone (M15).

**Table 6.1.1f: Quantitative distribution of metabolites in % of the recovered radioactivity after oral application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 (sum of urine and faeces, values for faeces are given in brackets)**

Test Description	High dose		Low dose		Multiple dose	
Sex	male	female	male	female	male	female
M21	7	7	6	3	8	2
M17	7 (2)	6 (1)	<1	(<1)	3	<1
M20	3	4	3	1	4	<1
M18	1	< 1	<1	<1	<1	<1
M11	< 1	< 1	nd	nd	nd	nd
M3, M31, M32	1	1	<1	<1	1	<1
M16	1	2	1	1	1	<1
M15	16 (2)	16 (2)	13 (1)	7	13 (1)	3
M29	(<1)	nd	(2)	nd	(2)	(1)
M2	0.5	0.5	<1	<1	<1	<1
M12	8	6	21	6	19	7
M6	3 (1)	4	3	3	3	3
M7	1	1	2 (1)	1	3 (1)	2 (1)
M10	8 (1)	22	17 (1)	53 (2)	15 (1)	55
M13	1	2	<13	<1	<1	<1
M19	(1)	nd	nd	nd	(<1)	(<1)
FOE 5043	(2)	nd	nd	(<1)	2	(<1)
Sum Identified	60.5	71.5	68	75	74	72

A major portion of the *N*-acetyl conjugate (M10), especially in male rats, was oxidised to the corresponding sulfoxide of des-isopropyl-fluorophenylacetanilide methylsulfone (M12) while other portions went through other transformation pathways. In contrast, a major metabolic pathway especially in female rats involved C-S bond cleavage, of either the cysteine or glutathione conjugate, possibly by  $\beta$ -lyase of the intestinal microflora to form a free thiol. The free thiol was methylated, oxidised, and des-isopropylated to form des-isopropyl fluorophenylacetanilide methylsulfone (M15).

2-Amino-5-fluoro phenol (M21) was probably produced by the amide hydrolysis with aryl amidase.

Sex-related metabolic differences were observed regarding the formation of the metabolites M10 and M12. respectively. Female rats excreted significantly higher amounts of M10 with urine compared to males, while male rats appeared to be more efficient in oxidation of metabolite M10 to the corresponding sulfoxide (M12).

The distribution of metabolites identified in rats after oral application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 is summarized in Table 5.1.1g. The three major metabolites in urine were the glucuronic acid of thiadone (M24) which accounted for 28 to 40% of the total radioactive residue, the oxalylacetic acid conjugate of thiadone (M26) with amounts between 6 and 11 % and free thiadone (M9) which accounted for 5 to 7% of the total radioactive residue. <sup>14</sup>CO<sub>2</sub> was a major metabolite and accounted for 22 to 32%. <sup>14</sup>CH was also detected and accounted for 12 to 22% of the total radioactive residue.

Application of [thiadiazolc-5-<sup>14</sup>C]FOE 5043 to rats showed comparable results to those found in the urine of [thiadiazole-2-<sup>14</sup>C]FOE 5043 treated rats. The metabolites found were identical to those with the label in 2-position and the quantitative distribution was in the same order of magnitude (Table 6.1.1g).

**Table 6.1.1g: Quantitative distribution of metabolites in % of the recovered radioactivity after oral application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 and of [thiadiazole-5-<sup>14</sup>C]FOE 5043**

Test description	[thiadiazole-2- <sup>14</sup> C]FOE 5043			[thiadiazolc-5- <sup>14</sup> C]FOE 5043		
	High dose	Low dose	Low dose	High dose	Low dose	Low dose
sex	male	male	female	male	male	female
M26	12	10	6	17	17	23
M24	40	32	28	44	33	21
M9	7	5	5	8	7	9
<sup>14</sup> CO <sub>2</sub>	22	27	32	---	---	---
<sup>14</sup> CH <sub>4</sub>	12	15	22	---	---	---
Sum identified	93	89	93	69	57	53

<b>New studies; not evaluated</b>	This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. (2010) <b>Evaluation of pharmacokinetic data. New EU data requirement</b>
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<b>Report:</b>	<b>KCA 5.1.1/01, [REDACTED], 2010</b>
<b>Title:</b>	Amendment No. 1 to the Final Report: The Metabolism of FOE 5043 in Rats
<b>Document No:</b>	<a href="#">M-384235-01-1</a>
<b>Report No:</b>	Bayer Report No. 106665-1
<b>Guidelines and data requirements:</b>	US EPA Ref.: 85-1, Rat Metabolism
<b>GLP</b>	Yes

## Material and Methods

In a basic ADME study<sup>1</sup>, groups of each five male and five female rats received a single oral dose of [fluorophenyl-UL-<sup>14</sup>C] and [thiadiazole-2-<sup>14</sup>C]flufenacet. The dose rates amounted to 1 mg/kg bw (single low dose) and to 150 mg/kg bw (single high dose). Additional groups of rats received 14 non-labelled doses followed by a single radiolabelled dose of flufenacet at the low dose level.

Intravenous administration or bile cannulation after oral administration for determination of the bioavailability were not necessary, since a high extent of renal excretion and a high amount of exhaled radiolabelled carbon dioxide and methane indicated an almost complete absorption of the oral dose.

The total radioactive residues (TRR) were measured in blood plasma at different time intervals after administration (10, 20, 40, 90, 90 min; 2, 4, 6, 8, 24, 32, 48, 72, 96 hr).

## Results and Discussion

Average plasma levels of each dose group are compiled in Table 6.1.1- 1 for the fluorophenyl label and in Table 6.1.1- 2 for the thiadiazole label. Peak levels are printed in bold type.

From the plasma levels the toxicokinetic parameters  $T_{max}$ ,  $C_{max}$ , and  $T_{1/2}$  for elimination of TRR from the plasma were derived visually. The AUC (area under the curve 0 – 72 or 96 hours) was estimated using the trapezoidal method (sum of all partial trapezoid areas under the plasma curve; trapezoid area = time difference between two adjacent concentration levels multiplied by half of the sum of two levels).

In addition, the computer program TOPFIT pharmacokinetic modelling was also used where it was possible (not possible for low dose plasma levels with the fluorophenyl label). The model integrates the areas under the curve by approximation of a continuous curve to the measured plasma levels. From this approximated curve AUC (0 -  $\infty$ ),  $T_{max}$  and  $C_{max}$  are derived.

The results of this pharmacokinetic evaluation are compiled in Table 6.1.1- 3. The peak maxima ( $C_{max}$ ) at the low dose were achieved at  $T_{max}$  of 1 – 2 hours after dosing for both radiolabels. Following administration of a high dose, the maxima of the fluorophenyl level were delayed and reached 24 – 32 hours after dosing indicating a slower GIT absorption due to saturation effects. The peak maximum of the thiadiazole label was reached after a similar time period of 1 -2 hours for the low dose and accounted for 24 hours for the high dose.

The plasma curve of male and female rats receiving a single and multiple low dose(s) of [fluorophenyl-UL-<sup>14</sup>C]flufenacet showed two peaks, the first peak one hour after dosing and a second lower peak 6 – 8 hours after dosing, indicating the presence of an enterohepatic circulation. In contrast, a low dose using the [thiadiazole-2-<sup>14</sup>C] label showed only one peak maximum 1 – 4 hours after administration.

For the fluorophenyl-label, the half-life of elimination  $T_{1/2}$  was relatively short at the low dose level amounting to 4 hours for a single dose. However,  $T_{1/2}$  was extended to 24 hours for male rats receiving the multiple doses and to 72 hours for the high dose. For the thiadiazole-label, the elimination half-lives were slightly longer for the low dose amounting to 6 – 8 hours and for the high dose amounting to 24 hours.

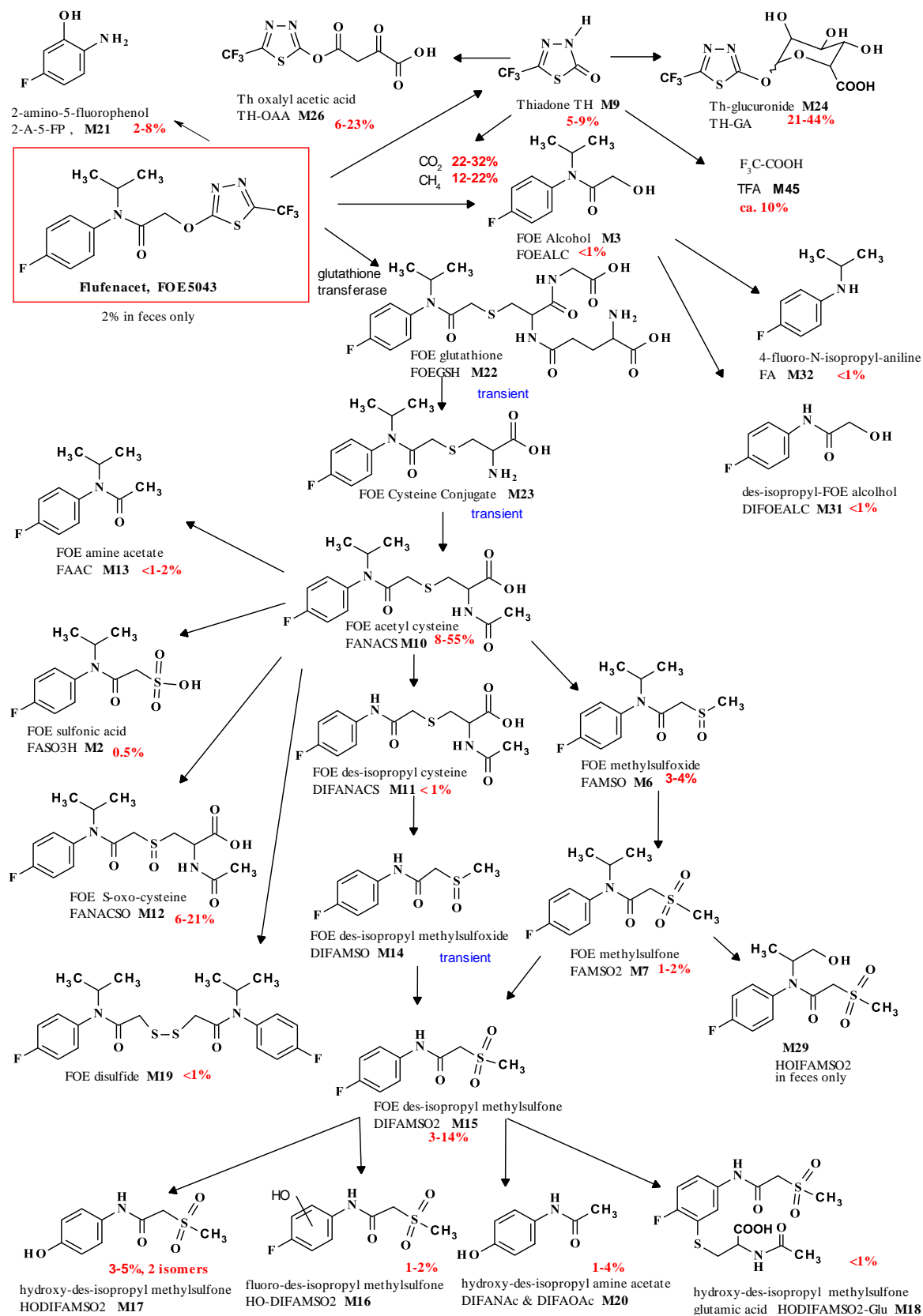
No sex difference and no influence of repeated dosing could be observed.

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<sup>1</sup> [REDACTED] (1995). The metabolism of FOE 5043 in rats. Unpublished report of Miles Inc. Stilwell, KS, USA, now Bayer CropScience, Comp. No. M-002247-01-1.

Metabolic pathways of Flufenacet in the rat according to [REDACTED] (1995), M-002247-01-1 is presented below:

Fig. B.6.1.1 Metabolic pathways of Flufenacet in the rat



Red numbers: maximum proportion of the administered dose in the urine.

**Table 6.1.1- 1: Averaged plasma levels in rats following oral administration of [fluorophenyl-UL-<sup>14</sup>C]flufenacet (mg equ/L, mean of each 5 animals)**

Time	Low Dose Males	Low Dose Females	High Dose Males	High Dose Females	Multiple Low Dose Males	Multiple Low Dose Females
10 min	0.055	0.035	2.124	2.050	0.026	0.041
20 min	0.201	0.141	5.295	4.727	0.154	0.166
40 min	0.302	0.290	7.722	6.447	0.300	0.354
60 min	<b>0.312</b>	<b>0.361</b>	7.709	6.554	<b>0.368</b>	<b>0.390</b>
90 min	0.236	0.259	6.100	5.804	0.314	0.286
2 hr	0.212	0.188	8.280	5.015	0.243	0.224
4 hr	0.154	0.121	7.797	8.701	0.198	0.136
6 hr	<b>0.240</b>	0.163	14.306	19.756	0.251	0.138
8 hr	0.239	<b>0.193</b>	22.085	27.874	<b>0.283</b>	<b>0.164</b>
24 hr	0.132	0.131	<b>36.833</b>	37.000	0.155	0.096
32 hr	0.093	0.100	32.549	<b>39.272</b>	0.127	0.078
48 hr	0.042	0.054	20.206	24.396	0.070	0.054
72 hr	0.018	0.026	14.328	8.311	0.025	0.015
96 hr	-	0.016	-	4.503	-	-

**Table 6.1.1- 2: Averaged plasma levels in rats following oral administration of [thiadiazole-2-<sup>14</sup>C]flufenacet (mg equ/L, mean of each 5 animals)**

Time	Low Dose Males	Low Dose Females	High Dose Males
10 min	1.103	0.083	34.018
20 min	2.385	0.264	111.593
40 min	3.104	0.659	160.034
60 min	<b>3.359</b>	1.438	165.230
90 min	3.341	1.786	173.790
2 hr	3.332	2.630	180.878
4 hr	2.430	<b>2.731</b>	<b>185.704</b>
6 hr	1.654	2.272	154.379
8 hr	1.174	1.793	116.928
24 hr	0.135	1.313	44.293
32 hr	0.070	0.246	34.363
48 hr	0.026	0.159	4.829
72 hr	0.022	0.041	-
96 hr	-	-	-

**Table 6.1.1- 3: Summary of toxicokinetic plasma parameters determined in rats following oral administration of [fluorophenyl-UL-<sup>14</sup>C] and [thiadiazole-5-<sup>14</sup>C]flufenacet**

	Calculated via Excel				Calculated via TOPFIT model			
	T <sub>max</sub> (h)	C <sub>max</sub> (mg/L)*	T <sub>1/2</sub> (h)	AUC** (mg/L*h)	T <sub>max</sub> (h)	C <sub>max</sub> (mg/L)*	T <sub>1/2</sub> (h)	AUC (mg/L*h)
<b>[fluorophenyl-UL-<sup>14</sup>C] label</b>								
Low Dose, Males	1	0.312	4	7.38	nc	nc	nc	nc
Low Dose, Females	1	0.361	4	7.63	nc	nc	nc	nc
High Dose, Males	24	36.8	72	1672.46	24.5	32.1	nc	2200
High Dose, Females	32	39.3	72	1980.04	21.9	37.4	nc	2230
Multiple Low Dose, Males	1	0.368	24	9.27	nc	nc	nc	nc
Multiple Low Dose, Females	1	0.390	4	6.14	nc	nc	nc	nc
<b>[thiadiazole-2-<sup>14</sup>C] label</b>								
Low Dose, Males	1	3.359	6	31.03	1	3.42	nc	30.9
Low Dose, Females	2	2.731	8	31.28	2.7	2.6	nc	28.4
High Dose, Males	4	185.7	24	3183.70	1.47	185	nc	3380

nc = not calculated

\* mg/L = mg parent equivalents per litre

\*\*AUC for 0 – 72 or 96 hours (0 – 48 hours for [thiadiazole-2-<sup>14</sup>C], low dose, females)**Rat metabolism study with [thiadiazole-5-<sup>14</sup>C]flufenacet**

A new supporting rat metabolism study is summarised here which was not submitted with the original dossier and therefore not evaluated in the former EU review of flufenacet. The objective of this study was the identification of systemic label-specific metabolites originating from [thiadiazole-5-<sup>14</sup>C]flufenacet, particularly the formation of trifluoroacetate (TFA).

<b>New studies; not evaluated</b>	A new supporting rat metabolism study (2012)
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<b>Report:</b>	<b>KCA 5.1.1/02, [REDACTED], 2012</b>
<b>Title:</b>	[Thiadiazole-5- <sup>14</sup> C]Flufenacet: Supportive Experiment for Identification of Metabolites in the Urine of the Rat
<b>Document No:</b>	<a href="#">M-441499-01-1</a>
<b>Report No:</b>	EnSa-12-0439
<b>Guidelines:</b>	OECD Guideline 417: Toxicokinetics, adopted 22-July-2010, US EPA OCSPH Health Effects Test Guideline OPPTS 870.7485
<b>GLP</b>	yes

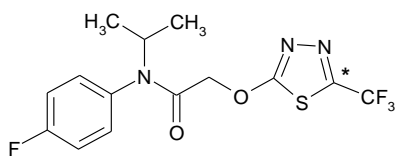
## Executive Summary

A supportive study was conducted on the metabolism of [thiadiazole-5-<sup>14</sup>C] in the rat to investigate polar metabolites. The test substance was orally given to four male rats per gavage at a dose rate of approximately 1 mg/kg bw. Urine and faeces were collected at different intervals up to 48 hours after dosing. Then, the animals were sacrificed and blood plasma was also sampled.

Excretion was almost complete at the end of the study with renal excretion being the dominant route of elimination. Chromatographic profiling of the urine samples was similar with that of a former metabolism study of flufenacet in the rat using all three radiolabels. In this study, free thiadone was detected at a portion of 6.5% of the dose in the urine. An additional polar chromatographic fraction increased with the collection interval and reached the dominant portion of excreted residues by the last collection period (24 - 48 hours after dosing). This fraction was identified as trifluoroacetate. If all urine samples were pooled the trifluoroacetate metabolite accounted for approximately 10% of the administered dose of flufenacet. This metabolite was also detected in the plasma. It is therefore concluded that this metabolite is covered in toxicology studies of the parent substance flufenacet in the rat.

## Material and methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluoro-phenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5- <sup>14</sup> C]Flufenacet
Specific radioactivity	3.81 MBq/mg (103.04 mCi/g)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

### Test Animals

Species	Rat ( <i>Rattus norvegicus domesticus</i> )
Strain	Wistar Unilever HsdCpb: WU
Breeder	
Sex, number	4 male rats
Body weight	Approx. 193 g at administration and 199 g at sacrifice
Age	6 – 7 weeks
Acclimatization	One week before administration



Housing	Individually in Makrolon metabolism cages allowing separation of urine and faeces, 21-23°C, 53-68% relative air humidity, 12/12 h light/dark cycle
Feed and water	Rat/mice long life diet from Promivi Kliba AG, Switzerland, <i>ad libitum</i> ; tap water, <i>ad libitum</i>

#### Preparation of the dosing mixtures and administration

The radiolabelled test substance was suspended in water containing 2% Chremophor EL and magnetically stirred in a cold chamber overnight. This suspension was administered orally using a syringe attached to an animal-feeding knob cannula (gavage) at a dose rate of approximately 1 mg/kg bw. The exact dose rate of 0.99 mg/kg bw was determined from radioactivity measurement of the dosing solution, the dosed volume and the body weight of the animals.

#### Collection of urine and faeces

Urine was collected 4, 8, 24 and 48 hours after administration individually from each animal in cryogenic traps cooled with dry ice. At each sampling period the collection funnels were rinsed with water and the water added to the respective urine sample. For chromatographic analysis the individual urine samples of the same collection interval were combined. Faeces were sampled individually 24 and 48 hours after dosing into cryogenic traps and homogenized using a highspeed stirrer after addition of water at a ratio of 1:1. Each sample was radioassayed by LSC.

#### Sacrifice and sampling of plasma

The animals were sacrificed 48 hours after administration by exsanguination following anesthesia by injection of Pentobarbital-Na. The blood was separated into blood cells and plasma by centrifugation. Proteins in the plasma were precipitated by addition of acetonitrile at a ratio of 1:1.

#### Storage, processing and radioassaying of samples

All samples were storage at  $\leq -18^{\circ}\text{C}$  until work-up. Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed  $^{14}\text{CO}_2$  was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC.

#### Radio-chromatography and mass spectrometry of samples

The residues in urine and plasma were separated by radio-HPLC equipped with a UV detector (254 nm) and a radiomonitor with a solid scintillator. A RP18 column (250 x 4.6 mm, 5  $\mu\text{m}$  particles) was operated with a gradient solvent mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v). Column recovery was proven to be complete by comparison of the eluted radioactivity with and without the separation column.

For confirmation, the radioactive samples were also investigated by thin layer chromatography and evaluation of the developed plates by radioluminography (radio-TLC). TLC separation was achieved on silica gel 60 plates (20 x 20 cm) developed by a mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/11/1, v/v/v/v).

Identification of the radioactive metabolites was performed by combined HPLC/MS using a RP18 column (250 x 2 mm, 3 µm particles) and a gradient mixture of water (containing 0.1% formic acid) and acetonitrile (containing 0.1% formic acid) for separation and an Orbitrap mass spectrometer using the electrospray ionization mode for identification. In some cases, NMR spectra were measured at 600 MHz. In addition, radiolabelled reference standards were co-chromatographed to support identification.

## Findings

### Excretion of radioactive residues and plasma level at sacrifice

Following oral administration of approximately 1 mg/kg bw of [thiadiazole-5-<sup>14</sup>C]flufenacet 89% of the radiolabelled dose had been excreted until sacrifice (48 hours after dosing). The predominant portion (83% of the dose) was excreted within 24 hours. 86.5% of the dose was excreted with the urine and approximately 2.8% of the dose with the faeces. These results are very similar to those of the former metabolism study using radiolabelled flufenacet with all three different label positions<sup>2</sup>. In the blood plasma at sacrifice the residue level amounted to 0.224 mg equivalents/kg (mg equ/kg).

### Metabolic profile in urine (Table 6.1.1- 4)

The radio-HPLC chromatograms of the urine samples collected at different intervals after dosing showed a similar metabolic profile. However, a very polar fraction (short elution time) comprising of three closely eluting peaks increased with the collection time, finally forming the predominant metabolite fraction at the latest sampling period (24 – 48 hours after dosing). This fraction was separately collected and re-analyzed by radio-TLC together with radiolabelled trifluoroacetate as reference standard. The TLC analysis showed only one radioactive spot for the mixture of the urine fraction and the reference standard. It is therefore concluded that the polar HPLC fraction consisted of only one metabolite, i.e. trifluoroacetate (TFA, M45). In radio-HPLC this fraction formed an artificial pattern of three peaks due to matrix effects of the urine. In a pooled urine sample collected 0 – 48 hours after administration, the trifluoroacetate metabolite accounted for approximately 10% of the oral dose. It can therefore be considered to be covered in toxicity tests of the parent substance in the rat.

Another metabolite (No. 15) could be identified as FOE-thiadone (M9) by co-chromatography with a respective (non-labelled) reference standard. It accounted for approx. 6.5% of the oral dose. A number of additional metabolites were detected in the radio-HPLC separations but not identified, because the objective of this study was to show the formation of trifluoroacetate from flufenacet in the rat

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<sup>2</sup> [REDACTED] (1995): The metabolism of FOE 5043 in rats. Unpublished report 106665 of Miles Inc., Stilwell, KS, USA, now Bayer CropScience, Comp. No. M-002247-01-1.

metabolism. However, identification of these unknown metabolites was conducted in the mentioned former study using radiolabelled flufenacet with the same (and other) label positions.

#### Metabolic profile in the plasma

Radio-HPLC of plasma samples showed a broad zone in the polar region and a relatively sharp peak in the non-polar region. The polar fraction was collected and re-analyzed by radio-TLC revealing that it mainly consisted of trifluoroacetate (M45). The non-polar peak could not be identified due to its low portion in the plasma.

#### **Conclusion**

Following oral administration of [thiadiazole-5-<sup>14</sup>C]flufenacet to rats most of the radioactivity was already excreted within 24 hours with renal excretion being the predominant route of elimination. The excretion pattern was similar to that of a former study on the metabolism of radiolabelled flufenacet in the rat. Thiadone (M09) was detected in the urine up to 6.5% of the dose. An additional polar metabolite detected in urine and blood plasma revealed to be trifluoroacetate (M45) reaching a level of approximately 10% of the administered dose. Therefore, it is concluded that this metabolite is covered in toxicological studies of the parent substance.

**Table 6.1.1- 4: Metabolic profile in urine samples collected at different intervals after oral administration of 1 mg/kg bw of [thiadiazole-5-<sup>14</sup>C]flufenacet to rats**

Peak	Metabolite/chromatographic region	Collection interval				
		0 - 4h	4 - 8h	8 - 24h	24 - 48h	0 – 48h
		% of dose administered				
Excreted with urine		16.37	34.87	30.13	5.10	86.48
1	Trifluoro acetate, TFA (M45)	---	---	0.25	0.08	0.33
2	Trifluoro acetate, TFA (M45)	0.37	0.96	3.51	3.24	8.08
3	Trifluoro acetate, TFA (M45)	0.09	0.39	0.39	0.07	0.94
	Trifluoro acetate, TFA (subtotal 1-3)	0.46	1.35	4.14	3.39	9.34
4	region 1	---	0.21	0.70	0.29	1.19
5	region 2	0.21	0.46	0.40	---	1.07
6	region 3	0.21	0.43	0.32	---	0.95
7	region 4	8.57	15.90	11.69	0.36	36.53
8	region 5	0.18	0.41	0.38	---	0.97
9	region 6	0.50	1.26	1.23	0.15	3.14
10	region 7	1.99	6.93	5.68	0.66	15.26
11	region 8	0.23	0.53	0.42	---	1.18
12	region 9	2.00	3.68	2.06	---	7.74
13	region 10	0.19	0.14	0.19	---	0.52
14	region 11	0.09	0.18	0.08	---	0.35
15	FOE-thiadone (M9)	1.49	2.77	2.13	0.09	6.48
16	region 12	0.26	0.62	0.71	0.15	1.75
Total		16.37	34.87	30.13	5.10	86.48

**Remark about formation of trifluoroacetate under physiological conditions**

Under physiological and environmental conditions metabolic formation of TFA does not result in trifluoroacetic acid (TFA-H), rather than in formation of a trifluoroacetate salt (consists of TFA anion and counter cation). This is because of the very high acidity of TFA-H as characterized by its low pKa of 1.3<sup>3</sup> (for comparison, pKa of acetic acid: 4.76) indicating a complete dissociation at a higher pH than 1.3

During metabolic formation of TFA the acidity of the analyzed matrix (e.g. plasma, urine) does not change indicating that TFA is not present as free trifluoroacetic acid TFA-H. This is due to the low amounts formed compared to the significantly higher buffer capacity of the physiological medium (e.g. organs, tissues and body fluids). It is rather formed as TFA anion with an undefined counter cation depending on the physiological medium. Since the counter cation is undefined the TFA is usually denoted by the name of its parent acid, trifluoroacetic acid, keeping in mind that their salts are meant.

<sup>3</sup> Winkler, S., 2011: Trifluoro acetic acid (AE C502988): Determination of the dissociation constant in water, unpublished report 20100672.02 of Siemens, Prozess-Sicherheit Frankfurt, Germany, for Bayer CropScience, Comp. No. M-4186298-01-1

While the acid TFA-H is known to be highly irritant due to its high acidity, the TFA anion combined with a physiologically appearing cation behaves like an inert salt. Therefore, toxicological evaluation must not be conducted with TFA-H, but with a TFA salt.

<b>New studies; not evaluated</b>	A new supporting studies. <i>Comparative in-vitro metabolism studies</i> ; <b>New EU registration requirement</b> [EC Regulation (EU) No 283/2013 of 1-March-2013]
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#### **Comparative in-vitro metabolism**

According to the data requirements published in the Commission Regulation (EU) No 283/2013 of 1-March-2013 a “comparative *in-vitro* metabolism study” should be performed “on animal species to be used in pivotal studies and on human materials (microsomes or intact cell systems) in order to determine the relevance of the toxicological animal data . ” However, no official test guideline of guidance exists at present. In these cases, waiving of this particular data requirement is considered acceptable according to the “Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of approval of the chemical active substance according to regulation (EU) No. 283/2013 and regulation (EU) No. 284/2013” (SANCO/10181/2013-rev.2 of 2-May-2013).

**The similarity of the metabolism of flufenacet in man and laboratory animal used to simulate human metabolism, i.e. the rat, was investigated by a first-tier approach using microsomes.**

<b>Report:</b>	<b>KCA 5.1.1/03, Solà, J., 2014</b>
<b>Title:</b>	[Thiadiazole-5- <sup>14</sup> C]Flufenacet: Metabolic stability of profiling in liver microsomes from rats and humans for inter-species comparison
<b>Document No:</b>	<a href="#">M-475336-01-1</a>
<b>Report No:</b>	S34338 of Harlan Laboratories, Barcelona, Spain, for Bayer CropScience, Germany
<b>Guidelines:</b>	No guideline available
<b>GLP</b>	yes

#### **Executive Summary**

The comparative metabolism of [Thiadiazole-5-<sup>14</sup>C]Flufenacet (<sup>14</sup>C-Flufenacet) was investigated in animal *in-vitro* systems by incubating the test substance with liver microsomes from male Wistar rats and humans in the presence of NADPH cofactor. The test substance concentration was 15 µM and the protein concentration 1 mg/mL. The temperature was 37°C and the incubation period 1 hour. The test duration of 1 hour was considered as reasonable because positive results were obtained from the enzymatic reaction of the reference substance testosterone to hydroxy-testosterone already after 10

minutes. Sampling of the test system was conducted at beginning and end of the incubation. Samples were radioassayed and analysed following protein precipitation by reversed phase radio-HPLC.

The recovery of radioactivity was measured in the microsome incubations at the end of incubation (1 hr) and amounted to > 100.5% of the applied radioactivity.

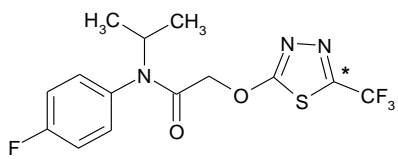
The metabolic activity of the microsomes was clearly demonstrated by determining 6 $\beta$ -hydroxy-testosterone formed from testosterone by testosterone 6 $\beta$ -hydroxylase (positive control). This biochemical reaction is well-known for a CYP3A microsomal enzyme.

<sup>14</sup>C-Flufenacet was found to be highly stable during *in-vitro* incubations with liver microsomes from either rats or humans. Three minor metabolites were detected after incubation at low amounts (> 4.5% of the applied radioactivity) with both, rat and human liver microsomes.

The conclusion of this *in-vitro* test with liver microsomes was that the metabolism of <sup>14</sup>C-Flufenacet is comparable in rat and human.

## Material and methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluoro-phenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5- <sup>14</sup> C]Flufenacet
Specific radioactivity	3.81 MBq/mg (103.04 mCi/g)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

### Test system

Microsomes	Pooled liver microsomes from male Wistar rats and humans
Further ingredients	Diluted aqueous solutions of sodium phosphate buffer (pH 7.4), magnesium chloride, and NADPH solution
Stop of the reaction	Addition of acetonitrile

### Preparation of the stock and working solutions

<sup>14</sup>C-Flufenacet was dissolved in acetonitrile (stock solution). The stock solution was diluted with different amounts of a mixture of 50 mM aqueous ammonium acetate (pH 5) and acetonitrile (9/1)

(working solution) to result in the test solution and a set of dilutions for establishment of the calibration curve for quantitative HPLC. The radioactive peak areas plotted against the injected radioactivity resulted in a linear calibration curve ( $r \geq 0.99$ ).

#### ***Sample preparation and incubation***

$^{14}\text{C}$ -Flufenacet solutions were incubated separately with rat and human liver microsomes. 50  $\mu\text{M}$  of flufenacet were mixed with 350  $\mu\text{L}$  of 100 mM sodium phosphate buffer (pH 7.4), 100  $\mu\text{L}$  of 100 mM magnesium dichloride and 25  $\mu\text{L}$  of rat liver microsomes (20 mg protein/mL) or 19.5  $\mu\text{L}$  of human liver microsomes (26 mg protein/mL). After 2-minutes pre-heating to 37°C 50  $\mu\text{L}$  of 6.4 mM NADPH ( $\pm 9\%$ ) were added to start the enzymatic reaction. The incubations were performed in a thermomixer device with shaking at 1000 rpm. The reaction was stopped after 1 hour by addition of 0.5 mL acetonitrile. The incubations with rat and human microsomes were conducted in triplicate.

For quality control, a stability test of  $^{14}\text{C}$ -flufenacet in sodium phosphate buffer (pH 7.4) without microsomes and positive control test with incubation the reference substance testosterone in the microsome system were conducted. The metabolic activity of the microsomes was thus determined by formation of 6 $\beta$ -hydroxytestosterone by the CYP3A microsomal system that is part of the liver microsomes. The quantitative determination of (non-labelled) 6 $\beta$ -hydroxytestosterone was performed via a calibration curve of the HPLC signal and the injected amount.

#### ***Sample processing***

After termination of the enzyme reaction the incubation mixture was centrifuged for 15 min at 16 000 g. The supernatant was removed and diluted with the starting mobile phase of radio-HPLC. These samples were directly chromatographed without further extraction.

#### ***Radioassaying and radio-chromatography***

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC). Prior to LSC of the incubated samples these samples were centrifuged. An aliquot of the supernatant was removed and radioassayed. The radioactive components after microsome incubation were separated by radio-HPLC equipped with a UV detector (235 nm) and a flow-through radiomonitor with an admixture cell and liquid scintillator. A RP18 column (150 x 4.6 mm, particle size 5  $\mu\text{m}$ ) was operated at 40 °C with a gradient mixture of 50 mM ammonium acetate solution (pH 5.0) and acetonitrile.

**Findings**Positive metabolism control

Formation of 6 $\beta$ -hydroxytestosterone from testosterone demonstrated sufficient metabolic capability of the microsome batches used in the study. Testosterone 6  $\beta$ -hydroxylase activity amounted to 1256.4 pmol/mg/minute (male rat liver microsomes) and 3102.8 pmol/mg/minute (pooled human liver microsomes).

Recovery of radioactivity

The mean recovery of radioactivity in the incubation mixtures was found to be 99.3% and 102.6% of the applied radioactivity in rat and human liver microsomes at the beginning and 100.5% in rat and 100.7% in human liver microsomes at the end of the incubation, respectively.

Metabolic profile after incubation with microsomes

The results of the tests demonstrated that <sup>14</sup>C-Flufenacet is highly metabolically stable due to *in-vitro* incubations with liver microsomes from either rats or humans, in which 94.4% and 95.5% of the initial <sup>14</sup>C-Flufenacet remained unchanged after 1-hour incubation, respectively.

The metabolism of flufenacet was very similar in the rat and human liver microsome system. Three minor metabolites were detected (Table 6.1.1- 5):

Flu-1: 4.5% of applied in the rat and 0.9% in the human system.

Flu-2: < LOQ in the rat and 1.7% in the human system

Flu-3: 1.0% in the rat and 2.0% in the human system.

Overall, the results of this comparative test suggest that phase-I metabolism is not significantly involved in the biotransformation of flufenacet in rat and human liver microsomes.

**Conclusion**

<sup>14</sup>C-labelled flufenacet was incubated in with rat and human liver microsomes for one hour at 37°C. This comparative *in-vitro* test suggested that flufenacet is highly metabolically stable with both rat and human liver microsomes. Three minor metabolites were formed similarly in both test systems not exceeding 4.5% of the applied radioactivity



**Table 6.1.1- 5: Metabolic profile of [thiadiazole-5-<sup>14</sup>C]flufenacet in rat and human liver microsomes**

Origin of the microsomes	Incubation period	Unchanged Flufenacet	Flu-1	Flu-2	Flu-3
	[min]	[% of applied <sup>14</sup> C-Flufenacet radioactivity]			
Rat	0	100	0	0	0
	60	94.4	4.5	< LOQ*	1.0
Human	0	100	0	0	0
	60	95.5	0.9	1.7	2.0
Buffer control	60	100	0	0	0

\* LOQ = 299 dpm corresponding to 0.001 µg flufenacet equivalents or 1.2% of applied

### B.6.1.2 Supplementary study in rats (bioavailability of metabolites)

This studies of FOE 5043 metabolites has been done for investigation of the bioavailability of selected plant metabolites in rats. The metabolite chosen to represent metabolites arising from the fluorophenylacetamide moiety was FOE 5043 oxalate. Thiadiazole-N-glucoside was chosen to represent the thiadiazole metabolites.

Previous evaluation	In DAR for original approval (1997);  Metabolism of FOE 5043 in soybeans studies were presented and evaluated during the EU process for Annex I listing.
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Report:	KIIIA, Krolski, ME., Bosnak, L.L., 1995
Title:	Metabolism of FOE 5043 in soybeans
Document No:	not available
Report No:	unpublished report no.: MR105187 of March 7, 1995(a)
Guidelines:	EPA 171-4(a) Nature of the Residue in Plants
GLP	yes

#### Material and methods:

[Fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate with a specific radioactivity of 50.2 mCi/mMole (purity 98%) as well as [thiadiazole-2-<sup>14</sup>C]-N-glucoside with a specific radioactivity of 1.39 mCi/mMole (purity 85%) was administered to 3 male rats at a single oral dose of 1 mg/kg bw.

#### Findings:

The average recovery was 80 % in the oxalate experiment and 92% in the N-glucoside experiment (see Table 6.1.2).

In the experiment with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate the majority of the radioactivity was excreted with faeces (70%), smaller amounts were found in the urine (28%). The tissues and carcass contained less than 1% of the total radioactivity with no single tissue having more than 0.02 mg/kg of residue. The only compound observed in urine and faeces was identified as the unchanged FOE 5043 oxalate. Although the majority of the residue was excreted with faeces, the material may have been absorbed; however, FOE 5043 oxalate clearly was not metabolized. The highest residue in any tissue was 0.02 mg/kg in liver, with all other tissues having radioactive residues <0.01 mg/kg. Using FOE 5043 oxalate as a typical residue in plants arising from the fluorophenyl portion of the active ingredient, there should be no detectable residues in animal tissues from the acetamide moiety of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues.

**Table 6.1.2: Excretion of total radioactivity and radioactive residues in the rat after oral application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate and [thiadiazole-2-<sup>14</sup>C]-N-glucoside (values are given in % of recovered radioactivity) [average values of 3 rats].**

Excretion of radioactive and radioactive residues	[fluorophenyl-UL- <sup>14</sup> C] FOE 5043 oxalate 1 mg/kg	[thiadiazole-2- <sup>14</sup> C]-N-glucoside 1 mg/kg
Urine	28	84
Faeces	70	12
Total excreted	98	96
Tissues/Carcass	<1	2
Cage Rinse	2	1
Total	100	100

In the experiment with [thiadiazole-2-<sup>14</sup>C]-N-glucoside the majority of the radioactivity was excreted with urine (84%) and a smaller amount with faeces (12%). The majority of the faecal radioactivity was excreted within 24 hours. The tissues and carcass contained only 2% of the total radioactive residue with no single tissue having > 0.01 mg/kg of residue. Analysis of urine and faeces samples identified 95% of the total radioactive residue as unmetabolized thiadiazole-N-glucoside. Since the majority of the residue was excreted in the urine, the material obviously was absorbed but not metabolized. As the only detectable residues in plants arising from the thiadiazole moiety of FOE 5043 are the thiadiazole-N-glucoside and similar conjugates, there should be no detectable residues in animal tissues from the thiadiazole portion of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues.

**B.6.1.2.1 Absorption, distribution, metabolism and excretion by other routes**

ADME studies using other than the oral intake route were not conducted and not deemed to be needed since a high degree of oral absorption was concluded from renal excretion of radiolabelled metabolites and the exhalation of radiolabelled carbon dioxide and methane following oral administration of fluorophenyl- and thiadiazole-2 and 5-<sup>14</sup>C labelled flufenacet.

## B.6.2 Acute toxicity

### Summary of acute toxicity studies

Flufenacet has a low to moderate order of acute toxicity by the oral route, and a low order of acute toxicity by the dermal and inhalation routes of exposure.

It is not irritating to the skin, and essentially non-irritating to the eyes. The results of the dermal sensitization studies revealed equivocal evidence of a sensitization potential. Both Maximization tests on guinea pigs were positive, the more practice relevant Buehler Patch Test on guinea pigs and the Local Lymph Node assay on mice were negative. Furthermore, flufenacet does not show a phototoxic potential.

**Table 6.2-1: Summary of acute toxicity studies\***

Route/Study	Species	Sex	Results	Reference
Oral	Mouse	M F	LD <sub>50</sub> : 1331 mg/kg bw 1756 mg/kg bw	██████████, 1991 <a href="#">M-004850-01-1</a>
Oral <sup>2)</sup>	Rat	M	LD <sub>50</sub> : 683 mg/kg bw	██████████, 1992a <a href="#">M-004864-01-1</a>
Oral <sup>1)</sup>	Rat	M F	LD <sub>50</sub> : 1617 mg/kg bw 589 mg/kg bw	██████████, 1993 <a href="#">M-004865-02-1</a>
Dermal	Rat	M F	LD <sub>50</sub> : >2000 mg/kg bw >2000 mg/kg bw	██████████, 1992 <a href="#">M-004843-01-1</a>
Inhalation (aerosol, 4h)	Rat	M F	LC <sub>50</sub> : >3740 mg/m <sup>3</sup> >3740 mg/m <sup>3</sup>	██████████, 1990 <a href="#">M-004844-01-1</a>
Skin irritation	Rabbit	M	Not irritating	██████████, 1992 <a href="#">M-004846-01-1</a>
Eye irritation	Rabbit	M	Not irritating	██████████, 1992 <a href="#">M-004847-01-1</a>
Skin sensitisation Buehler method	Guinea pig	M	Not sensitizing	██████████, 1992 <a href="#">M-004845-01-1</a>
Skin sensitisation M&K method	Guinea pig	M	Sensitizing	██████████, 1994 <a href="#">M-004637-01-1</a>
<b>Skin sensitisation M&amp;K method</b>	<b>Guinea pig</b>	<b>F</b>	<b>Sensitizing</b>	██████████, 1995 <a href="#">M-004677-01-1</a>
<b>Skin sensitization Local lymph node assay</b>	<b>Mouse</b>	<b>F</b>	<b>Not sensitizing</b>	██████████, 2004 <a href="#">M-090513-01-1</a>
<b><i>In vitro</i> 3T3 NRU phototoxicity test</b>	<b>BALB/c 3T3 cells</b>		<b>Not phototoxic</b>	Heppenheimer, 2013 <a href="#">M-464615-01-1</a>

\* New studies, i.e. studies previously not submitted, are written in bold [highlighted in green]

M = male, F = female; <sup>1)</sup> animals were fasted (overnight); <sup>2)</sup> animals were non-fasted

### Conclusion:

Based on the acute toxicity studies the following classification results for flufenacet:

Oral: Acute Tox 4, H302

Dermal: none

Inhalation: none

Skin & eye irritation: none

Skin sensitization: Skin Sens 1, H317

Phototox: none

**B.6.2.1 Oral toxicity**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Acute oral toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>6.2.1/01 [REDACTED] (1991)</b>
<b>Title:</b>	Experimental acute oral toxicity study with technical grade FOE 5043 in mice
<b>Document No:</b>	M-004850-01-1
<b>Report No:</b>	unpublished report no. 6013 of Sept. 25, 1991
<b>Guidelines:</b>	FIFRA § 81-1; TSCA 40 CFR Section 798. 1175; OECD guideline 401: MAFF 59 NohSan No. 4200; EU-guideline 84/449 B1
<b>GLP</b>	yes

**Material and methods:**

FOE 5043, batch number: NLL 3643-5, purity: 98.1%. formulated in aqueous Cremophor EL, 2% v/v; single oral administration to fasted mice (CD-I); application volume: 20 ml/kg

**Findings:**

Acute oral toxicity with fasted mice (see table below)

Dose; mg/kg	toxicological result*	onset of death after	duration of clinical signs	LD <sub>50</sub> (14 days) mg/kg bw
<b>Male</b>				
474	0/2/5	--	1d-4d	
663	0/1/5	--	1d-2d	
669	1/1/5	0d	1d-4d	
1388	2/3/5	1d	1d-8d	
2850	4/0/4	0d-1d	--	1331 (908-2611)
<b>Female</b>				
663	0/0/4	--	--	
669	0/0/5	--	--	
1032	2/1/5	1d	1d-2d	
1388	1/2/5	1d	1d-3d	
2850	4/2/5	1d	0d-2d	1756 (1218-4322)

\*1st figure - number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals used

**Clinical signs:**

decreased activity, increased reactivity, convulsions, unkempt, ungroomed, salivation, lacrimation, various stains about the head, forepaws and ventrum.

**Body weight:**

Not affected in animals surviving to day 14

**Gross necropsy:**

- animals which died: salivation, urine stain
- which were killed at termination: no treatment-related lesions

**Conclusion:**

FOE 5043 is of low acute oral toxicity;

Male LD<sub>50</sub> = 1331 mg/kg bw; Female LD<sub>50</sub> = 1756 mg/kg bw

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Acute oral toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>6.2.1/02 [REDACTED]; 1992a</b>
Title:	Acute oral toxicity study with FOE 5043 in nonfasted male rats,
Document No:	M-004864-01-1
Report No:	Miles Inc., unpublished report no. 6686 of April 23. 1992
Guidelines:	FIFRA § 81-1: TSCA 40 CFR Section 798.1175; OECD guideline 401; MAFF guideline 59 NohSan No. 4200; EU-guideline 84/449 B1
GLP	yes

**Material and methods:**

FOE 5043, batch number: NLL 3643-5, Purity: 98.0% - 98.1% formulated in aqueous Cremophor EL, 2%; single oral administration to rats (Sas:CD[SD]BR); application volume: 20 mL/kg bw

**Findings:**

Acute oral toxicity with nonfasted male rats

Dose; mg/kg	toxicological result*	onset of death after	duration of clinical signs	LD <sub>50</sub> (14 days) mg/kg bw.
312	0/4/5	--	1d-2d	
625	3/5/5	1d	0d-3d	
1250	4/1/5	0d-1d	0d-1d	
2500	5/0/5	0d-1d	--	683 (350 - 1150)

\*1st figure - number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals used

**Clinical signs:** decreased activity, clear lacrimation, red lacrimal stain, red nasal stain, salivation, urine stain, red anal discharge, perianal stain.

**Body weight:**

Not affected in animals surviving to day 14.

**Gross necropsy:**

- animals which died: salivation, lacrimation, ventral urine stain, nasal stain
- animals which were killed at termination: lacrimation, enlarged cervical lymphnodes

**Conclusion:**

FOE 5043 is of low acute oral toxicity; Male LD<sub>50</sub> = 589 mg/kg bw

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Acute oral toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>6.2.1/03 [REDACTED] (1993)</b>
Title:	Acute Oral Toxicity Study with Technical Grade FOE 5043 in Rats
Document No:	M-004865-02-1
Report No:	Bayer Corporation, unpublished report no. 6916 of Jan. 20, 1993
Guidelines:	FIFRA § 81-1; TSCA 40 CFR Section 798.1175; OECD guideline 401: MAFF guideline 59 NohSan No. 4200; EU-guideline 84/449 B 1
GLP	yes

**Material and methods:**

FOE 5043, batch number: NLL 3643-5 (for 1990 study). F1.036 (for 1992 study) purity: 98.0% - 99.0%,) formulated in aqueous Cremophor EL, 2% single oral administration to fasted rats (Sprague-Dawley) application volume: 20 mL/kg.

**Findings:**

Acute oral toxicity with fasted rats

Dose; mg/kg	toxicological result*	onset of death after	LD <sub>50</sub> (14 days) mg/kg bw
Males			
0	0/0/10	-	

46**	0/0/5	-	
138	0/3/5	-	
600	0/4/5	-	
1146	3/4/5	0-1 days	
4560	4/3/5	0-5 days	1617
Female			
0	0/0/5	-	
46**	0/0/5	-	
325	0/4/5	-	
514	2/4/5	1 day	
664	4/3/5	1 day	
1292	5/5/5	1 day	589

\*1st figure - number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals used

\*\* maximum dosage without clinical signs

#### **Clinical signs:**

salivation, lacrimation, head and ventrum stains

#### **Gross necropsy:**

no treatment-related findings

#### **Conclusion:**

FOE 5043 is of low to moderate acute toxicity;

Male LD<sub>50</sub> = 1617 mg/kg bw; Female LD<sub>50</sub> = 589 mg/kg bw



**B.6.2.2 Dermal toxicity**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Acute dermal toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	██████████; 1992b
<b>Title:</b>	Acute Dermal Toxicity Study with Technical Grade FOE 5043 in Rats
<b>Document No:</b>	M-004843-01-1
<b>Report No:</b>	Bayer Corporation, unpublished report no 5477 of March 31. 1992
<b>Guidelines:</b>	FIFRA § 81-2, November 1984; TSCA 40 CFR Section 798.1100; OECD guideline 402; MAFF guideline 59 NohSan No. 4200; EU-guideline 84/449 B.3
<b>GLP</b>	yes

**Material and methods:**

FOE 5043, Batch no.: NLL 3643-5, Purity: 98.1% - 98.0%, moistened with tap water; single dermal application to the shorn skin of Sprague-Dawley rats (16 cm<sup>2</sup>), covered with an occlusive patch for 24 hours.

**Findings:**

Acute dermal toxicity with rats (see Table below)

Dose mg/kg	toxicological result*	onset of death after	LD <sub>50</sub> (14days) mg/kg b.w.
Males			
2000	0/0/5	--	> 2000
Females			
2000	0/2/5	--	> 2000

\*1st figure - number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals used

**Clinical signs:**

Treatment-related clinical signs of toxicity were not observed in males. Females exhibited treatment-related urine stains that began on day 0 (day of treatment) and ended within day 6 of the post-treatment observation period. In the absence of mortality in either sex at the dermal limit dose (2000 mg/kg bw). additional dose levels were not tested and the LD<sub>50</sub> values were not determined.

**Gross necropsy:**

No treatment-related findings

**Conclusion:**

FOE 5043 shows a low dermal toxicity.

Male LD<sub>50</sub> > 2000 mg/kg bw; Female LD<sub>50</sub> > 2000 mg/kg bw

**B.6.2.3 Acute inhalation toxicity**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Acute inhalation toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	██████████; 1990
<b>Title:</b>	Acute Four-Hour Inhalation Toxicity Study with Technical Grade FOE 5043 in Rats
<b>Document No:</b>	M-004844-01-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 5362 of Oct. 26, 1990
<b>Guidelines:</b>	FIFRA § 81-3; TSCA 40 CFR Section 798.1150; OECD guideline 403; MAFF guideline 59 NohSan No. 4200; EU-guideline 84/449 B.2
<b>GLP</b>	yes

**Material and methods:**

FOE 5043, batch number: 17001/90. purity: 94.8%, mixed with acetone/polyethylene glycol 400 (50%/50, generated as liquid aerosol; single inhalation (nose-only inhalation) administration to Sprague-Dawley rats (Sas:CD|SD|BR) for 4 hours. The particle's mass median aerodynamic diameter (MMAD) and geometric standard deviations GSD were similar in each group. The mean achieved concentration were 3260 and 3740 mg/m<sup>3</sup>, with MMADs of 1.84 and 1.68 µm. The corresponding GSDs were 1.56 and 1.53, respectively. 3740 mg/m<sup>3</sup> was the maximum technically attainable concentration. The percentage of particles of 2 µm at 3260 and 3740 mg/m<sup>3</sup> was 56% and 64%, respectively.

**Findings:**

Acute inhalation toxicity with rats (exposure: 4 hours) (see table below)

concentration mg/m <sup>3</sup>	toxicological result*	onset of death after	LC <sub>50</sub> mg/m <sup>3</sup>
Male			
0 (air control)	0/4/6	--	
0 (vehicle control)	0/6/6	--	
3260	0/6/6	--	
3740	0/6/6	--	> 3740
Female			
0 (air control)	0/1/6	--	
0 (vehicle control)	0/6/6	--	
3260	0/6/6	--	
3740	0/6/6	--	> 3740

\*1st figure - number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals used

**Clinical signs:**

After a 4 hour exposure, clinical signs of toxicity were observed in both sexes at 3260 mg/m<sup>3</sup> air, the lowest concentration tested. These symptoms included ataxia tilted head, lacrimation, moribundity, nasal discharge, rales, urine stain and rough coat. Signs were first observed shortly after exposure (day 0) and a complete recover}-' for both sexes was observed by day 4 of the post-treatment observation period. No mortality occurred in either sex at the highest concentration tested, therefore, the LC<sub>50</sub> is demonstrated to be >3740 mg/m<sup>3</sup> air.

#### Body weights:

groups exposed to test material: no significant decrements from rates of body weight gains of vehicle-exposed groups.

#### Gross necropsy:

no lesions considered to be related to exposure to the test material.

#### Conclusion:

FOE 5043 shows a low inhalation toxicity.

Male LD<sub>50</sub> > 3740 mg/m<sup>3</sup>; Female LD<sub>50</sub> > 3740 mg/m<sup>3</sup>

#### B.6.2.4 Skin Irritation

Previous evaluation	In DAR for original approval (1997);  Skin irritation toxicity studies were presented and evaluated during the EU process for Annex I listing.
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Report:	██████████;1992a
Title:	Primary dermal Irritation Stud with Technical Grade FOE 5043 in Rabbits
Document No:	M-004846-01-1
Report No:	Bayer Corporation, unpublished report no. 6350 of Jan 23, 1992
Guidelines:	FIFRA § 81-5; TSCA 40 CFR Section 798.4470; OECD guideline 404; MAFF guideline 59 NohSan No. 4200; EU-guideline 84/449 B.4
GLP	yes

#### Material and methods:

FOE 5043, batch number: 17001/90, purity: 92.6% - 93.8% moistened with tap water; single dermal application to New Zealand White Rabbits for 4 hours, dosage: 0.5 grams.

**Findings:**

Skin irritation in rabbits (contact time: 4 hours)

Animal No.	Local Finding	Toxicological Result	primary irritation score			
			1h	24h	48h	72h
004	ER	0	0	0	0	0
	ED	0	0	0	0	
005	ER	0	0	0	0	0
	ED	0	0	0	0	
006	ER	0	0	0	0	0
	ED	0	0	0	0	
007	ER	0	0	0	0	0
	ED	0	0	0	0	
009	ER	0	0	0	0	0
	ED	0	0	0	0	
010	ER	0	0	0	0	0
	ED	0	0	0	0	

ER = erythema; ED - edema

**Conclusion:**

FOE 5043 is not irritating to the skin.

**B.6.2.5 Eye Irritation**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Eye irritation toxicity studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>██████████; 1992b</b>
<b>Title:</b>	Primary Eye Irritation Study with Technical Grade FOE 5043 in Rabbits
<b>Document No:</b>	M-004847-01-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 6349 of Jan. 27, 1992
<b>Guidelines:</b>	FIFRA § 81-4; TSCA 40 CFR Section 798.4500; OECD guideline 405. MAFF guideline 59 NohSan No. 4200; EU-Guideline 84/449 B 5
<b>GLP</b>	yes

**Material and methods:**

FOE 5043, batch number: 17001/90, purity: 92.6% - 93.K%; single administration into the conjunctival sac of left eye of New Zealand White Rabbits; dosage: 54 mg (0.1 mL), for 7 days; scoring according to Draize.

**Findings:** Eye irritation in rabbits (contact time: 72 hours or 7 days)

<b>Animal Number</b>	<b>examined organs</b>	<b>Toxicological Results of hours/days post-exposure</b>					<b>Reaction animal</b>
		<b>1h</b>	<b>24h</b>	<b>48h</b>	<b>72h</b>	<b>7d</b>	
008	Cornea	0	0	0	0		none
	Iris	0	0	0	0		
	Conj. R	1	1	0	0		
	Conj. C	0	0	0	0		
	Conj. D	0	0	0	0		
011	Cornea	0	0	0	0	0	none
	Iris	0	0	0	0	0	
	Conj. R	1	1	1	1	0	
	Conj. C	1	1	0	0	0	
	Conj. D	1	0	0	0	0	
013	Cornea	0	0	0	0	0	none
	Iris	0	0	0	0	0	
	Conj. R	1	1	1	1	0	
	Conj. C	1	1	0	0	0	
	Conj. D	1	0	0	0	0	
014	Cornea	0	0	0	0		none
	Iris	0	0	0	0		
	Conj. R	1	1	1	0		

	Conj. C	1	1	0	0		
	Conj. D	0	0	0	0		
015	Cornea	0	0	0	0		none
	Iris	0	0	0	0		
	Conj. R	1	1	1	0		
	Conj. C	1	1	0	0		
	Conj. D	0	0	0	0		
016	Cornea	0	0	0	0		none
	Iris	0	0	0	0		
	Conj. R	1	1	1	0		
	Conj. C	1	1	0	0		
	Conj. D	3	0	0	0		

Conj. = Conjunctiva; R = redness; C = chemosis; D = discharge

#### Conclusion:

The test-article was essentially non-irritating to the eye

#### B.6.2.6 Skin Sensitization

Previous evaluation	<b>In DAR for original approval (1997);</b>  <b>Skin Sensitization studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report 01:</b>	<b>[REDACTED]; 1992c</b>
Title:	Dermal Sensitization Study with Technical Grade FOE 5043 in Guinea Pigs,
Document No:	M-004845-01-1
Report No:	Bayer Corporation, unpublished report no. 3991 of March 21, 1992
Guidelines:	FIFRA § 81-6; TSCA 40 CFR Section 798.4100; OECD guideline 406; MAFF guideline 59 NohSan No. 4200; EU guideline 84/449
GLP	yes

#### Material and methods:

FOE 5043, batch number: 17001/90, purity: 92.6% - 93.8% moistened with deionized water; dosage (a.i.): 0.4 grams; administration: 6-hour induction exposures on study day 0, 7 and 14; a 24-hour challenge application on study day 27 to Hartley Guinea Pigs using the Buehler topical closed-patch technique. The Buehler test used was conducted using 3 induction treatments.

**Findings:** Sensitization in guinea pigs

	Erythema scores			
	Induction			Challenge
Treatment	1 st	2 nd	4 rd	24 & 48 hr
Test	0/0*	0/0	0/0	0/0
Control	--	--	0/0	

\* incidence/severity

**Clinical signs:** None observed: 0% of the animals were sensitized.**Conclusion:**

The test-article does not cause a dermal sensitization reaction in Buehler 3 test (without adjuvant).

<b>Report 02:</b>	██████████ (1994)
Title:	FOE 5043 - Study for the skin sensitising effect in guinea pigs (Maximization Test of Magnusson and Kligman).
Document No:	M-004637-01-1
Report No:	Bayer AG. unpublished report no. 23560 of Dec. 16, 1994
Guidelines:	OECD Guideline no. 406; EU guideline 92/69 B.6.; FIFRA § 81-6
GLP	yes

**Material and methods:**

FOE 5043, batch no.:898313105, purity: 96.8%, formulated in 0.9% NaCl solution/Cremophor EL (2% w/v); intracutaneous and dermal administration to guinea pigs (Hsd Win:DH); dosage (a.i.): 5% (intradermal application). 50% (topical application, 1 week after intradermal injection), 50%, 25% (1st challenge, dermal application, 3 weeks after intradermal induction) application volume: 0.1 ml/injection (intradermal application). 0.5 ml/application (dermal application). Flufenacet concentrations were 5% for intradermal induction and 50% for topical induction.

**Findings:**

Skin reddening/challenge (concentration 50%)				Skin reddening/challenge (concentration 25%)			
Test substance patch		Control patch		Test substance patch		Control patch	
48h*	72h*	48h*	72h*	48h*	72h*	48h*	72h*
1st control group				1st control group			
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
Test substance group				Test substance group			
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1	1	0	0	1	0	0	0
0	0	0	0	1	0	0	0
2	2	0	0	1	1	0	0
1	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0
0	2+	0	0	0	1+	0	0
1	1	0	0	1	0	0	0
1	1	0	0	1	1+	0	0
0	0	0	0	0	0	0	0
0	1	0	0	0	1	0	0
1	1	0	0	1	1	0	0
1	1	0	0	1	1	0	0
0	0	0	0	0	0	0	0
1	1	0	0	1	0	0	0
1	1+	0	0	1	1	0	0
1	1	0	0	1	1	0	0
2	2	0	0	1	1	0	0

\* findings made 48h and 72h after start of exposure

+ treatment areas squamous in places

Number of animals exhibiting skin reddening (48 and 72 hours after initiation of challenge)

	Test substance group (20 animals)					1st control group (10 animals)			
	Test substance patch			Control patch		Test substance patch		Control patch	
Time	48h	72h	total	48h	72h	48h	72h	48h	72h
Challenge									
50%	11	12	13	0	0	0	0	0	0
25%	12	9	14	0	0	0	0	0	0



**Conclusion:**

FOE 5043 has a definite skin sensitizing potential under the conditions of the Maximization Test.

<b>New studies; not evaluated</b>	<p>This study was not presented at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. (2010).</p> <p><b>Confirmation of end point/study result.</b></p> <p>This test (Maximization test Magnusson and Kligman) was conducted using a different (pure) flufenacet batch (920902ELB01; 99.5%; see Table 6-1: Overview of flufenacet batches used for toxicity studies; page 6).</p>
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<b>Report 03:</b>	<b>██████████; 1995</b>
Title:	FOE 5043 - Study for the skin sensitization effect in guinea pigs (Maximization test Magnusson and Kligman)
Document No:	M-004677-01-1
Report No:	23924
Guidelines:	OECD 406; EC Guideline 92/69, Method B.6.; US-EPA-FIFRA §81-; Deviations: none
GLP	yes

**Materials and methods:****A. Materials****1. Test materials:**

Name:	FOE 5043
Description:	white powder
Lot/Batch no:	920902ELB01
Purity:	99.5% (w/w)
Stability of test compound:	guaranteed for study duration; expiry date: 1995-04-30

<b>2. Vehicle:</b>	physiological saline solution containing 2% v/v Cremophor EL®
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**3. Test animals:**

Species:	Guinea pig
Strain:	Hsd Win:HD
Age:	5 – 7 weeks
Weight at dosing:	279 – 374 g
Source:	██ ██
Acclimatisation period:	At least seven days
Diet:	"Altromin®3020 - Maintenance Diet for Guinea Pigs" (Altromin GmbH, Lage, Germany), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	conventionally in type IV Makrolon® cages; adaptation: in groups of five; study period: in groups of two or three per cage; Bedding: low-dust wood shavings (Ssniff Spezialdiaeten GmbH,

Soest, Germany)

**Results and discussion:**

Appearance and behaviour of the test substance group were not different from the control group with the following exceptions:

After the 2<sup>nd</sup> induction, on day 9 four animals showed encrustations on the treatment areas, on day 10 eight animals showed encrustations on the treatment areas. The encrustations had healed by day 14 in five animals, by day 15 in one animal, by day 16 in two animals, by day 17 in two animals, by day 20 in two animals.

After the 1<sup>st</sup> challenge, 6 (30%) of the test substance animals responded with "slight localized" to "moderate confluent" redness to the 50% test substance formulation, 7 (35%) of the test substance animals responded with "slight localized" to "severe" redness to the 25% test substance formulation. There were no skin reactions in the control group.

After the 2<sup>nd</sup> challenge, 5 (25%) and 6 (30%) of the test substance animals responded with "slight localized" to "moderate confluent" redness to the 12% and 6% test substance formulation, respectively. There were no skin reactions in the control group.

No mortalities occurred. The body weight development of the treatment group animals corresponded to that of the first control group.

**Table 6.2.6: Number of animals exhibiting skin effects**

	Test item group (20 animals)					Control group (10 animals)				
	Test item patch			Control patch		Test item patch			Control patch	
Hours	48	72	Total	48	72	48	72	Total	48	72
1 <sup>st</sup> Challenge										
50%	6	5	6	0	0	0	0	0	0	0
25%	6	6	7	0	0	0	0	0	0	0
2 <sup>nd</sup> Challenge										
12%	5	3	5	0	0	0	0	0	0	0
6%	6	4	6	0	0	0	0	0	0	0

**Conclusions**

After the 1<sup>st</sup> challenge the 50% and 25% test substance formulations led to skin redness in 30% and 35% of the test animals, respectively. There were no skin reactions in the control group.

After the 2<sup>nd</sup> challenge the 12% and 6% test substance formulations led to skin redness in 25% and 30% of the test animals.

**Flufenacet exhibits a skin-sensitization potential under the conditions of the maximization test**

<b>New studies; not evaluated</b>	<p>This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. (2010) <b>Confirmation of end point/study result</b></p> <p><b>Local Lymph Node Assay was conducted according to the new testing guideline.</b></p>
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<b>Report 04:</b>	<b>[REDACTED]; 2004</b>
Title:	FOE 5043 - Local lymph node assay in mice (LLNA/IMDS)
Document No:	M-090513-01-1
Report No:	AT01491
Guidelines:	OECD 406 and 429 Guidelines, 96/54/EC, Method B.6; US-EPA 712-C-03-197, OPPTS 870.2600; Deviations: none
GLP	yes

## Materials and methods:

### A. Materials

#### 1. Test materials:

Name:	FOE 5043
Description:	Beige-brown solid
Lot/Batch no:	EDHB001715
Purity:	97.5% (w/w)
Stability of test compound:	guaranteed for study duration; expiry date: 2004-12-22

#### 2. Vehicle:

Acetone/olive oil, 4:1

#### 3. Test animals:

Species:	NMRI mouse
Strain:	Hsd Win:NMRI
Age:	10 – 11 weeks
Weight at dosing:	26 – 33 g
Source:	[REDACTED]
Acclimatisation period:	At least seven days
Diet:	"PROVIMI KLIBA SA 3883 maintenance diet for rats and mice (Provimi Kliba SA, Kaiseraugst, Switzerland), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Adaptation: conventional in Makrolon type III cages, up to 8 mice per cage; study period: in type II cages, one animal per cage; bedding: low-dust wood granulate ( J. Rettenmaier & Soehne Fuellstoff-Fabriken, Rosenberg, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose	0%-2%-10%-50%
Application route:	Epicutaneously onto the dorsal part of both ears
Application volume:	25 µL/ear
Duration:	Three consecutive days
Group size:	6 females/group

## Observations:

Local lymph node weight, cell count determination, ear swelling, ear weight, body weight (at beginning and termination of study)

**Results and discussion:**

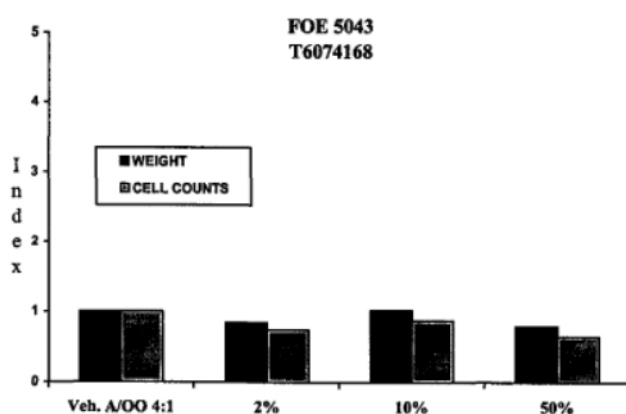
The body weights of the animals were not affected by any treatment.

Slight significant decreases compared to vehicle treated animals regarding cell counts and ear weight were detected in the highest dose group. The "positive level" of ear swelling which is  $2 \times 10^{-2}$  mm increase, i.e. about 10% of the control values, has also not been exceeded in any dose group. No substance specific effects were determined for ear weights, too.

Overall the NMRI mice did not show an increase in the stimulation indices for cell counts or for weights of the draining lymph nodes after application of the test item flufenacet. The "positive level" which is 1.35 for the cell count index was never reached or exceeded in any dose group.

The study indicates that the LLNA/IMDS does neither point to a non-specific (irritating) nor to a specific immuno-stimulating (sensitizing) potential of the test item.

**Figure 6.2.6: Bar charts (weight and cell count) for the LLNA**

**Conclusions:**

No activation of the cells of the immune system via dermal route was determined after application of up to and including 50% flufenacet. Therefore, the concentration of 50% turned out to be the NOEL for the parameters investigated in this study.

**Flufenacet shows neither an irritating, nor a sensitizing potential in mice after dermal application.**

**B.6.2.7 Phototoxicity**

New studies; not evaluated	<p><b>New EU registration requirement</b> [EC Regulation (EU) No 283/2013 of 1-March-2013]</p> <p>According to the new data requirements (COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013) (1), the conduct of a phototoxicity study is required under certain conditions.</p> <p>The Circumstances in which a phototoxicity study, according to the new data requirements is required is “<i>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 <math>10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}</math>, no toxicity testing is required.</i>”</p> <p>As the Ultraviolet/visible molar extinction/absorption coefficient of the active substance exceeds the trigger of <math>10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}</math> a cytotoxicity assay in vitro with BALB/c 3T3 cells has been performed.</p>
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<b>Report:</b>	<b>Heppenheimer, A.; 2013</b>
Title:	Flufenacet TC: Cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight
Document No:	M-464615-03-1
Report No:	1561200
Guidelines:	Commission Regulation (EC) No. 440/2008, B41; Committee for Proprietary Medicinal Products (CPMP) CPMP/SWP/398/01; OECD 432; Deviations: none
GLP	yes

**Materials and methods:****A. Materials****1. Test materials:**

Name:	Flufenacet TC
Synonyms:	FOE 5043, AE F133402
Description:	Light beige powder
Lot/Batch no:	NK61CK0650
Purity:	98.18% (w/w)
Stability of test compound:	guaranteed for study duration; expiry date: 2014-07-16

**2. Vehicle and or positive control:**

Solvent control: Earle's Balanced Salt Solution (EBSS) containing 1% (v/v) dimethylsulfoxide (DMSO).  
Positive control: chlorpromazine (Sigma) dissolved in EBSS

**3. Test system:**

Culture medium: Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% (v/v) NCS.

Cell cultures: BALB/c 3T3 cell clone 31 (supplied by [REDACTED]).

Large stocks (Master Cell Stock) of the BALB/c 3T3 31 cell line are stored in liquid nitrogen in the cell bank of Harlan CCR. A working cell stock is produced by multiplying from the master cell stock. Thawed stock cultures were propagated at  $37 \pm 1.5$  °C in 75 cm<sup>2</sup> plastic flasks. Seeding was done with about  $1 \times 10^6$  cells per flask in 15 mL DMEM, supplemented with 10% NCS. Cells were sub-cultured twice weekly. The cell cultures were incubated at  $37 \pm 1.5$  °C in a  $7.5 \pm 0.5\%$  carbon dioxide atmosphere.

**B. Study design and methods****1. Treatment**

Dose:

Test item	+/- UV	Final concentrations in µg/mL
Flufenacet	+/-	1.95, 3.91, 7.81, 15.63, 62.50, 125.0, 250.0
Positive control	+	6.25, 12.5, 25, 37.5, 50, 75, 100, 200
	-	0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0
Solvent control	+/-	EBSS containing 1 % (v/v) DMSO

The test item flufenacet was dissolved in DMSO. The final concentration of the solvent in EBSS was 1% (v/v).

Solar simulator:

Irradiation was performed with a Dr. Hönle Sol 500 solar simulator. The filter H1 was used to keep the UVB irradiation as low as possible. The produced wavelength of the solar simulator with the filter was  $> 320$  nm. Due to the inhomogeneous distribution of irradiation intensity the UVA intensity was measured at the complete area with a UV-meter. The homogeneous area was marked and the cultures were irradiated in this area. The solar simulator was switched on about 30 minutes prior to the start of experiment. The absorption spectrum of the test item was determined in the range from 270-800 nm. The test item showed absorption below 330 nm.

Seeding of cultures:  $2 \times 10^4$  cells per well were seeded in 100 µL culture medium in two 96 well plates

Replicates: 2 (one for exposure to irradiation, one for treatment in the dark)

Treatment & irradiation: 24 h after seeding the cultures were washed with EBSS. 100 µL of solved test item were added/well and the plates were pre-incubated for 1 hour in the dark. Afterwards one plate was irradiated at  $2.55 - 2.7$  mW/cm<sup>2</sup> ( $7.65 - 8.1$  J/cm<sup>2</sup>) for  $50 \text{ min} \pm 2 \text{ min}$  at  $20-30$  °C, the other plate was stored for  $50 \text{ min} \pm 2 \text{ min}$  at  $20-30$  °C in the dark. Test item was removed and both plates were washed with EBSS. Fresh culture medium was added and the plates were incubated about 21.5 hours at  $37 \pm 1.5$  °C and  $7.5 \pm 0.5\%$  CO<sub>2</sub>.

Cytotoxicity determination: For measurement of Neutral Red uptake the medium was removed and 0.1 mL serum-free medium containing 50 µg Neutral Red / mL were added to each well. The plates were

incubated for another 3 hours at 37 °C, before the medium was removed completely and the cells were washed with EBSS. For extraction of the dye 0.15 mL of a solution of 49% (v/v) deionized water, 50% (v/v) ethanol and 1% (v/v) acetic acid were added to each well. After approximately 10 minutes at room temperature and a brief agitation, the plates were transferred to a microplate reader (Versamax®, Molecular Devices) equipped with a 540 nm filter to determine the absorbance of the extracted dye. This absorbance showed a linear relationship with the number of surviving cells.

Number of measurements: Flufenacet and positive control: 6 times per concentration  
Solvent control: 12 times

## 2. Evaluation

The mean absorption ( $OD_{540}$ ) value per concentration was calculated. The  $ED_{50}$  values were determined by curve fitting by software. The Photo-irritancy factor (PIF), as well as the Mean Phototoxic effect (MPE) was calculated according to OECD guideline 432.

Evaluation criteria:

PIF <2 or MPE <0.1	→ no phototoxic potential
PIF >2 and <5 or	
MPE >0.1 and <0.15	→ probable phototoxic potential
PIF >5 or MPE >0.15	→ phototoxic potential

## Results and discussion:

In the range finding experiment (RFE) no cytotoxic effects were observed after exposure of the cells to the test item flufenacet, neither in the presence nor in the absence of irradiation to artificial sunlight. Therefore,  $ED_{50}$ -values and PIF could not be calculated. The resulting MPE-value was 0.054.

In the main experiment (ME) the highest test item concentration of 250 µg/mL caused a cytotoxic effect in the presence and absence of light. The cell viabilities decreased below the threshold for cytotoxicity of 70% (11.60% and 27.72%). The calculated PIF- and MPE-values were 1.08 and 0.040, respectively.

In the confirmatory experiment (CE) the cytotoxic effect at the highest concentration was confirmed. The cell viability was not reduced below 50% without irradiation. Thus an  $ED_{50}$ -, as well as the PIF-value could not be determined. The MPE was -0.032.

MPE-values in all experiments were <0.1. In the main experiment where a PIF-value could be calculated, the PIF was <2. Thus, flufenacet does not possess any phototoxic potential.

The mean of solvent control values of the irradiated versus the non-irradiated group met the acceptance criteria. The positive control chlorpromazine induced phototoxicity in the expected range in the presence of irradiation.

The results are summarised in the tables below.

**Table 6.2.7/01: OD<sub>540</sub> values Neutral Red assay of the main experiment**

Con- centration [µg/mL]	OD <sub>540</sub> with artificial sunlight			Con- centration [µg/mL]	OD <sub>540</sub> without artificial sunlight		
	Mean	SD	% of solvent control		Mean	SD	% of solvent control
Treatment with flufenacet							
Solvent control	0.8965*	0.0913	100.00	Solvent control	0.8988*	0.1105	100.00
1.95	0.9317	0.0712	103.93	1.95	0.8871	0.0889	98.70
3.91	0.9391	0.0996	104.75	3.91	0.8987	0.0896	99.98
7.81	0.9556	0.0992	106.59	7.81	0.9101	0.0893	101.25
15.63	0.9719	0.0612	108.41	15.63	0.8897	0.0729	98.98
31.25	0.9637	0.0738	107.50	31.25	0.8761	0.0706	97.47
62.50	0.9037	0.0809	100.81	62.50	0.8671	0.0816	96.47
125.0	0.8373	0.0855	93.39	125.0	0.8035	0.0470	89.39
250.0	0.1040	0.0216	11.60	250.0	0.2491	0.0246	27.72
Treatment with positive control chlorpromazine							
Solvent control	0.7585*	0.0337	100.00	Solvent control	0.8954*	0.1066	100.00
0.125	0.6415	0.0260	84.57	6.25	0.9295	0.0879	103.80
0.250	0.3976	0.0362	52.41	12.50	0.5612	0.1113	62.67
0.500	0.0748	0.0138	9.87	25.00	0.0677	0.0058	7.56
0.750	0.0671	0.0064	8.85	37.50	0.0507	0.0022	5.66
1.000	0.0660	0.0013	8.70	50.00	0.0492	0.0037	5.49
1.500	0.0705	0.0079	9.29	75.00	0.0507	0.0025	5.66
2.000	0.0726	0.0213	9.57	100.00	0.0483	0.0016	5.40
4.000	0.0725	0.0094	9.56	200.00	0.0493	0.0016	5.50

\* mean OD<sub>540</sub> out of 12 wells



**Table 6.2.7/02: OD<sub>540</sub> values Neutral Red assay of the confirmatory experiment**

Con- centration [µg/mL]	OD <sub>540</sub> with artificial sunlight			Con- centration [µg/mL]	OD <sub>540</sub> without artificial sunlight		
	Mean	SD	% of solvent control		Mean	SD	% of solvent control
Treatment with flufenacet							
Solvent control	0.6524*	0.0527	100.00	Solvent control	0.7331*	0.0500	100.00
1.95	0.6786	0.0545	104.01	1.95	0.7310	0.0394	99.70
3.91	0.6947	0.0706	106.48	3.91	0.7518	0.0238	102.55
7.81	0.7241	0.0627	110.99	7.81	0.7561	0.0259	103.14
15.63	0.7191	0.0390	110.23	15.63	0.7559	0.0336	103.10
31.25	0.7162	0.0434	109.78	31.25	0.7924	0.0153	108.08
62.50	0.7008	0.0526	107.42	62.50	0.7474	0.0301	101.94
125.0	0.6430	0.0272	98.56	125.0	0.7503	0.0205	102.35
250.0	0.1082	0.0125	16.59	250.0	0.4207	0.0210	57.39
Treatment with positive control chlorpromazine							
Solvent control	0.7221*	0.0461	100.00	Solvent control	0.6932*	0.0297	100.00
0.125	0.6192	0.0437	85.75	6.25	0.6504	0.0322	93.82
0.250	0.0684	0.0055	9.47	12.50	0.5628	0.0328	81.19
0.500	0.0894	0.0194	12.38	25.00	0.1239	0.0430	17.87
0.750	0.0778	0.0142	10.77	37.50	0.0512	0.0023	7.38
1.000	0.0812	0.0172	11.25	50.00	0.0492	0.015	7.09
1.500	0.0749	0.016	10.37	75.00	0.0540	0.0087	7.78
2.000	0.0653	0.0052	9.04	100.00	0.0493	0.0022	7.11
4.000	0.0770	0.0237	10.66	200.00	0.0521	0.0055	7.51

\* mean OD<sub>540</sub> out of 12 wells**Table 6.2.7/03: Summary of results of the Neutral Red assay**

	Substance	ED <sub>50</sub> (+UV) [µg/mL]	ED <sub>50</sub> (-UV) [µg/mL]	PIF	MPE	% viability of solvent control of irradiated vs. non-irradiated plate
Range finding experiment	Flufenacet	--	--	--	0.054	93.4
	Positive control	0.46	11.33	24.92	0.594	108.0
Main experiment	Flufenacet	175.7	188.0	1.08	-0.040	99.7
	Positive control	0.25	13.38	54.40	0.740	84.7
Confirmatory experiment	Flufenacet	181.5	--	--	-0.032	89.0
	Positive control	0.16	17.85	110.09	0.690	104.2

**Conclusions:**Based on the study results flufenacet **does not possess** a phototoxic potential.

### B.6.3 Short-term toxicity

#### Summary of short-term toxicity studies

Short term oral toxicity of flufenacet was investigated in the rat (90-day toxicity study), in the mouse (90-day toxicity study) and in the dog (90-day and 1-year toxicity studies). In all three species, the main target organs were liver, thyroid, kidney, the hematopoietic and nervous systems indicated by changes in clinical chemistry, organ weights and/or histopathological findings. The comparative species differences in toxicological profile, find the rat and the mice similar in primary and secondary target organs, but a sensitivity of certain cell types was observed in the dog as evidenced by histopathological lesions of vacuoles in the brain after 90-day exposure. After 1-year exposure of flufenacet to dogs minimal to moderate vacuolization of the ciliary body epithelium and cystic vacuolization of the peripheral optic retina was observed and a minimal to moderate axonopathy was noted in the brain, spinal cord and sciatic nerve of dogs. Specialized testing such as computerized electrocardiograms, clinical neurological examinations, and quantitative electroencephalography revealed a number of compound-related effects.

Alterations in circulating serum thyroid hormones thyroxine (T4) and triiodothyronine (T3) were observed in each species and were considered indicative of hepatic interference. Primary haematological parameters affected by treatment in each species included changes in erythrocytes, platelets, haemoglobin, and haematocrit concentrations. Histopathological findings generally correlated with alterations in organ weights.

**A decrease in body weight gain was observed at higher dose levels only in the 90-day rat study at 191/127 mg/kg bw/day in males/females. There were no meaningful body weight changes in mice and dogs. However, decreased terminal body weights were noted in the 1-year dog study at 62/27 mg/kg bw/day in males/females.**

In a subacute dermal toxicity study in rats, findings included a decrease in thyroxine (T4) and free thyroxine (FT4) levels, an increase in liver weights, and histopathological findings of the liver. A high-dose recovery group treated similarly with flufenacet demonstrated a complete recovery from all responses to treatment by two weeks after the final application.

**The liver was also the primary target organ after subacute (5x 6hours and 20x 6hours) inhalation exposure with secondary effects on the thyroid hormone levels. Increased liver weights with correlating clinical- and histopathological findings were observed. The inhalation toxicity studies revealed also alterations in the nasal cavity and larynx, in kidney-, hematologic/spleen-, and thyroid-related endpoints.**

**Table 6.3-1: Summary of short-term toxicity studies**

Study	Sex	NO(A)EL mg/kg bw/day	LO(A)EL mg/kg bw/day	Main findings seen at LO(A)EL	Reference
Rat 21-day dermal	M F	1000 1000	-- --	No adverse effects noted. T4 ↓, liver findings considered adaptive response to treatment.	██████████, 1995 M-004981-01-1
Rat 1-week inhalation (see page	M, F	~14 48 mg/m <sup>3</sup>	~66 225 mg/m <sup>3</sup>	T4 ↓ Liver: rel. weight ↑	██████████, 2008 <a href="#">M-300005-01-1</a>
Rat 4-week inhalation (see page:	M, F	~7 19 mg/m <sup>3</sup>	~81 220 mg/m <sup>3</sup>	HB ↓, HCT ↓, RETI ↑, HEINZ ↑, AP ↓, TG ↓, Liver: enzymes ↑, rel. weight ↑, spleen: weight ↑, histopathological changes in nasal cavity and larynx, spleen, testes, thyroid, liver	██████████, 2008 <a href="#">M-302961-01-1</a>
Rat 90-day feeding	M F	-- <sup>a)</sup> 7.2	6.0 29	HB ↓, T4 ↓, GLUC ↓, Liver: weight ↑, hepatocellular swelling, cell degeneration or necrosis; spleen: brown granular pigment accumulation within red pulp; kidney: mild renal proximal tubule injury	██████████, 1995 <a href="#">M-004999-01-1</a>
Mouse 90-day feeding	M F	18 25	64 91	T4 ↓ Liver: rel. weight ↑	██████████, 1995 <a href="#">M-004985-01-1</a>
Dog 90-day feeding	M F	1.7 1.7	7.2 6.9	ALAT ↓, LDH ↑, albumin ↓, globulin ↑, T4 ↓, GLUC ↓, Spleen: pigment, kidney: rel. weight ↑	██████████, 1995 <a href="#">M-004977-02-1</a>
Dog 1-year feeding	M F	1.3 1.1	28 27	Hb ↓, Hct ↓, MCV ↓, MCH ↓, MCHC ↓, CHOL ↑, GLUC ↓, T4/T3 ↓, ALAT ↓, AP ↑, albumin ↓, Liver, heart, kidney: abs. + rel. weight ↑ BW gain ↓, Haematology effect: alterations and compound related changes in hematologic endpoints, Neurology effects: abnormal behavior, postural abnormalities, optic nystagmus/strabismus/ placement Necropsy: ↑ increased relative heart weights; ↑ increased relative kidney weights ↑ increased relative and absolute liver weights ↑ increased relative adrenal weights ↑ increased relative thyroid compound-related changes were limited to the 800 and/or 1600 ppm dose levels in both males and females	██████████, 1995, 1997 <a href="#">M-005001-02-2</a>

<sup>a)</sup> The subchronic NOEL for males was established on the basis of the toxicity profile which emerged through the first year of the 2-year rat study.

M = male, F = female, ↑ = increase, ↓ = decrease

### B.6.3.1 Oral 28-day study

The 28-day toxicity studies were performed as range-finding studies cited in the 90-day studies which were evaluated during the EU process for Annex I listing. See point 6.3.2

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

<b>Previous evaluation</b>	<b>In DAR for original approval (1997)</b>
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**B.6.3.2.1 Rat**

<b>Report:</b>	<b>[REDACTED]; 1995b</b>
Title:	Technical grade FOE 5043: A subchronic toxicity testing study in the rat.
Document No:	M-004999-01-1
Report No:	Bayer Corporation, unpublished report no. 7733
Guidelines:	FIFRA § 82-1; TSCA guideline no. 798.2650; OECD guideline no. 408; MAFF guideline 59 NoSan no. 4200; EU-guideline 87/302
GLP	yes

**Material and methods:**

FOE 5043, batch number: 17001/90, purity: 92.6% - 94.8%. corn oil at 1% by weight of the diet; acetone/corn oil mixture *was* used to dissolve the test article prior to mixing with the dietary carrier. The control diet was prepared the same way, excluding only the test substance. Administration: Oral by feeding for approximately 13 weeks to Fischer 344 rats (CDF[F-344]/BR)

dosage (a.i.): 0 - 100 - 400 - 1600 - 3000 ppm.  
corresponding to: 0, 6.0, 24.3, 109.1, or 191.2 mg/kg bw/day in males,  
0, 7.2, 28.8, 127.2. or 224.5 mg/kg bw/day in females

**Clinical signs:**

With the exception of an increased frequency of food spillage noted in 1600 and 3000 ppm males and females, no other differences with respect to physical appearance, activity, behavior, condition of coat, appetite or thirst between dosed and control animals were seen.

**Body weight:**

Body weight gain (BWG) was evaluated for each chemically-treated group in terms of a grand or overall mean of weekly averages which was then compared to the grand mean of the untreated control group. Based on this criteria, BWG remained unaffected in 100 and 400 ppm males and females and 1600-ppm males. A 6% decline in BWG was noted in 1600 ppm females while 5 and 8% declines in BWG, respectively, were measured in 3000 ppm males and females.

**Table B.3.2-1 Summary of body weight**

Dose [ppm]	Mean body weights in g									
	Males					Females				
	0	100	400	1600	3000	0	100	400	1600	3000
BW Day 0	186.6	184.4	188.8	190.3	193.9	127.6	125.1	126.2	126.8	128.3
BW Day 7	200.7	203.3	206.7	203.3	199.9	131.2	131.4	130.2	129	128.7
BW Day 14	215.5	217.3	220.1	217.3	209.1	136.3	137.9	136.9	135.3	131.4
BW Day 21	227.5	232.8	234.3	229.9	221.5	144.1	142.9	141.4	139.8	136*
BW Day 28	239.9	246.2	244.1	230.1	238.8	148.2	148	145.3	140.5	140.7
BW Day 35	253.6	258.9	257.6	244.8	245.1	152.5	152.7	158.8	143.7*	139.7*
BW Day 42	264	270.5	267.3	253.3	254.4	156.8	155.6	151.6	145.1*	145*
BW Day 49	274.5	279	277.4	261.8	257.1	160.8	159.6	155.3	147.2*	144.2*
BW Day 56	281	286.8	282.8	267.3	264.7	163.8	162	157.1	149.7*	147.4*
BW Day 63	288.6	294.3	291.4	273.9	267.6*	167.1	165	161.2	152.1*	147.1*
BW Day 70	296.1	301.9	297	279.8	275.8*	171	168.8	162.7	154.3*	151*
BW Day 75/76/77	304.7	304.6	309.3	291.7	279	172.6	167.9	167.3	158.1	153.1
BW Day 84	293.3	295.4	294.5	272.8*	256.1*	167.4	161.9	159.4	147.7*	136.3*

BW = body weight

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

**Table B.3.2-2 Summary of body weights of male and female rats (% of control)**

Dose [ppm]	Males				Females			
	100	400	1600	3000	100	400	1600	3000
BW Day 0	99%	101%	102%	104%	98%	99%	99%	101%
BW Day 7	101%	103%	101%	100%	100%	99%	98%	98%
BW Day 14	101%	102%	101%	97%	101%	100%	99%	96%
BW Day 21	102%	103%	101%	97%	99%	98%	97%	94%*
BW Day 28	103%	102%	96%	100%	100%	98%	95%	95%
BW Day 35	102%	102%	97%	97%	100%	104%	94%*	92%*
BW Day 42	102%	101%	96%	96%	99%	97%	93%*	92%*
BW Day 49	102%	101%	95%	94%	99%	97%	92%*	90%*
BW Day 56	102%	101%	95%	94%	99%	96%	91%*	90%*
BW Day 63	102%	101%	95%	93%*	99%	96%	91%*	88%*
BW Day 70	102%	100%	94%	93%*	99%	95%	90%*	88%*
BW Day 75/76/77	100%	102%	96%	92%	97%	97%	92%	89%
BW Day 84	101%	100%	93%*	87%*	97%	95%	88%*	81%*

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

**Table B.3.2-3 Summary of body weight gain (BWG) in g**

	Males					Females				
Dose (ppm)	0	100	400	1600	3000	0	100	400	1600	3000
BWG D0-7	14.1	18.9	17.9	13	6	3.6	6.3	4	2.2	0.4
BWG D7-14	14.8	14	13.4	14	9.2	5.1	6.5	6.7	6.3	2.7
BWG D14-21	12	15.5	14.2	12.6	12.4	7.8	5	4.5	4.5	4.6
BWG D21-28	12.4	13.4	9.8	0.2	17.3	4.1	5.1	3.9	0.7	4.7
BWG D28-35	13.7	12.7	13.5	14.7	6.3	4.3	4.7	13.5	3.2	-1
BWG D35-42	10.4	11.6	9.7	8.5	9.3	4.3	2.9	-7.2	1.4	5.3
BWG D42-49	10.5	8.5	10.1	8.5	2.7	4	4	3.7	2.1	-0.8
BWG D 49-56	6.5	7.8	5.4	5.5	7.6	3	2.4	1.8	2.5	3.2
BWG D 56-63	7.6	7.5	8.6	6.6	2.9	3.3	3	4.1	2.4	-0.3
BWG D63-70	7.5	7.6	5.6	5.9	8.2	3.9	3.8	1.5	2.2	3.9
BWG D70-75/76/77	8.6	2.7	12.3	11.9	3.2	1.6	-0.9	4.6	3.8	2.1
BWG D75/76/77-84	-11.4	-9.2	-14.8	-18.9	-22.9	-5.2	-6	-7.9	-10.4	-16.8

**Food consumption:**

Food consumption, assessed in terms of both g consumed/animal/day and g consumed/kg body wt/day, remained unaffected in 100- and 400-ppm males and females. Alterations in food efficiency were suggested at higher doses: however, an increased incidence of spillage was also observed.

**Table B.3.2-4 Summary of food consumption**

	Mean food consumption (g/animal/day)									
	Males					Females				
Dose (ppm)	0	100	400	1600	3000	0	100	400	1600	3000
Food cons. Day 7	15.6	15.91	16.04	16.14	15.78	11.35	11.48	10.99	10.68	11.58
Food cons. Day 14	15.99	15.99	15.89	15.91	15.92	11.31	11.52	11.38	11.35	11.45
Food cons. Day 21	15.37	15.58	15.78	15.86	15.37	11.54	11.18	11.17	11.87	11.64
Food cons. Day 28	15.68	16.32	15.52	16.62	15.62	11.54	11.43	10.88	12.04	10.91
Food cons. Day 35	15.4	15.83	15.85	15.89	16.4	11.35	11.2	10.88	10.58	10.97
Food cons. Day 42	15.39	15.72	15.96	15.72	15.58	11.24	11.03	10.81	10.58	10.9
Food cons. Day 49	16.03	16.42	15.74	15.53	15.86	11.35	11.25	10.55	10.03* (-12%)	10.33
Food cons. Day 56	15.77	15.99	15.95	16.33	16.49	11.4	11.06	10.94	10.81	10.5
Food cons. Day 63	15.49	15.82	15.88	15.94	16.6	11.15	11.13	11.16	10.77	10.72
Food cons. Day 70	16.19	16.58	16.15	15.98	16.94	11.83	11.67	11.09	10.85	11
Food cons. Day 77	15.76	15.77	15.5	15.23	16.66	11.08	10.81	10.25* (-7%)	10.2	10.94
Food cons. Day 84	13.82	14.16	14.48	14.65	13.67	9.94	9.71	9.74	9.6	8.74*

										(-12%)
--	--	--	--	--	--	--	--	--	--	--------

\*  $p \leq 0.05$  significant by Anova + Dunnetts tests (two-sided)

() = percent change from controls

### Ophthalmology:

All ophthalmologic findings were considered incidental and not related to exposure to FOE 5043.

### Hematology:

Hematological changes in erythrocyte-related parameters included: 1) an increase in reticulocyte number in both sexes at 1600 ppm; 2) a decrease in erythrocyte count (-3%) and hematocrit (-4%) for males at 400 ppm and for both sexes at 1600 ppm and greater; and 3) a decrease in hemoglobin for males at 100 ppm (-3%) and for both sexes at 400 ppm and greater. Increases in platelet count were noted for both sexes at 3000ppm. Leukocyte count was increased for males at 400 ppm and greater and females at 3000 ppm.

**Table B.3.2-5 Summary of haematological examinations**

	Male					Female				
	0	100	400	1600	3000	0	100	400	1600	3000
Hb [g/dL]	17.5	16.9*	16.8*	16.2*	15.8*	17.3	16.9	16.7*	15.5*	14.9*
% Δ	--	-3	-4	-7	-10	--	-2	-3	-10	-14
RETI [%]	1.4	1.4	1.4	2.4*	2.8*	1.7	1.8	1.7	3.0*	2.7*
% Δ	--	0	0	+71	+100	--	+6	0	+76	+59
RETI # [abs, calculated]	0.1376	0.1351	0.1331	0.2167	0.2422	0.1528	0.1595	0.1474	0.2409	0.2071
Hematocrit [%]	48	47.2	46.3*	45.2*	44.4*	47.1	46.7	45.9	43*	41.5*
% Δ	--	-2	-4	-6	-7	--	-1	-3	-9	-12
RBC [ $10^6/\text{mm}^3$ ]	9.83	9.65	9.51*	9.03*	8.65*	8.99	8.86	8.67*	8.03*	7.67*
% Δ	--	-2	-3	-8	-12	--	-1	-4	-11	-15
Platelets [ $10^3/\text{mm}^3$ ]	819	789	817	915*	937*	847	841	801	923	960*
% Δ	--	-4	0	+12	+14	--	-1	-5	+9	+13
MCV [ $\mu\text{m}^3$ ]	48.9	48.9	48.7	50.1*	51.3*	52.4	52.7	52.9*	53.5*	54.1*
% Δ	--	0	0	+2	+5	--	+1	+1	+2	+3
MCHC [g/dL]	36.4	35.8*	36.2	35.8*	35.5*	36.7	36.2	36.4	36.1*	35.8*
% Δ	--	-2	-1	-2	-2	--	-1	-1	-2	-2
MCH [pg]	17.8	17.5*	17.6	17.9	18.2*	52.4	52.7	52.9*	53.5*	54.1
% Δ	--	-2	-1	+1	+2	--	+1	+1	+2	+3
LEU	7.9	8.7	9*	9.8*	9.9*	6.4	6.7	6.4	6.9	7.3
% Δ	--	+10	+14	+24	+25	--	+5	0	+8	+14
Heinz bodies	0	0	0	0	0	0	0	0	0	0

\*  $p \leq 0.05$  significant by Anova + Dunnetts tests (two-sided) and Kruskal-Wallis + Mann Whitney-U tests

% Δ = percent change from control

# Values for absolute reticulocytes were calculated

**Table B.3.2-6 Overview of historical control data for selected hematological parameters**

Parameter	Males			Females		
	Range Min - Max	Mean range	No	Range Min - Max	Mean range	No
Hemoglobin (g/dL)	12.92 – 18.8	15.75 – 16.37	707	12.8 – 18.6	15.2 – 16.37	647
Hematocrit (%PCV)	35.96 – 51.0	43.48 – 47.7	726	36.12 – 51.4	43.39 – 47.7	666
RBC [ $10^6/\text{mm}^3$ ]	7.58 – 10.56	8.53 – 9.87	726	6.44 – 9.71	8.07 – 9.04	666
PLT [ $10^3/\text{mm}^3$ ]	625 - 1089	749 - 909	726	440 - 1088	755 - 911	666

**Clinical chemistry:**

Clinical chemistry parameters exhibiting changes following exposure to FOE 5043 included:

- 1) increased potassium for females at 3000 ppm;
- 2) decreased glucose for females at 400 ppm and greater and for males at 3000 ppm;  
for females at 400 ppm and greater and for males at 3000 ppm;
- 3) increased uric acid for females at 400 ppm and greater and for males at 1600 ppm and greater;
- 4) increased phosphate for males at 3000 ppm;
- 5) increased cholesterol for both sexes at 1600 ppm and greater;
- 6) increased protein, albumin and globulin for males at 3000 ppm;
- 7) decreased triglyceride for males at 400 ppm and greater;
- 8) increased  $\gamma$ -glutamyltransferase for females at 1600 ppm and greater;
- 9) decreased thyroxine for males at 100 ppm and greater and for females at 400 ppm and greater;
- 10) decreased triiodothyronine for males at 400 ppm and greater, and increased triiodothyronine for females at 3000 ppm.

**Table B.3.2-7 Summary of clinical chemistry – selected parameters**

Parameter	Dose group (ppm) males					Dose group (ppm) females				
	0	100	400	1600	3000	0	100	400	1600	3000
means (unit)										
K (mmol/L)	5.0	5.1	5.2	5.2	5.2	5.2	5.6*	5.7*	5.8*	6.0*
K (% $\Delta$ )	--	+2	+4	+4	+4	--	+8	+10	+12	+15
Gluc (mg/dL)	105	103	106	100	85*	99	94	88*	86*	81*
Gluc (% $\Delta$ )	--	-2	+1	-5	-19	--	-5	-11	-13	-18
Uric acid (mg/dL)	0.6	0.7	0.7 <sup>s</sup>	0.8 <sup>s</sup>	0.9 <sup>s</sup>	0.9	1.0	1.2*	1.2	1.2*
Uric acid (% $\Delta$ )	--	+17	+17	+33	+50	--	+11	+33	+33	+33
Trig (mg/dL)	146	147	123*	45*	45*	36	54*	62*	45*	45*
Trig (% $\Delta$ )	--	+1	-14	-69	-69	--	+50	+72	+25	+25
Chol (mg/dL)	55	60	57	66*	92*	80	90*	94*	114*	130*
(% $\Delta$ )	--	+9	+4	+20	+67	--	+13	+18	+43	+63
GGT (U/L)	1	1	2*	3*	2	2	1	2	4*	5*



Parameter	Dose group (ppm) males					Dose group (ppm) females				
	0	100	400	1600	3000	0	100	400	1600	3000
means (unit)										
GGT (% Δ)	--	0	+100	+200	+100	--	-50	0	+100	+150
T-Prot (g/dL)	7.4	7.4	7.5	8.0*	8.4*	7.2	7.0	7.1	7.5*	7.5
T-prot (% Δ)	--	0	+1	+8	+14	--	-3	-1	+4	+4
Alb (g/dL)	3.5	3.5	3.6	3.7*	3.7*	3.6	3.4*	3.4*	3.5	3.4
(% Δ)	--	0	+3	+6	+6	--	-6	-6	-3	-6
Glob (g/dL)	3.9	3.8	3.9	4.3*	4.6*	3.6	3.6	3.6	4.0*	4.0*
Glob (% Δ)	--	-3	0	+10	+18	--	0	0	+11	+11
Phos (mg/dL)	8.1	8.7*	8.5	8.7*	9.2*	8.5	8.5	9.2	8.9	8.8
Phos (% Δ)	--	+7	+5	+7	+14	--	0	+8	+5	+4
T4 (µg/dL)	6.3	5.4*	4.2*	3.5*	2.4*	3.9	3.4	3.2*	2.6*	3.2*
T4 (% Δ)	--	-14	-33	-44	-62	--	-13	-18	-33	-18
T3 (ng/mL)	1.2	1.1	0.9*	0.8*	0.8*	0.9	0.9	0.9	0.9	1.1*
T3 (% Δ)	--	-8	-25	-33	-33	--	0	0	0	+22

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided) and Kruskal-Wallis + Mann Whitney-U tests

\$ p ≤ 0.05 significant by Kruskal-Wallis + Mann Whitney-U tests

% Δ = percent change from control

**Table B.3.2-8 Overview of historical control data for T3 and T4**

Parameter	Males			Females		
	Range Min - Max	Mean range	No	Range Min - Max	Mean range	No
T3 [ng/mL]	0.5 – 1.54	1.02 – 1.06	63	0.78 – 1.44	1.1	20
T4 [µg/dL]	2.19 – 10.76	5.55 – 6.48	93	2.2 – 6.90	4.38 – 4.55	50

The decrease of haemoglobin in males at 100 ppm is only □3% when compared to the concurrent control value. It is also within the historical control range of 12.92-18.8 g/dL of the testing facility. In addition, comparison of the control value of this study with the historical control range demonstrates that the control value in this study of 17.5 g/dL is at the upper limit of the HCD-range. This would explain the observed statistical differences already observed at the low dose. Overall, the slight decrease of haemoglobin of -3% observed in low dose males is considered to be within the normal variation and thus not considered an adverse effect.

The decreased T4 value observed in low dose males is only 14% lower when compared to the control. Thyroid hormone changes below 20% are considered to be a normal background variation and thus not adverse. In addition, the observed mean T4 value in low dose males of 5.4 µg/dL are within the HCD range of the testing facility (2.19 – 10.76 µg/dL). In addition, there are no correlated histopathological findings in the thyroids in this dose group, as well as other dose groups in males and females. In males relative thyroid weights were significantly increased at 1600 ppm and above, while liver weights were significantly increased at 400 ppm and above.

Overall, the observed changes of haemoglobin and T4 in low dose males are not considered to be adverse findings.

Thus, the NOAEL of this study should be 100 ppm for males and females, corresponding to 6.0 and 7.2 mg/kg bw/day, respectively.

Table 6.3.2.1-1 Clinical chemistry summary data - males

RAT SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

Species : Rat

All subsets

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## MEAN Serum Chemistry

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M A L E S		Na mmol/L	K mmol/L	Cl mmol/L	UN mg/dL	Gluc mg/dL	Creat mg/dL	Uric-A <sup>§</sup> mg/dL	Trig <sup>§</sup> mg/dL	Chol mg/dL
Control	Mean	147	5.0	100	17	105	0.7	0.6	146	55
	SD	2	0.3	1	2	10	0.1	0.2	25	6
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	145	5.1	99	18	103	0.8	0.7	147	60
	SD	2	0.4	1	2	7	0.1	0.2	31	6
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	144*	5.2	98*	18	106	0.7	0.7 <sup>§</sup>	123*	57
	SD	1	0.3	1	1	8	0.1	0.1	28	8
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	143*	5.2	98*	18	100	0.7	0.8 <sup>§</sup>	45*	66*
	SD	2	0.3	1	3	9	0.1	0.2	12	8
	n	15	15	15	15	15	15	15	15	15
3000 ppm	Mean	145*	5.2	98*	17	85*	0.7	0.9 <sup>§</sup>	45*	92*
	SD	1	0.4	1	2	9	0.1	0.4	9	12
	n	15	15	15	15	15	15	15	15	15

URIC ACID, TRIGLYCERIDE, LD WERE HETEROGENEOUS BY BARTLETT'S TEST; NONPARAMETRIC ANALYSIS

Statistics: Anova + Dunnetts tests (two-sided): \* P&lt;0.05

(Exp.Unit = Animal)

§ = Nonhomogeneous by Bartlett's test; Kruskal-Wallis + Mann Whitney-U tests performed:

\*# = P&gt;0.05, not significant by Kruskal-Wallis + Mann Whitney-U tests

§ = P&lt;0.05, significant by Kruskal-Wallis + Mann Whitney-U tests

\* = P&lt;0.05, significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann Whitney-U) tests

Blank = P&gt;0.05, not significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann Whitney-U) tests

Table 6.3.2.1-2 Clinical chemistry summary data - males

## RAT SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

Species : Rat

All subsets

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## MEAN Serum Chemistry

Nominal days in study 84

M A L E S		CK U/L	LD <sup>§</sup> U/L	AST U/L	ALT U/L	GGT U/L	ALP U/L	T-Bili mg/dL	T-Prot g/dL	Alb g/dL
Control	Mean	157	186	83	49	1	139	0.1	7.4	3.5
	SD	104	133	9	6	1	13	0.1	0.2	0.1
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	168	281	80	49	1	133	0.2*	7.4	3.5
	SD	94	232	10	5	1	11	0.1	0.2	0.1
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	120	113	69*	40*	2*	125*	0.1*	7.5	3.6
	SD	34	43	4	3	1	8	0.1	0.3	0.1
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	124	170	67*	38*	3*	127*	0.2*	8.0*	3.7*
	SD	61	116	7	3	2	7	0.0	0.3	0.1
	n	15	15	15	15	15	15	15	15	15
3000 ppm	Mean	154	219	63*	41*	2	116*	0.1	8.4*	3.7*
	SD	80	256	7	3	1	5	0.1	0.3	0.1
	n	15	15	15	15	15	15	15	15	15

URIC ACID, TRIGLYCERIDE, LD WERE HETEROGENEOUS BY BARTLETT'S TEST; NONPARAMETRIC ANALYSIS

Statistics: Anova + Dunnetts tests (two-sided): \* P&lt;0.05

(Exp.Unit = Animal)

§ = Nonhomogeneous by Bartlett's test; Kruskal-Wallis + Mann Whitney-U tests performed:

\*# = P&gt;0.05, not significant by Kruskal-Wallis + Mann Whitney-U tests

\$ = P&lt;0.05, significant by Kruskal-Wallis + Mann Whitney-U tests

\* = P&lt;0.05, significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann Whitney-U) tests

Blank = P&gt;0.05, not significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann Whitney-U) tests

Table 6.3.2.1-3 Clinical chemistry summary data - males

RAT SUBCHRONIC DIETARY STUDY WITH FOR 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

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Species: Rat

Sex : Male All sub-sets

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Mean Serum Chemistry

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Nominal days in study 84

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M A L E S		Glob g/dL	Phos mg/dL	Calc mg/dL	T4 ug/dL	T3 ng/mL
Control	Mean	3.9	8.1	11.8	6.3	1.2
	SD	0.2	0.4	0.2	0.8	0.2
	n	15	15	15	15	15
100 ppm	Mean	3.8	8.7*	11.7	5.4*	1.1
	SD	0.2	0.5	0.2	0.6	0.2
	n	15	15	15	15	15
400 ppm	Mean	3.9	8.5	11.5*	4.2*	0.9*
	SD	0.2	0.4	0.2	0.7	0.3
	n	15	15	15	15	15
1600 ppm	Mean	4.3*	8.7*	11.4*	3.5*	0.8*
	SD	0.2	0.6	0.2	0.5	0.3
	n	15	15	15	15	15
3000 ppm	Mean	4.6*	9.2*	12.0	2.4*	0.8*
	SD	0.3	0.9	0.3	0.7	0.4
	n	15	15	15	15	15

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Statistics: Anova + Dunnetts tests (two-sided): \* P&lt;=5%

(Exp.Unit = Animal)

Table 6.3.2.1-4 Clinical chemistry summary data - females

RAT SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

Species : Rat

All subsets

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## MEAN Serum Chemistry

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F E M A L E S		Na mmol/L	K mmol/L	Cl mmol/L	UN mg/dL	Gluc mg/dL	Creat mg/dL	Uric-A mg/dL	Trig mg/dL	Chol mg/dL
Control	Mean	145	5.2	103	21	99	0.8	0.9	36	80
	SD	3	0.4	1	2	6	0.1	0.3	6	9
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	146	5.6*	103	18*	94	0.7	1.0	54*	90*
	SD	2	0.4	1	2	9	0.1	0.2	8	7
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	145	5.7*	101*	19*	88*	0.8	1.2*	62*	94*
	SD	2	0.4	2	2	10	0.1	0.3	10	8
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	146	5.8*	101*	19*	86*	0.7	1.2	45*	114*
	SD	3	0.3	2	3	11	0.1	0.4	8	13
	n	15	15	15	15	15	15	15	15	15
3000 ppm	Mean	145	6.0*	101*	19*	81*	0.7	1.2*	45*	130*
	SD	2	0.5	2	2	7	0.1	0.5	10	17
	n	15	15	15	15	15	15	15	15	15

Statistics: Anova + Dunnetts tests (two-sided): \* P&lt;0.05

(Exp.Unit = Animal)

Table 6.3.2.1-5 Clinical chemistry summary data - females

## RAT SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

Species : Rat

All subsets

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## MEAN Serum Chemistry

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F E M A L E S		CK <sup>§</sup> U/L	LD U/L	AST U/L	ALT <sup>§</sup> U/L	GGT U/L	ALP U/L	T-Bili mg/dL	T-Prot g/dL	Alb g/dL
Control	Mean	85	230	88	44	2	119	0.0	7.2	3.6
	SD	24	124	16	11	1	10	0.0	0.3	0.1
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	121	239	80	40	1	100*	0.2*	7.0	3.4*
	SD	117	118	16	6	1	13	0.1	0.3	0.2
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	127	286	84	39	2	100*	0.1*	7.1	3.4*
	SD	92	124	7	4	1	13	0.1	0.3	0.1
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	236* <sup>§</sup>	317	77	36*	4*	100*	0.2*	7.5*	3.5
	SD	275	261	13	4	2	11	0.1	0.4	0.2
	n	15	15	15	15	15	15	15	15	15
3000 ppm	Mean	106	293	76	37*	5*	106*	0.1	7.5	3.4
	SD	44	113	13	5	2	18	0.1	0.3	0.1
	n	15	15	15	15	15	15	15	15	15

CK AND ALT WERE HETEROGENEOUS BY BARTLETT'S TEST; NONPARAMETRIC ANALYSIS

Statistics: Anova + Dunnetts tests (two-sided): \* P&lt;0.05

(Exp.Unit = Animal)

§ = Non-homogeneous by Bartlett's Test; Kruskal-Wallis + Mann-Whitney U Tests performed:

\*# = P&gt;0.05, not significant by Kruskal-Wallis + Mann-Whitney U Tests

\$ = P&lt;0.05, significant by Kruskal-Wallis + Mann-Whitney U Tests

\* = P&lt;0.05, significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann-Whitney U) tests

Blank = P&gt;0.05, not significant by (Anova + Dunnetts) and (Kruskal-Wallis + Mann-Whitney U) tests

Table 6.3.2.1-6 Clinical chemistry summary data - females

RAT SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-172-GC

Species: Rat

Sex : Female All sub-sets

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Mean Serum Chemistry

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Nominal days in study 84

## F E M A L E S

		Glob g/dL	Phos mg/dL	Calc mg/dL	T4 ug/dL	T3 ng/mL
Control	Mean	3.6	8.5	11.6	3.9	0.9
	SD	0.3	0.8	0.4	0.8	0.1
	n	15	15	15	15	15
100 ppm	Mean	3.6	8.5	11.6	3.4	0.9
	SD	0.2	1.2	0.4	0.6	0.2
	n	15	15	15	15	15
400 ppm	Mean	3.6	9.2	11.4	3.2*	0.9
	SD	0.2	0.9	0.2	0.6	0.2
	n	15	15	15	15	15
1600 ppm	Mean	4.0*	8.9	11.3	2.6*	0.9
	SD	0.3	1.4	0.3	0.8	0.2
	n	15	15	15	15	15
3000 ppm	Mean	4.0*	8.8	11.6	3.2*	1.1*
	SD	0.2	1.4	0.4	0.6	0.3
	n	15	15	15	15	15

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Statistics: Anova + Dunnetts tests (two-sided): \* P<=5%

(Exp.Unit = Animal)



**Urinalysis:**

Not indicated subchronic oral rodent study; 90-day urine collection and analysis was performed in combined chronic toxicity/oncogenicity study with the test material

**Gross necropsy:**

Gross pathological evidence of toxicity was not observed in this study.

**Organ weights:**

Organ weight changes included increased liver and thyroid weights for both sexes at 400 ppm and greater, and increased spleen weights for males at 3000 ppm.

**Table B.3.2-9 Summary organ weight - males**

	<b>Males</b>				
<b>Dose [ppm]</b>	<b>0</b>	<b>100</b>	<b>400</b>	<b>1600</b>	<b>3000</b>
terminal BW [g]	307.4	304	305	284.3* –8%	266.5* –13%
Liver weight (rel.) [%]	4.04	4.125 (+2%)	4.355* (+8%)	5.112* (+27%)	5.606* (+39%)
Thyroid weight (abs.) [g]	0.0190	0.0190	0.0210	0.02100	0.0220*
Thyroid weight (rel.) [%]	0.0063	0.0063	0.0067 (+6%)	0.0074* +17%	0.0084* (+33%)
Kidney weight (rel.) [%]	0.819	0.791* (–3%)	0.79* (–4%)	0.785* (–4%)	0.804
Spleen weight (rel.) [%]	0.224	0.239	0.223	0.251* +12%	0.303* (+35%)
Adrenal weight (rel.) [%]	0.015	0.015	0.016	0.016	0.018*
Testes weight (rel.) [%]	0.946	0.948	0.957	1.008* (+7%)	1.076* (+14%)

**Table B.3.2-10 Summary organ weight - females**

	<b>Females</b>				
<b>Dose [ppm]</b>	<b>0</b>	<b>100</b>	<b>400</b>	<b>1600</b>	<b>3000</b>
terminal BW [g]	169.8	168.6	161.5	153.6* (–10%)	142* (–16%)
Liver weight (rel.) [%]	3.7	3.834	4.066	4.845* (+31%)	5.158* (+39%)
Thyroid weight (abs.) [g]	0.01400	0.01500	0.01500	0.01400	0.0150
Thyroid weight (rel.) [%]	0.00820	0.00870	0.00910	0.00910	0.0105* (+28%)
Kidney weight (rel.) [%]	0.833	0.85	0.824	0.817	0.825
Spleen weight (rel.) [%]	0.302	0.307	0.311	0.299	0.343 (+14%*)
Adrenal weight (rel.) [%]	0.032	0.032	0.034	0.034	0.038*
Ovary weight (rel.) [%]	0.054	0.062	0.053	0.055	0.051

**Histopathology:**

Histopathological findings were observed for both sexes at 400 ppm and greater. These included increased incidences of: 1) hepatocellular swelling characterized by both cytomegaly and karyomegaly; 2) individual liver cell degeneration or necrosis; 3) brown granular pigment accumulation within the red pulp of the spleen; and 4) mild renal proximal tubule injury.

**Table B.3.2-11 Summary histopathology**

	Males					Females				
	0	100	400	1600	3000	0	100	400	1600	3000
Eyes										
Cataract	0	0	0	0	0	0	0	1	0	0
Liver										
hepatocytomegaly	0	0	15* (1.7)	15* (2.3)	15* (3.0)	0	0	15* (1.0)	15* (3.1)	15* (3.1)
necrosis, individual cell	0	0	1 (1.0)	12* (1.0)	15* (1.0)	0	0	7* (1.0)	13* (1.2)	15* (1.6)
Thyroid	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
thyroid foll.-cell hypertrophy	0	0	0	0	0	0	0	0	0	0
Parathyroids	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Pituitary	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Kidney										
degeneration, hyaline	0	0	5* (1.0)	15* (1.7)	15* (1.8)	0	0	1 (1.0)	0	0
hyperplasia of epithelial cells	0	0	2 (1.0)	11* (1.4)	10* (1.5)	0	2 (1.0)	2 (1.0)	4 (1.5)	3 (1.0)
pigmentation	0	0	0	0	0	0	0	0	12* (1.1)	15* (1.7)
Spleen										
pigment accumulation	0	1	13* (1.0)	15* (1.3)	15* (2.3)	4 (1.5)	6 (1.3)	12* (1.8)	15* (3.1)	15* (3.0)
Hematopoiesis, extramedullary	1 (2.0)	1 (4.0)	0	0	2 (1.5)	1 (2.0)	2 (1.0)	1 (1.0)	2 (1.0)	0
Adrenal	NE	NE	NE	NE	NE					
Pancreas	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Thymus	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Testes	NE	NE	NE	NE	NE					
Seminal vesicle	NE	NE	NE	NE	NE					
Epididymides	NE	NE	NE	NE	NE					
Prostate	NE	NE	NE	NE	NE					
Ovary						NE	NE	NE	NE	NE
Uterus						NE	NE	NE	NE	NE
Mammary gland						NE	NE	NE	NE	NE
Urinary bladder	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE

NE; no treatment-related effects

() = average severity of animals with lesion: 1 (minimal) to 5 (severe)

<b>Conclusion</b>	<b>The study is in line with current guidelines.</b> NOAEL of this study should be 100 ppm for males and females, corresponding to 6.0 and 7.2 mg/kg bw/day, respectively.
-------------------	---

<b>Previous evaluation</b>	<b>In DAR for original approval (1997)</b>
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### B.6.3.2.2 Mouse

<b>Report:</b>	██████████ ; 1995a
<b>Title:</b>	Technical grade FOE 5043: a 13-week range-finding toxicity study in the mouse
<b>Document No:</b>	M-004985-01-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7720 of July 17, 1995
<b>Guidelines:</b>	FIFRA § 82-1; TSCA 798.2650; OECD guideline no. 408; MAFF guideline 59 NoSan no. 4200; EU-guideline 87/302
<b>GLP</b>	yes

#### Study summary:

The principal objective of this 90-day range-finding toxicity bioassay was to establish, under conditions of prolonged and repeated exposure, a definitive subchronic toxicological profile for the FOE 5043-exposed mouse in order to predict appropriate doses at which to conduct a subsequent oncogenicity testing study in the same animal. For the duration of this subchronic study, the technical grade of FOE 5043 (FOE 5043) was administered to the CD-I mouse (15 animals/dose/sex) at constant nominal dietary concentrations of 0, 100, 400, 1600, or 4000 ppm relative to the analytically determined percentage of purity of the chemical. Both the control diet and the chemically-treated test diets were available for ad libitum consumption at all times. Determination of body weight and food consumption as well as a detailed clinical examination of each animal were conducted weekly. Standard hematologic and selected clinical chemistry endpoints were evaluated on blood collected just prior to termination of the in-life segment of the study. All animals placed on study were subject to a postmortem examination, which included (1) documenting and saving all gross lesions, (2) weighing designated organs, and (3) collecting representative tissue specimens for histopathologic evaluation.

#### Material and methods:

FOE 5043, batch number: 17001/90, purity: 92.6% - 94.8%: acetone/corn oil mixture was used to dissolve the test article prior to mixing with the dietary carrier; the control diet was prepared the same way excluding only the test substance. Dosage (a.i.): 0 - 100 - 400 - 1600 - 4000 ppm. equivalent to: 0, 18.2, 64.2, 275.1, 823.8 ing/kg bw/day (males), 0, 24.5, 91.3, 431.7, 1133.8 mg/kg bw/day (females.) administration: oral by the diet for 90 days to mice (CD-1|ICR|BR).

**Mortality:** no abnormalities detected

**Body weight:** no abnormalities detected

**Food consumption:** no abnormalities detected

**Hematology:** 4000 ppm: red blood cells, hemoglobin, platelets, hematocrit decreased

Table 6.3.2.2-1 Hematology summary data – males

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043 MILES Agriculture Division - Toxicology Study : 90-171-HK Species : Mouse All subsets								TABLE HE1-SUM Hematology Data - Summary	Date : 15-JUN-92 Time : 11:42:41 Page : 1
-----									
M E A N   H e m a t o l o g y									
-----									
M A L E S		Day 84 PLTS 10*3/mm3	Day 84 WBC 10*3/mm3	Day 84 RBC 10*6/mm3	Day 84 Hgb g/dL	Day 84 Hct %	Day 84 MCV um3	Day 84 MCH pg	Day 84 MCHC g/dL
Control	Mean	1559	8.4	9.72	15.4	46.3	47.7	15.9	33.3
	SD	166	3.1	0.49	0.7	1.7	1.2	0.3	0.5
	n	15	15	15	15	15	15	15	15
100 ppm	Mean	1533	8.4	9.55	15.0	45.5	47.7	15.8	33.0
	SD	155	3.4	0.41	0.5	1.5	1.2	0.4	0.3
	n	15	15	15	15	15	15	15	15
400 ppm	Mean	1594	9.5	9.55	15.0	45.8	47.9	15.7	32.7
	SD	140	2.4	0.43	1.0	2.0	0.8	0.7	1.2
	n	15	15	15	15	15	15	15	15
1600 ppm	Mean	1557	7.8	9.43	15.1	45.7	48.5	16.1	33.1
	SD	141	2.8	0.29	0.5	1.6	2.0	0.6	0.5
	n	15	15	15	15	15	15	15	15
4000 ppm	Mean	1368*	7.7	8.82*	14.4*	42.7*	48.6	16.3*	33.7
	SD	144	2.2	0.70	1.0	2.4	2.1	0.6	0.7
	n	15	15	15	15	15	15	15	15
-----									
Statistics: * = significant by initial ANOVA + Dunnett's test (p<=0.05). @ = heterogenous by Bartlett's test (p<=0.001); nonparametric analysis with Kruskal-Wallis ANOVA + Mann-Whitney U-tests (p<=0.05). *# = not significant by nonparametric tests. \$ = significant by nonparametric tests. * = remains significant after nonparametric tests. blank = not significant by any test.									
PLTS	= Platelet Count				WBC	= Leukocyte Count			
RBC	= Erythrocyte Count				Hgb	= Hemoglobin			
Hct	= Hematocrit				MCV	= Mean Cell Volume			
MCH	= Mean Cell Hemoglobin				MCHC	= Mean Cell Hemoglobin Concentration			

Table 6.3.2.2-2 Hematology summary data – females

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043							Date : 16-JUN-92	
MILES Agriculture Division - Toxicology							Time : 12:44:48	
Study : 90-171-HK								
Species : Mouse								
All subsets							Page : 1	
TABLE HE1-SUM								
Hematology Data -								
Summary								
-----								
M E A N   H e m a t o l o g y								
-----								
F E M A L E S		Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84
		PLTS	WBC	RBC	Hgb	Hct	MCV	MCH
		10*3/mm3	10*3/mm3	10*6/mm3	g/dL	%	um3	pg
Control	Mean	1341	7.5	9.33	15.4	44.5	47.7	16.9
	SD	259	4.5	1.36	0.9	6.4	1.5	3.0
	n	14	14	14	14	14	14	14
100 ppm	Mean	1390	6.7	9.47	15.4	46.1	48.7	16.3
	SD	139	2.5	0.49	0.8	2.2	1.8	0.5
	n	15	15	15	15	15	15	15
400 ppm	Mean	1408	6.9	9.26	14.8	44.8	48.4	16.0
	SD	203	3.1	0.58	0.9	3.3	1.9	0.5
	n	14	14	14	14	14	14	14
1600 ppm	Mean	1411	8.9	9.37	15.4	46.1	49.3	16.4
	SD	112	2.5	0.61	0.9	2.7	1.7	0.5
	n	15	15	15	15	15	15	15
4000 ppm	Mean	1162*	5.3	8.59§	14.2*	41.8§	48.8	16.5
	SD	175	2.1	1.33	2.2	6.1	2.0	0.7
	n	14	14	14	14	14	14	14
-----								
Statistics: * = significant by initial ANOVA + Dunnett's test (p<=0.05).								
# = heterogenous by Bartlett's test (p<=0.001); nonparametric analysis								
with Kruskal-Wallis ANOVA + Mann-Whitney U-tests (p<=0.05).								
*# = not significant by nonparametric tests.								
§ = significant by nonparametric tests.								
* = remains significant after nonparametric tests.								
blank = not significant by any test.								
PLTS	= Platelet Count			WBC	= Leukocyte Count			
RBC	= Erythrocyte Count			Hgb	= Hemoglobin			
Hct	= Hematocrit			MCV	= Mean Cell Volume			
MCH	= Mean Cell Hemoglobin			MCHC	= Mean Cell Hemoglobin Concentration			

**Clinical chemistry:**

> 400 ppm, males: thyroxine decreased

4000 ppm. females: thyroxine decreased, triiodothyronine decreased, aspartate aminotransferase increased

4000 ppm. males: alkaline phosphatase increased



Table 6.3.2.2-3 Clinical chemistry summary data – males

## MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species : Mouse

All subsets

## TABLE CCl-SUM

Clinical Chemistry -  
Summary

Date : 16-JUN-92

Time : 14:17:21

Page : 1

## MEAN Serum Chemistry

M A L E S		Day 84 UN mg/dL	Day 84 Chol mg/dL	Day 84 AST U/L	Day 84 ALT U/L	Day 84 ALP U/L	Day 84 T4 ug/dL	Day 84 T3 ng/mL
Control	Mean	41	134	60	49	64	4.42	0.47
	SD	7	46	29	34	23	0.94	0.08
	n	15	15	15	15	15	15	15
100 ppm	Mean	35	133	49	42	60	4.63	0.45
	SD	8	22	11	20	17	1.34	0.10
	n	15	15	15	15	15	15	15
400 ppm	Mean	38	155	49	36	57	3.87	0.46
	SD	9	34	11	27	14	0.80	0.09
	n	15	15	15	15	15	15	15
1600 ppm	Mean	39	147	53	52	92	3.18*	0.44
	SD	9	34	6	18	54	0.80	0.14
	n	15	15	15	15	15	15	15
4000 ppm	Mean	34	148	53	59	106*	2.05*	0.48
	SD	8	52	11	21	76	0.58	0.14
	n	15	15	15	15	15	15	15

Statistics: \* = significant by initial ANOVA + Dunnett's test ( $p \leq 0.05$ ).@ = heterogenous by Bartlett's test ( $p \leq 0.001$ ); nonparametric analysiswith Kruskal-Wallis ANOVA + Mann-Whitney U-tests ( $p \leq 0.05$ ).

\*# = not significant by nonparametric tests.

\$ = significant by nonparametric tests.

\* = remains significant after nonparametric tests.

blank = not significant by any test.

UN = Urea Nitrogen

AST = Aspartate Aminotransferase

ALP = Alkaline Phosphatase

T3 = Triiodothyronine

Chol = Cholesterol

ALT = Alanine Aminotransferase

T4 = Thyroxine

Table 6.3.2.2-4 Clinical chemistry summary data – females

MOUSE SUBCHRONIC DIETARY STUDY WITH FOR 5043

MILES Agriculture Division - Toxicology

Study : 90-171-BK

Species : Mouse

All subsets

TABLE CC1-SUM

Clinical Chemistry -

Summary

Date : 16-JUN-92

Time : 14:17:21

Page : 2

## MEAN Serum Chemistry

F E M A L E S		Day 84 UN mg/dL	Day 84 Chol mg/dL	Day 84 AST U/L	Day 84 ALT U/L	Day 84 ALP U/L	Day 84 T4 ug/dL	Day 84 T3 ng/mL
Control	Mean	32	94	60	37	81	3.91	0.40
	SD	6	22	17	20	22	1.07	0.07
	n	15	15	15	15	15	15	15
100 ppm	Mean	34	104	60	29	90	3.96	0.47*
	SD	4	28	11	6	14	1.04	0.09
	n	14	14	14	14	14	13	13
400 ppm	Mean	31	100	55	27	94	3.41	0.43
	SD	6	21	11	11	29	0.63	0.06
	n	14	14	14	14	14	14	14
1600 ppm	Mean	33	103	68	35	94	3.12	0.42
	SD	7	26	10	14	23	1.19	0.09
	n	15	15	15	15	15	14	15
4000 ppm	Mean	32	105	80*	51§	99	1.61*	0.30*
	SD	7	32	21	24	25	0.43	0.07
	n	14	14	14	14	14	14	14

Statistics: \* = significant by initial ANOVA + Dunnett's test ( $p \leq 0.05$ ).@ = heterogenous by Bartlett's test ( $p \leq 0.001$ ); nonparametric analysiswith Kruskal-Wallis ANOVA + Mann-Whitney U-tests ( $p \leq 0.05$ ).

\*# = not significant by nonparametric tests.

§ = significant by nonparametric tests.

\* = remains significant after nonparametric tests.

blank = not significant by any test.

UN = Urea Nitrogen

AST = Aspartate Aminotransferase

ALP = Alkaline Phosphatase

T3 = Triiodothyronine

Chol = Cholesterol

ALT = Alanine Aminotransferase

T4 = Thyroxine

**Clinical signs:**

> 1600 ppm: increased activity, swaying movements of the head;

4000 ppm: food spillage, circling increased

**Table 6.3.2.2-5 Clinical signs summary data – males**

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species: Mouse

Sex : Male All sub-sets

TABLE CO-SUM

CLINICAL OBSERVATIONS SUMMARY

---

C l i n i c a l   S u m m a r y   I n c i d e n c e   R e p o r t (Full report)

---

Weeks 1 - 15

With reference to nominal day zero

Dose Group	A	B	C	D	E
Dose	Control	100 ppm	400 ppm	1600 ppm	4000 ppm
Total animals	15	15	15	15	15

**GENOBS****WITHIN NORMAL LIMITS**

Incidence (%)	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)
Mean onset (days)	0	0	0	0	0
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

**BEHAVIOR****SWAYING MOVEMENT OF HEAD**

Incidence (%)	0 ( 0)	0 ( 0)	0 ( 0)	9 ( 60)	15 (100)
Mean onset (days)	0	0	0	72	27
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

**SPILLER**

Incidence (%)	5 ( 33)	8 ( 53)	5 ( 33)	9 ( 60)	14 ( 93)
Mean onset (days)	19	22	29	14	13
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

**CIRCLING**

Incidence (%)	0 ( 0)	0 ( 0)	0 ( 0)	1 ( 6)	6 ( 40)
Mean onset (days)	0	0	0	77	63
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

**INCREASED ACTIVITY**

Incidence (%)	0 ( 0)	0 ( 0)	0 ( 0)	9 ( 60)	15 (100)
Mean onset (days)	0	0	0	75	33
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

## MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species: Mouse

Sex : Male All sub-sets

## TABLE CO-SUM

## CLINICAL OBSERVATIONS SUMMARY

## Clinical Summary Incidence Report

(Full report)

Weeks 1 - 15

With reference to nominal day zero

Dose Group	A	B	C	D	E
Dose	Control	100 ppm	400 ppm	1600 ppm	4000 ppm
Total animals	15	15	15	15	15
TOTALS					
Incidence (%)	5( 33)	8( 53)	5( 33)	13( 86)	15(100)
Mean onset(days)	19	22	29	32	11
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0

Table 6.3.2.2-6 Clinical sings summary data – females

## MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species: Mouse

Sex : Female All sub-sets

## TABLE CO-SUM

## CLINICAL OBSERVATIONS SUMMARY

## Clinical Summary Incidence Report

(Full report)

Weeks 1 - 15

With reference to nominal day zero

Dose Group	A	B	C	D	E
Dose	Control	100 ppm	400 ppm	1600 ppm	4000 ppm
Total animals	15	15	15	15	15
GENOBS					
WITHIN NORMAL LIMITS					
Incidence (%)	15(100)	15(100)	15(100)	15(100)	15(100)
Mean onset(days)	0	0	0	0	0
Deaths	0	0	1	0	1
(mean day)	0	0	7	0	70
DEAD.					
Incidence (%)	0( 0)	0( 0)	0( 0)	0( 0)	1( 6)
Mean onset(days)	0	0	0	0	70
KILLED (UNSCHEDULED).					
Incidence (%)	0( 0)	0( 0)	1( 6)	0( 0)	0( 0)
Mean onset(days)	0	0	7	0	0
ABNORMAL BODY POSITION					
HUNCHED BACK					
Incidence (%)	0( 0)	0( 0)	1( 6)	0( 0)	0( 0)
Mean onset(days)	0	0	7	0	0
Deaths	0	0	1	0	0
(mean day)	0	0	7	0	0
BEHAVIOR					
SWAYING MOVEMENT OF HEAD					
Incidence (%)	0( 0)	0( 0)	0( 0)	11( 73)	15(100)
Mean onset(days)	0	0	0	67	27
Deaths	0	0	0	0	1
(mean day)	0	0	0	0	70

## MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-BK

Species: Mouse

Sex : Female All sub-sets

## TABLE CO-SUM

## CLINICAL OBSERVATIONS SUMMARY

## Clinical Summary Incidence Report (Full report)

Weeks 1 - 15

With reference to nominal day zero

Dose Group	A	B	C	D	E
Dose	Control	100 ppm	400 ppm	1600 ppm	4000 ppm
Total animals	15	15	15	15	15
<b>SPILLER</b>					
Incidence (%)	11 ( 73)	11 ( 73)	12 ( 80)	15 (100)	15 (100)
Mean onset(days)	18	12	21	21	13
Deaths	0	0	0	0	1
(mean day)	0	0	0	0	70
<b>CLOGGED FEEDER</b>					
Incidence (%)	1 ( 6)	0 ( 0)	0 ( 0)	0 ( 0)	0 ( 0)
Mean onset(days)	84	0	0	0	0
Deaths	0	0	0	0	0
(mean day)	0	0	0	0	0
<b>CIRCLING</b>					
Incidence (%)	0 ( 0)	0 ( 0)	0 ( 0)	4 ( 26)	14 ( 93)
Mean onset(days)	0	0	0	77	59
Deaths	0	0	0	0	1
(mean day)	0	0	0	0	70
<b>INCREASED ACTIVITY</b>					
Incidence (%)	0 ( 0)	0 ( 0)	0 ( 0)	11 ( 73)	15 (100)
Mean onset(days)	0	0	0	68	33
Deaths	0	0	0	0	1
(mean day)	0	0	0	0	70

**Organ weight:**

400 ppm, males: relative liver weight increased

≥ 1600 ppm: absolute liver weight increased

≥ 1600 ppm, males: absolute kidney weight decreased

≥ 1600 ppm, females: relative liver weight increased

4000 ppm. males: absolute liver weight increased,

4000 ppm. females: absolute and relative spleen weight increased

relative kidney weight increased, absolute liver weight increased.

absolute and relative ovary weight decreased

Table 6.3.2.2-7 Absolutec organ weight summary data – males

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043 MILES Agriculture Division - Toxicology Study : 90-171-HK Species : Mouse All subsets										Date : 17-JUN-92 Time : 15:12:53 Page : 1
TABLE OWLK-SUM										
Terminal Body and Absolute										
Organ Weights - Summary										
-----										
M E A N   O r g a n   W e i g h t s										
-----										
M A L E S		Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84
		TermBW	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Spleen	Testes
		g	g	g	g	g	g	g	g	g
Control	Mean	36.1	0.007	0.500	0.179	0.688	2.079	0.228	0.078	0.246
	SD	3.8	0.002	0.019	0.019	0.079	0.254	0.031	0.013	0.025
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	34.8	0.008	0.499	0.178	0.645	1.996	0.216	0.077	0.251
	SD	2.7	0.003	0.020	0.011	0.046	0.226	0.020	0.014	0.025
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	34.3	0.008	0.487	0.178	0.647	2.250	0.225	0.080	0.234
	SD	2.8	0.001	0.028	0.019	0.064	0.275	0.036	0.014	0.020
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	33.8	0.009	0.491	0.169	0.590*	2.614*	0.207	0.080	0.247
	SD	3.1	0.002	0.029	0.018	0.058	0.295	0.013	0.012	0.025
	n	15	15	15	15	15	15	15	15	15
4000 ppm	Mean	32.3*	0.009	0.495	0.183	0.600*	3.285*	0.223	0.094*	0.238
	SD	3.5	0.003	0.025	0.016	0.073	0.487	0.025	0.020	0.034
	n	15	15	15	15	15	15	15	15	15
-----										
Statistics: * = significant by initial ANOVA + Dunnett's test (p<=0.05). @ = heterogenous by Bartlett's test (p<=0.001); nonparametric analysis with Kruskal-Wallis ANOVA + Mann-Whitney U-tests (p<=0.05). *# = not significant by nonparametric tests. \$ = significant by nonparametric tests. * = remains significant after nonparametric tests. blank = not significant by any test.										

Table 6.3.2.2-8 Absolute organ weight summary data – females

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species : Mouse

All subsets

TABLE OW1K-SUM

Terminal Body and Absolute

Organ Weights - Summary

Date : 17-JUN-92

Time : 15:17:25

Page : 1

## MEAN Organ Weights

F E M A L E S		Day 84	Day 84	§ Day 84	Day 84	Day 84	§ Day 84	Day 84	Day 84	§ Day 84
		TermSW	Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Ovaries	Spleen
		g	g	g	g	g	g	g	g	g
Control	Mean	27.4	0.011	0.496	0.151	0.438	1.597	0.198	0.034	0.093
	SD	1.5	0.002	0.012	0.015	0.035	0.141	0.016	0.007	0.009
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	27.6	0.011	0.503	0.151	0.444	1.614	0.212	0.039	0.087
	SD	1.9	0.002	0.014	0.014	0.042	0.169	0.018	0.009	0.010
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	27.8	0.011	0.502	0.157	0.453	1.717	0.223*	0.036	0.091
	SD	1.7	0.002	0.027	0.016	0.038	0.148	0.034	0.006	0.019
	n	14	14	14	14	14	14	14	14	14
1600 ppm	Mean	27.0	0.012	0.494	0.154	0.440	1.787§	0.203	0.033	0.096
	SD	3.2	0.003	0.037	0.021	0.050	0.208	0.022	0.009	0.022
	n	15	15	15	15	15	15	15	15	15
4000 ppm	Mean	23.6*	0.010	0.494	0.145	0.417	2.101*	0.200	0.026*	0.093
	SD	2.0	0.002	0.034	0.012	0.046	0.416	0.023	0.006	0.023
	n	14	14	14	14	14	14	14	14	14

Statistics: \* = significant by initial ANOVA + Dunnett's test ( $p \leq 0.05$ ).§ = heterogenous by Bartlett's test ( $p \leq 0.001$ ); nonparametric analysis with Kruskal-Wallis ANOVA + Mann-Whitney U-tests ( $p \leq 0.05$ ).

\*§ = not significant by nonparametric tests.

§ = significant by nonparametric tests.

\* = remains significant after nonparametric tests.

blank = not significant by any test.



Table 6.3.2.2-9 Relative organ weight summary data – males

## MOUSE SUBCHRONIC DIETARY STUDY WITH POE 5043

MILES Agriculture Division - Toxicology

Study : 90-171-HK

Species : Mouse

All subsets

## TABLE OW2K-SUM

Relative Organ Weights -  
Summary

Date : 17-JUN-92

Time : 15:22:59

Page : 1

## MEAN Organ / Body Weight Ratios

M A L E S		Day 84 TermBW g	Day 84 Adr/BW %	Day 84 Brn/BW %	Day 84 Hrt/BW %	Day 84 Kid/BW %	Day 84 Liv/BW %	Day 84 Lun/BW %	Day 84 Spl/BW %	Day 84 Tes/BW %
Control	Mean	36.1	0.021	1.400	0.497	1.914	5.769	0.632	0.218	0.687
	SD	3.8	0.005	0.160	0.047	0.198	0.504	0.053	0.042	0.081
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	34.8	0.024	1.443	0.514	1.861	5.732	0.623	0.223	0.726
	SD	2.7	0.008	0.118	0.038	0.156	0.388	0.071	0.043	0.095
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	34.3	0.024	1.426	0.521	1.891	6.538*	0.654	0.235	0.684
	SD	2.8	0.004	0.122	0.055	0.204	0.393	0.090	0.042	0.065
	n	15	15	15	15	15	15	15	15	15
1600 ppm	Mean	33.8	0.027	1.459	0.502	1.750	7.721*	0.614	0.238	0.734
	SD	3.1	0.006	0.131	0.052	0.158	0.489	0.045	0.027	0.082
	n	15	15	15	15	15	15	15	15	15
4000 ppm	Mean	32.3*	0.027	1.552	0.572*	1.869	10.166*	0.696*	0.292*	0.740
	SD	3.5	0.009	0.185	0.066	0.222	0.722	0.080	0.060	0.100
	n	15	15	15	15	15	15	15	15	15

Statistics: \* = significant by initial ANOVA + Dunnett's test ( $p \leq 0.05$ ).§ = heterogeneous by Bartlett's test ( $p \leq 0.001$ ); nonparametric analysiswith Kruskal-Wallis ANOVA + Mann-Whitney U-tests ( $p \leq 0.05$ ).

\*§ = not significant by nonparametric tests.

§ = significant by nonparametric tests.

\* = remains significant after nonparametric tests.

blank = not significant by any test.

TermBW = Terminal Body Weight

Brn/BW = Brain/BW

Kid/BW = Kidneys/BW

Lun/BW = Lungs/BW

Tes/BW = Testes/BW

Adr/BW = Adrenals/BW

Hrt/BW = Heart/BW

Liv/BW = Liver/BW

Spl/BW = Spleen/BW

Table 6.3.2.2-10 Relative organ weight summary data – females

MOUSE SUBCHRONIC DIETARY STUDY WITH FOE 5043										Date : 17-JUN-92
MILES Agriculture Division - Toxicology										Time : 15:27:29
Study : 90-171-HK										
Species : Mouse										
All subsets										Page : 1
TABLE 0W2K-SUM										
Relative Organ Weights - Summary										
-----										
M E A N   O r g a n / B o d y   W e i g h t   R a t i o s										
-----										
F E M A L E S		Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	Day 84	
		TermBW	Adr/BW	Brn/BW	Hrt/BW	Kid/BW	Liv/BW	Lun/BW	Ova/BW	Spl/BW
		g	%	%	%	%	%	%	%	%
Control	Mean	27.4	0.041	1.816	0.553	1.604	5.840	0.722	0.124	0.341
	SD	1.5	0.007	0.084	0.061	0.128	0.473	0.039	0.028	0.035
	n	15	15	15	15	15	15	15	15	15
100 ppm	Mean	27.6	0.040	1.831	0.551	1.611	5.852	0.773§	0.142	0.317
	SD	1.9	0.008	0.130	0.059	0.110	0.380	0.069	0.028	0.039
	n	15	15	15	15	15	15	15	15	15
400 ppm	Mean	27.8	0.040	1.813	0.565	1.636	6.187	0.806§	0.130	0.326
	SD	1.7	0.008	0.109	0.051	0.147	0.400	0.126	0.020	0.063
	n	14	14	14	14	14	14	14	14	14
1600 ppm	Mean	27.0	0.044	1.847	0.574	1.641	6.640*	0.759	0.121	0.354
	SD	3.2	0.010	0.197	0.073	0.185	0.465	0.099	0.030	0.065
	n	15	15	15	15	15	15	15	15	15
4000 ppm	Mean	23.6*	0.043	2.096*	0.616*	1.767*	8.835*	0.852*	0.110	0.390
	SD	2.0	0.009	0.142	0.044	0.155	1.190	0.122	0.023	0.081
	n	14	14	14	14	14	14	14	14	14
-----										
Statistics: * = significant by initial ANOVA + Dunnett's test (p<=0.05).										
§ = heterogenous by Bartlett's test (p<=0.001); nonparametric analysis										
with Kruskal-Wallis ANOVA + Mann-Whitney U-tests (p<=0.05).										
*# = not significant by nonparametric tests.										
§ = significant by nonparametric tests.										
* = remains significant after nonparametric tests.										
blank = not significant by any test.										
TermBW	= Terminal Body Weight				Adr/BW	= Adrenals/BW				
Brn/BW	= Brain/BW				Hrt/BW	= Heart/BW				
Kid/BW	= Kidneys/BW				Liv/BW	= Liver/BW				
Lun/BW	= Lungs/BW				Ova/BW	= Ovaries/BW				
Spl/BW	= Spleen/BW									

**Histopathology**

≥ 1600 ppm: liver: hepatocytomegaly

≥ 1600 ppm, males: thyroid: hyperplasia

4000 ppm, males: liver - individual cell necrosis, spleen-hematopoiesis: thyroid-colloid increased

4000 ppm, females: spleen: pigmentation; thyroid: hyperplasia

**Table 6.3.2.2-11 Histopathology - summary**

	<b>Dietary Dose Levels (ppm)</b>				
	<b>Control</b>	<b>100</b>	<b>400</b>	<b>1600</b>	<b>4000</b>
<b>Histopathology</b>					
<b>Liver</b>					
Hepatocytomegaly	-	-	M:1/15 F:2/15	M:15/15* F:14/15*	M:15/15* F:14/15*
Necrosis					
Individual Cell	-	M:1/15 F: -	M: - F: -	M:3/15 F: -	M:5/15* F: -
Degeneration	-	-	M: -	M: -	M: -
Vacuolar	-	-	F:1/15	F: -	F: -
<b>Spleen</b>					
Hematopoiesis, E.M.	M:5/15 F:12/15	M:6/15 F:10/15	M:10/15 F:10/15	M:10/15 F:14/15	M:15/15* F:14/15
Pigmentation	M: - F:1/15	M: - F:2/15	M: - F:5/15	M: - F:8/15*	M: - F:13/15*
<b>Thyroids</b>					
Colloid Increased	M:2/15 F: -	M:1/15 F: -	M:3/14 F: -	M:7/15 F: -	M:10/15* F: -
Hyperplasia					
Foll. Cell	M: - F: -	M: - F: -	M:2/14 F: -	M:9/15* F:2/15	M:12/15* F: 8/15*

<sup>1</sup> Percent changes for body weight and food consumption calculated by comparing the "grand means" of control and treatment groups for the 13 weekly determinations taken for each dose group on study.

<sup>2</sup> Abbreviations: M, male; F, female; -, no change.

\* Indicates a statistically significant difference from control;  $p \leq 0.05$ .

<b>Conclusion</b>	<p><b>The study is in line with current guidelines.</b></p> <p>Through approximately 13 weeks of continuous dietary exposure to the chemical, the toxicological response of the mouse could be broadly characterized as involving structural and/or functional alterations in liver, hematologic/spleen, and thyroid-related endpoints.</p> <p><b>NOAEL: 100 ppm (male); 100 ppm (female) equal to 18.2 mg/kg bw/day (males) or 24.5 mg/kg bw/day (females)</b></p>
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<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>FOE 5043: 13-week subchronic feeding study in beagle dogs studies were presented and evaluated during the EU process for Annex I listing.</b>
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### B.6.3.2.3 Dog

<b>Report:</b>	<b>[REDACTED]; 1995a</b>
<b>Title:</b>	<b>FOE 5043: 13-week subchronic feeding study in beagle dogs</b>
<b>Document No:</b>	M-004977-02-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7563 of March 28, 1995
<b>Guidelines:</b>	FIFRA § 83-5; TSCA 40 CFR Section 798.3320; OECD guideline 453; MAFF guideline 59 NohSan No. 4200; EU-guideline 87/302
<b>GLP</b>	yes

#### Material and methods:

This study was conducted to obtain information on the toxic effects of FOE 5043 when fed subchronically to Beagle dogs, and to provide data for selection of chronic study dose levels.

Technical grade FOE 5043 (batch number: 17001/90. formulated in corn oil at 1% by weight of the diet, served as a vehicle for the test article (acetic-solvent) as it was mixed with the feed) was administered in the diet to Beagle dogs (4 animals per sex and treatment level) at nominal concentrations of 0, 50, 200, 800 and 2400 ppm corresponding to:

- 1.67, 7.2, 27.2 or 96.9 mg/kg body weight/day in males

-1.7, 6.9, 28.0 or 93.2 mg/kg body weight/day in females for thirteen weeks.

#### Findings and the summary of the toxicodynamic effects are as follows:

1. In-life body weights and feed consumption were not affected by compound administration.
2. Soft feces and thin body condition were considered treatment-related clinical signs at the 2400 ppm level in both sexes. There were no compound-related ophthalmic lesions due to compound administration.
3. Clinical chemistry:
  - changes in liver parameters were an increased lactate dehydrogenase (LD) and alkaline phosphatase (ALP) levels, and decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The LD was increased in male dogs at 2400 ppm and in females at 200, 800 and 2400 ppm. The ALP was increased in males and females at 2400 ppm. The ALT was decreased in both sexes at 200, 800 and 2400 ppm. The AST was decreased, but only as a compound related trend in both sexes at 800 and 2400 ppm. Further supportive of hepatic-related effects was a decrease in albumin in males at doses of 200, 800 and 2400 ppm, and in females at 800 and 2400 ppm. Only female globulin levels were considered increased in a compound-related manner at doses of 200, 800 and 2400 ppm.

- A treatment related decrease in serum thyroxine (T4) was found in both sexes at 200, 800 and 2400 ppm. A decreased triiodothyronine (T3) was likely to be compound-related in both sexes at 800 and 2400 ppm. This decline in both hormones, without corresponding cholesterol and triglyceride increases, and without accompanying myxedema-like clinical signs, suggests an extrathyroidal mechanism due to increased hepatic clearance.

- Calcium and glucose levels were decreased due to the metabolic effect of the compound on the liver, and the ubiquitous secondary effects of thyroid hormone loss. Serum glucose was decreased in males at 800 and 2400 ppm, and females at 200, 800 and 2400 ppm. Calcium was decreased in males at 2400 ppm, and in females at 800 and 2400 ppm, which often accompanies hypoalbuminemia in the dog.

#### 4. Hematology:

- Decreased red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC) in both sexes at the 2400 ppm dose, which was compensated for by study termination, with a responsive increase in platelets in both sexes at the 2400 ppm dose on day 87.

#### 5. Urinalysis:

There were no urinalysis findings or gross pathology observations related to compound administration.

#### 6. Necropsy, gross, pathology, histology:

- There was a compound-related decrease in terminal body weights in the 2400 ppm males and a similar biological trend in females. Treatment-related organ weight increases were found in absolute and relative liver weights in both sexes at 2400 ppm, and in the 800 ppm females. The relative kidney weights were increased in the 2400 ppm males and in the 200 and 2400 ppm females, with a similar trend in the 800 ppm group females. Principle micropathology findings consisted of:

a. Bone marrow hyperplasia in 2400 ppm males and females.

b. Spleen pigment in 200, 800 and 2400 ppm females and in 800 and 2400 ppm males

c. Kidney hyperplasia of epithelial cells in 800 and 2400 ppm males and in 2400 ppm females, and cytoplasmic vacuolization in collecting tubules in 800 and 2400 ppm females,

d. Moderate diffuse hepatomegaly in 2400 ppm males and females., and pigment in liver in 2400 ppm females,

e. Vacuolization of the cerebral cortex in 2400 ppm males and females;

f. Hypertrophy of thyroid follicular cells in 2400 ppm female

**Table 6.3.2.3 Findings and the summary of the toxicodynamic effects of FOE 5043: 13-week subchronic feeding study in beagle dogs:**

Flufenacet	Male						Female					
90-day oral dog	0	50	200	800	2400		0	50	200	800	2400	
Hb [g/dL] (day -6)	12.4	13.1	12.7	12.8	12.2		14.2	13.6	13.3	13.9	14	
Hb [g/dL] (day 31)	13.6	13.6	13.9	13.8	11.5		14.7	15.4	14.2	14.7	13.2	
Hb [g/dL] (day 57)	14.4	15.1	15.2	14.6	11.9	*	16.6	16.3	15.3	16.1	13.1	*
Hb [g/dL] (day 87)	14.3	15.7	16	15.2	13.8		15.8	16.5	16.8	16.6	13.7	
HCT [%] (day -6)	35.8	37.7	37	37.2	35		38.8	38.9	38.3	39.6	40.1	
HCT [%] (day 31)	40	39.3	40.5	40.6	34.2		42.5	44.2	41.3	43	39	
HCT [%] (day 57)	41	43.4	44	42.5	35.3	*	47.4	46.3	43.9	46.8	38.8	*
HCT [%] (day 87)	40.8	44.3	45.3	43.8	41		44.9	47	47.6	47.7	40.6	
RBC [ $10^6/\text{mm}^3$ ] (day -6)	5.58	5.83	6	6.15	5.78		5.85	6.23	6.03	6.28	6.28	
RBC [ $10^6/\text{mm}^3$ ] (day 31)	6.05	5.93	6.38	6.5	5.38		6.25	6.75	6.43	6.65	6.05	
RBC [ $10^6/\text{mm}^3$ ] (day 57)	6.18	6.43	6.77	6.76	5.36	*	6.84	7	6.76	7.23	5.87	*
RBC [ $10^6/\text{mm}^3$ ] (day 87)	6.13	6.58	7	7.03	6.28	*	6.45	7.03	7.35	7.48	6	
Platelets [ $10^3/\text{mm}^3$ ] (day -6)	400	400	303	308	378		348	380	373	388	343	
Platelets [ $10^3/\text{mm}^3$ ] (day 31)	388	328	310	320	430		315	350	338	388	340	
Platelets [ $10^3/\text{mm}^3$ ] (day 57)	382	338	312	355	459		318	341	326	384	444	*
Platelets [ $10^3/\text{mm}^3$ ] (day 87)	358	333	293	353	473	*	308	320	340	370	490	*
RETi [%] (day 87)	0.6	0.9	1.2	0.9	1.1		0.5	0.5	0.5	1.1	0.8	
MCV [ $\mu\text{m}^3$ ] (day 87)	66.6	67.4	64.9	62.5	65.5		69.5	66.9	64.7	63.8	67.7	
MCHC [g/dL] (day 87)	34.9	35.5	35.3	34.6	33.7	*	35.2	35.1	35.3	34.7	33.7	*
MCH [pg] (day 87)	23.3	23.9	22.9	21.6	22.1		24.5	23.5	22.8	22.2	22.8	*
ASAT (GOT) [U/L] (day 87)	32	34	31	25	27		37	40	36	27	33	

Flufenacet	Male								Female									
90-day oral dog	0	50		200		800		2400		0	50		200		800		2400	
ALAT (GPT) [U/L] (day 87)	50	35		27	*	18	*	12	*	37	36		29	*	17	*	15	*
Alkaline Phosphatase [U/L] (day 87)	104	109		99		137		352	*	92	94		104		117		277	*
Lactate dehydrogenase [U/L] (day 87)	127	170		156		192		258		140	234	*	248	*	215	*	398	*
Triglycerides (TG) [mg/dL] (day -6)	51	39		30		42		34		62	49		53		57		54	
Triglycerides (TG) [mg/dL] (day 31)	46	35		41		42		42		37	34		44		48		43	
Triglycerides (TG) [mg/dL] (day 57)	38	32		35		39		36		41	49		48		49		46	
Triglycerides (TG) [mg/dL] (day 87)	62	49		53		57		54		56	50		58		63		61	
CHOL [mg/dL] [mg/dL] (day -6)	186	158		164		166		136		135	130		158		157		140	
CHOL [mg/dL] (day 31)	197	148	*	182		189		172		163	176		198		182		202	
CHOL [mg/dL] (day 57)	187	149		172		188		184		152	176		180		186		161	
CHOL [mg/dL] (day 87)	205	163		180		185		208		141	160		171		193		210	
Total bilirubin [mg/dL] (day 87)	0.2	0.1	*	0.1	*	0.2		0.1	*	0.2	0.2		0.1		0.1		0.1	
ALB [g/dL] (day 87)	3.2	3.4		3	*	2.8	*	2.6	*	3.3	3.3		3.2		3	*	2.6	*
GLOB [g/dL] (day 87)	2.8	2.8		3		2.8		3.1		2.5	2.5		3.1	*	2.8	*	3.2	*
GLUC [mg/dL] (d -6)	108	114		102		105		104		101	105		102		108		106	
GLUC [mg/dL] (d 31)	99	105		99		83	*	81	*	89	102	*	97	*	89		80	*
GLUC [mg/dL] (d 57)	95	94		85		84	*	81	*	91	94		86		80		74	*
GLUC [mg/dL] (d 87)	104	99		90	*	83	*	79	*	96	97		82	*	78	*	69	*
Ca [mg/dL] (d 87)	11.4	11.2		11.2		11.2		10.7	*	11.4	11.2		11.1		10.9	*	10.4	*
T3 [ng/mL] (day -6)	0.58	0.67		0.71		0.59		0.66		0.54	0.61		0.80	*	0.72	*	0.65	
T3 [ng/mL] (day 31)	0.77	0.71		0.66		0.51	*	0.38	*	0.69	0.70		0.67		0.53	*	0.24	*
T3 [ng/mL] (day 57)	0.63	0.67		0.66		0.50		0.44	*	0.59	0.69		0.69		0.57		0.36	*
T3 [ng/mL] (day 87)	0.79	0.69		0.65		0.50	*	0.52	*	0.68	0.67		0.67		0.55		0.40	*
T4 [µg/mL] (day -6)	2.07	3.02	*	2.92	*	2.47		2.77	*	2.22	2.22		3.13		2.30		2.97	



Flufenacet	Male								Female									
90-day oral dog	0	50		200		800		2400		0	50		200		800		2400	
T4 [µg/mL] (day 31)	3.05	2.49		1.83	*	0.95	*	0.87	*	2.62	2.07		1.90	*	1.23	*	0.84	*
T4 [µg/mL] (day 57)	2.42	2.37		1.60		0.72	*	0.93	*	2.11	1.45		1.41		1.81		1.43	
T4 [µg/mL] (day 87)	3.25	2.40	*	1.70	*	0.99	*	0.81	*	2.39	2.25		1.62	*	1.13	*	0.69	*
BW at terminal kill [g]	11643	10093		11991		11574		9593.5	*	9681	9619		10044		10058		7931.5	
Liver weight (rel.) [%]	3.192	3.348		2.987		3.614		5.298	*	2.888	3.005		3.22		3.71	*	5.683	*
hepatomegaly	1	2		1		2		4	*	0	0		3		2		3	
pigmentation										0	0		0		0		4	*
Pituitary weight (abs.) [g]	0.0660	0.0710		0.0690		0.07900		0.0630		0.05600	0.06500		0.06800		0.05800		0.06500	
Pituitary weight (rel.) [%]	0.0006	0.0007		0.0006		0.00070		0.0007		0.00060	0.00070		0.00070		0.00060		0.00080	
Thyroid weight (abs.) [g]	0.8540	1.0210		0.9260		1.06900		1.2050		0.83000	0.62300		0.88800		0.82100		0.92800	
Thyroid weight (rel.) [%]	0.0074	0.0101		0.0078		0.00920		0.0126		0.00870	0.00650	*	0.00880		0.00830		0.01170	
thyroid foll.-cell hypertrophy										0	0		0		0		2	
Kidney weight (rel.) [%]	0.519	0.575		0.477		0.518		0.676	*	0.469	0.493		0.524	*	0.53		0.751	*
cytopl. vacuol. collecting tub.	0	0		0		0		0		0	0		0		1		4	*
hyperplasia of epithelial cells	0	1		0		2		4	*	1	0		3		1		3	
Bone marrow																		
Bone marrow hyperplasia	0	0		0		0		2		0	0		0		0		2	
Spleen																		
Spleen pigment (hemosiderin)	0	0		0		1		4	*	0	0		2		2		4	*
Brain																		
Cerebral cortex vacuol.	0	0		0		0		4 (2.3)	*	0	0		0		0		4 (2.5)	*

Flufenacet	Male						Female					
90-day oral dog	0	50	200	800	2400		0	50	200	800	2400	
Multifocal slight-moderate												
gliosis	0	0	0	0	1 (2.0)		0	0	1 (2.0)	0	0	
mineralisation	0	0	0	0	0		0	0	0	1 (2.0)	0	
Eye												
cyst	0	1 (2.0)	0	0	1 (2.0)		0	1 (1.0)	0	1 (1.0)	0	
Spinal cord												
mineralisation	0	0	2 (2.0)	1 (2.0)	1 (2.0)		2 (2.0)	2 (2.0)	2 (2.0)	0	2 (1.5)	
vacuolisation	0	0	0	0	0		0	0	1 (1.0)	0	0	
edema	0	0	0	1 (2.0)	0		0	0	0	0	0	
Nerves												
sciatic	0	0	0	0	0		0	0	0	0	0	
optic, pigmentation	0	0	0	0	0		0	1 (2.0)	0	0	0	
% pretreatment												

In the following summary tables statistical significances given as follows:

\* statistically significantly different at  $p < 0.05$

\*\* statistically significantly different at  $p < 0.01$

<b>Conclusion</b>	<p><b>The study is in line with current guidelines</b></p> <p>Through approximately 13 weeks of continuous dietary exposure to the chemical, the toxicological response of the dog could be broadly characterized as involving structural and/or functional alterations in liver, hematologic/spleen, and thyroid-related endpoints.</p> <p><b>NOAEL: 50 ppm (male); 50 ppm (female) equal to 1.67 mg/kg bw/day (males) or 1.7 mg/kg bw/day (females)</b></p>
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**B.6.5.3.4 Dog**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Technical Grade FOE 5043: A Chronic Toxicity Feeding Study in the Beagle Dog (1995b) (1997) studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>[REDACTED]; 1995b (1997)</b>
Title:	Technical Grade FOE 5043: A Chronic Toxicity Feeding Study in the Beagle Dog.
Document No:	M-005001-02-2.
Report No:	Bayer Corporation, unpublished report no. 7779 of Sept. 20, 1995; <i>Addendum: July 18, 1997</i>
Guidelines:	FIFRA § 83-1; TSCA 40 CFR Section 798.3320; OECD guideline 452; MAFF guideline 59 NohSan No. 4200; US-FDA. Appendix I I Guidelines of Toxicological Testing, October 1982; EU-guideline 87/302 Part B
GLP	Yes

**Material and methods:**

FOE 5043, batch number: Fl. 036 from 7/4/91. purity: 95.2% - 99.0% Corn oil, at 1% by weight of the diet, served as a vehicle for the test article as it was mixed with the feed. Acetone was used as a solvent. Administration: orally mixed in feed, to dogs (*Canis familiaris*) for at least one year. Dosage (a.i.): 0 - 40 - 800 - 1600 ppm, the concentrations were equal to 1.29, 27.75 or 62.24 mg/kg bw/day for males. 1.14, 26.82 or 58.79 mg/kg bw/day for females.

**Findings - General observations:**

A clinical observation of head tilt (1600 ppm, both sexes) was found, but there were no ophthalmologic examination findings by traditional methods.

**Body weight development:**

There were no meaningful changes among in-life body weights, however a compound-related terminal body weight decrease was noted (1600 ppm. both sexes; 800 ppm. females).

**Hematology:**

Principle hematology target parameters considered compound-related were: (1) decreased erythrocyte count (1600 ppm. both sexes). (2) decreased hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration (800 and 1600 ppm, both sexes), and (3) increased platelet count (1600 ppm, males).

**Liver enzyme induction:**

Nonspecific enzymatic induction (see below).

**Clinical Chemistry:**

Principle clinical chemistry target parameters considered compound-related were: (1) decreased glucose (800 and 1600 ppm, both sexes). (2) increased cholesterol (800 and 1600 ppm, both sexes), (3) decreased thyroxine and triiodothyronine (800 and 1600 ppm, both sexes), (4) decreased alanine aminotransferase (800 and 1600 ppm, both sexes), increased alkaline phosphatase (800 and 1600 ppm, both sexes), and (5) decreased albumin (800 ppm, females; 800 and 1600 ppm, males).

**Urinalysis:**

There were no urinalysis parameters affected in this study.

**Computer ECG & BP:**

Computerized electrocardiography found premature ventricular complexes, and mechanical abnormalities of notched ventricular R waves, and elevated ST segments (1600 ppm, both sexes).

**Clinical Neurology:**

Clinical neurology examination deficits detected, just prior to termination, that were regarded as meaningful were: (1) abnormal behavior - hypo-reactivity in two high dose animals, reduced reaction to movement and sound in a high dose animal and hyper-reactivity/hypertonia in high dose animals; (2) postural abnormalities - deficits in wheelbarrowing, hemiwalking, hemihopping, hemistanding and proprioception (1600 ppm, both sexes); (3) gait/body position - deficits in stride width, being stiff legged and reluctant to walk (1600 ppm, both sexes), with stride width deficits in two mid-dose dogs. Body position deficits in the high dose dogs consisted of wide stance, head tilt, being flat footed and being unsteady; and (4) optic nystagmus/strabismus/ placement - abnormal physiologic nystagmus, with one dog having placement deficits (1600 ppm group).

**Quantitative EEG:**

Quantitative electroencephalography measured during test conditions of defined naturalistic stimuli found compound-related target parameters of: relative delta, relative theta, relative beta contributions; and median spectral edge frequency within the theta-beta range, median spectral edge frequency and 90<sup>th</sup> percentile spectral edge frequencies. The diagnostic interpretation was that these parameter increases along with paroxysmal activity, in the mid- and high doses, suggested generalized central neuronal damage with no cortical localization.

**Toxicokinetics:**

Toxicokinetic analysis of urine metabolite and brain extract data found the pattern, of elimination of the metabolites thiadone, thiadone-glucuronide and the conjugates of cysteine, and mercapturic acid were not linear among dose groups. Thiadone displayed a typical plateau pattern of high dose saturation and a lack of parallelism; its excretion appearing to be saturated at the mid- and high doses. Thiadone or an unstable thiadone conjugate was found in brain extract samples which suggests penetration across the blood brain barrier, and an increased residence time within this body compartment.

**Necropsy, gross pathology, histopathology:**

There were no gross necropsy' observations considered to be compound-related. Organ histopathology: weight differences were found which included: (1) increased relative heart weights (800 and 1600 ppm, both sexes) and increased absolute heart weights (800 ppm, males; 1600 ppm, both sexes), (2) increased relative kidney weights (800 ppm, males; 1600 ppm, both sexes) and absolute kidney weights (800 ppm, males; 1600 ppm, both sexes), (3) increased relative liver weights (800 and 1600 ppm, both sexes) and increased absolute liver weights (800 and 1600 ppm, both sexes), (4) increased relative adrenal weights (1600 ppm, females), and (5) increased relative thyroid (1600 ppm, both sexes) and increased absolute thyroid weights (1600 ppm, both sexes) Numerous micropathological observations were considered to be compound-related that occurred in the liver, kidney, eye, brain, spinal cord and sciatic nerve. These compound-related changes were limited to the 800 and/or 1600 ppm dose levels in both males and females. The principle observations in the liver were minimal to slight hepatocytomegaly and slight hepatocyte vacuolization. In the kidney, minimal to slight hyperplasia of the epithelium lining the renal pelvis was noted. In the eye, minimal to moderate vacuolization of the ciliary body epithelium and cystic vacuolization of the peripheral optic retina was noted. In the brain, spinal cord and sciatic nerve, a minimal to moderate axonopathy was noted. Ultra-structural examination by electron microscopy characterized the axons as enlarged myelin and/or non-myelinated membrane-lined round organelles (primarily mitochondria).

**Table B.6.5.3.4 -1 Summary results of Technical Grade FOE 5043: A Chronic Toxicity Feeding Study in the Beagle Dog**

<b>Flufenacet</b>	<b>Male</b>						<b>Female</b>					
<b>chronic oral dog</b>	<b>0</b>	<b>40</b>		<b>800</b>		<b>1600</b>	<b>0</b>	<b>40</b>		<b>800</b>		<b>1600</b>
Hb [g/dL] (day -14)	14.3	13.9		13.8		12.8	13.9	13.9		14.4		13.6
Hb [g/dL] (day 0)	14.4	14.0		14.2		13.2	14.1	14.3		14.9		14.0
Hb [g/dL] (day 91)	17.3	17		14.8	*	12.9	16.8	17		14.9	*	13.3
Hb [g/dL] (day 189)	18	16.7		15.4	*	14.3	17.1	17.2		15.2		14.5
Hb [g/dL] (day 280)	16.9	17.3		15.7	*	14.3	16	16.8		15.9		15.7
Hb [g/dL] (day 373)	17.4	17		15	*	15.0	16.7	18		16.1		16.4
Hematocrit [%] (day 91)	48.8	48		42.7	*	37.7	47.5	48		43	*	38.3
Hematocrit [%] (day 189)	50.3	46.3		43.9	*	41	47.6	47.8		43		41.5
Hematocrit [%] (day 280)	47.3	48.6		44.7		40.6	45.2	47.2		45.3		44.9
Hematocrit [%] (day 373)	47.4	46.2		42.1	*	41.8	45.3	49.2		44.2		45.3
RBC [ $10^6/\text{mm}^3$ ] (day 91)	7.43	7.33		6.88		6.03	7.05	7.33		6.85		6.03
RBC [ $10^6/\text{mm}^3$ ] (day 189)	7.63	7.05		7.18		6.58	7.1	7.15		6.9		6.48
Platelets [ $10^3/\text{mm}^3$ ] (day 91)	278	280		378	*	385	348	333		370		428
Platelets [ $10^3/\text{mm}^3$ ] (day 189)	268	260		353	*	398	345	298		363		448
Platelets [ $10^3/\text{mm}^3$ ] (day 280)	245	248		343	*	383	338	315		345		420
Platelets [ $10^3/\text{mm}^3$ ] (day 373)	263	250		350	*	285	353	280		385		470
Met-Hb [%] (day 91)	0.6	0.6		0.6		0.9	0.8	0.7		0.6		0.8
Met-Hb [%] (day 189)	1.7	0.8		1.1		1.4	1.1	1		1.1		1.2
Met-Hb [%] (day 280)	1	0.5		0.7		1.5	0.8	0.8		0.9		1.1
Met-Hb [%] (day 373)	0.6	0.5		0.8		1	0.8	0.7		1.1		1.4
MCV [ $\mu\text{m}^3$ ] (day 91)	65.8	65.5		62.1	*	62.6	67.3	65.5		62.7	*	63.8
MCHC [g/dL] (day 91)	35.5	35.5		34.6	*	34.1	35.4	35.3		34.6		34.6
MCH [pg] (day 91)	23.4	23.2		21.5	*	21.4	23.9	23.1		21.7	*	22
ALAT [U/L] (day -14)	39	32		27		29	32	38		32		28
ALAT [U/L] (day 0)	38	35		28		30	34	33		32		29
ALAT [U/L] (day 91)	48	34		16	*	15	38	34		20	*	12

Flufenacet	Male							Female									
chronic oral dog	0	40			800			1600			0	40		800		1600	
ALAT [U/L] (day 189)	49	43			28	*		21	*		42	39		20	*	17	*
ALAT [U/L] (day 280)	56	52			24	*		33	*		43	40		33		23	
ALAT [U/L] (day 373)	45	33			25	*		22	*		37	33		22	*	23	*
AP [U/L] (day -14)	183	192			199			177			213	178		169		193	
AP [U/L] (day 0)	152	172			174			159			166	144		143		166	
AP [U/L] (day 91)	99	119			163	*		170	*		102	91		112		181	*
AP ([U/L] day 189)	83	87			165	*		175	*		79	87		107		197	*
AP [U/L] (day 280)	73	86			137			155	*		69	80		104	*	211	*
AP [U/L] (day 373)	56	64			106	*		156	*		86	62		92		219	*
GGT [U/L] (day -14)	3	2			3			2			4	3		2		3	
GGT ([U/L] day 0)	2	2			1			2			2	2		3		1	
GGT [U/L] (day 91)	5	3	*		3	*		2	*		3	3		3		3	
CHOL [mg/dL] (day -14)	176	162			177			162			185	154		138		171	
CHOL [mg/dL] (day 0)	169	158			173			169			193	161	*	132	*	180	
CHOL [mg/dL] (day 91)	184	160			216			220			211	187		159		213	
CHOL [mg/dL] (day 189)	174	132	*		206			233	*		213	171		180		215	
CHOL ([mg/dL] day 280)	149	132			196	*		223	*		232	203		175		232	
CHOL [mg/dL] (day 373)	147	132			168			254	*		198	172		211		274	*
ALB (day 91)	3.5	3.1	*		3.2	*		2.8	*		3.5	3.6		3.1	*	2.9	*
GLUC [mg/dL] (day -14)	106	100			106			102			104	107		110		107	
GLUC [mg/dL] (day 0)	97	97			104			94			92	99		105	*	93	
GLUC [mg/dL] (day 91)	83	87			91			88			93	95		91		87	
T3[ng/mL] (day -14)	0.8	0.8			0.8			0.7			0.8	0.8		0.7		0.8	
T3 [ng/mL] (day 0)	0.8	0.8			0.8			0.7			0.8	0.8		0.7		0.8	
T3 [ng/mL] (day 91)	0.8	0.8			0.7			0.5	*		0.9	0.8		0.6	*	0.5	*
T3 [ng/mL] (day 189)	0.8	0.7			0.6	*		0.6	*		0.7	0.7		0.5	*	0.5	*
T3 [ng/mL] (day 280)	0.7	0.7			0.6			0.5			0.6	0.6		0.5		0.4	
T3 [ng/mL] (day 373)	0.7	0.6			0.6			0.5			0.7	0.6		0.6		0.6	
T4 [µg/dL] (day -14)	2.6	2.4			2.5			2.4			3	2.6		2.6		2.8	
T4 [µg/dL] (day 0)	2.5	2.4			2.5			2			2.9	1.9		1.9		2.3	
T4 [µg/dL] (day 91)	2.6	2.3			1.9			1.5			3.5	2.6		1.8	*	1.6	*



Flufenacet	Male							Female							
chronic oral dog	0	40			800		1600		0	40		800		1600	
T4 [µg/dL] (day 189)	2.5	2			1.3	*	1	*	2.8	2.5		1.4	*	0.9	*
T4 [µg/dL] (day 280)	2.5	2.4			1.9		1.4		3.1	2.1	*	1.5	*	1.1	*
T4 [µg/dL] (day 373)	2.4	1.7			1.5		1.7		3.6	2.6		2.3		2.1	
BW at terminal kill [g]	12291	11464			11842		10939		10566	9928		9047.3		8495.3	
Liver weight (rel.) [%]	3.112	3.120			3.877	*	5.442	*	3.207	3.075		3.875	*	5.258	*
Hepatocytomegaly	0	0			0		4	*	0	0		2		3	
Vacuolization	0	0			2		2		0	1		2		1	
Pituitary weight (abs.) [g]	0.0610	0.0720			0.0820		0.079		0.062	0.073		0.067		0.057	
Pituitary weight (rel.) [%]	0.0005	0.0006			0.0007		0.0007		0.0006	0.0007		0.0007		0.0007	
Thyroid weight (abs.) [g]	1.0160	1.0610			0.9920		1.419		0.787	0.771		0.739		1.028	
Thyroid weight (rel.) [%]	0.0083	0.0095			0.0084		0.0128		0.0074	0.0077		0.0081		0.0123	*
thyroid foll.-cell hypertrophy minimal	0	0			0		3		0	0		0		0	
Kidney weight (rel.) [%]	0.508	0.597			0.653	*	0.688	*	0.554	0.513		0.656		0.727	*
Pigmentation	0	0			1		3		0	0		0		2	
Hyperplasia of epithelial cells	0	1			3		4	*	0	0		3		3	
Heart weight (rel.) [%]	0.835	0.87			1.013	*	1.075	*	0.832	0.844		0.956	*	1.069	*
Adrenal weight (rel.) [%]	0.013	0.012			0.012		0.013		0.013	0.015		0.014		0.02	*
Eye															
vacuolization cytoplasm ciliary body epithelium	0	0			4 (1.8)	*	4 (2.0)	*	0	0		4 (1.8)	*	4 (2.3)	*
Cyst	3 (1.0)	3 (1.0)			4 (2.0)	*	4 (2.0)	*	4 (1.0)	3 (1.3)		4 (1.8)		4 (2.5)	*
Cataract	0	0			1 (1.0)		0		0	0		0		2 (1.0)	
Brain															
axonal degeneration	2 (1.0)	4 (1.0)			3 (2.7)		4 (2.8)	*	2 (1.0)	4 (1.3)		4 (2.5)	*	4 (2.8)	*
vacuolization	1 (1.0)	2 (1.0)			0		1 (3.0)		0	0		0		1 (2.0)	
vacuolar degeneration	0	0			0		1 (1.0)								
gliosis	0	0			0		0		0	0		0		1 (2.0)	
Spinal cord															
axonal degen.	2 (1.0)	0			4 (2.0)		4 (2.8)	*	2 (1.0)	1 (1.0)		4 (2.0)	*	4 (3.0)	*
vacuolisation	0	0			0		0		0	0		0		2 (1.5)	
Nerves															
Sciatic n., axonal degen.	0	0			0		4 (1.3)	*	0	0		0		4 (1.3)	*

Flufenacet	Male				Female			
chronic oral dog	0	40	800	1600	0	40	800	1600
Optic n., degeneration	0	0	0	0	0	0	0	1 (2.0)
Optic n., degen. hyaline	0	0	0	0	0	0	0	1 (2.0)

\* statistically significantly different at  $p < 0.05$

\*\* statistically significantly different at  $p < 0.01$

<b>Conclusion</b>	<p><b>The study is in line with current guidelines. RMS supports the proposal of the previous evaluation.</b></p> <p><b><u>NOAEL was established in the 40 ppm dose group</u></b>, Intermediate level of toxicity was found at 800 ppm and the high dose of 1600 ppm established the maximum tolerated dose. Dietary exposures of parent FOE 5043 and of thiadone metabolite will be essentially zero according to plant and animal metabolism studies. The Toxicological profile suggests a sensitivity in the dog relative to thiadone excretion. The preponderance of scientific mode of action data support the contention that limitations in glutathione interdependent pathways and antioxidant stress, resulted in metabolic lesions or abiotrophism in the eye, brain and kidney of these dogs.</p>
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The following expert statement refers to results of the chronic feeding study in beagle dogs which was previously presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and baseline dossier of flufenacet, KCA 5.3.2, M-005001-02-2.

**According to the evaluator (RMS), the data presented are additional**

<b>Report:</b>	KCA 5.3.2 /05; Bongartz, R.; 2012; M-430840-02; Amended: 2012-07-04
<b>Title:</b>	Expert statement (non GLP) - Flufenacet (FOE 5043): Explanation of the chromatographic behaviour of FOE-thiadone in the extract of brain from dogs of the chronic feeding study
<b>Report No:</b>	EnSa-12/0266
<b>Document No:</b>	M-430840-02-1
<b>Guidelines:</b>	<b>not applicable;</b>
	<b>Deviations: not applicable</b>
<b>GLP/GEP:</b>	<b>no</b>

In the chronic dog study the metabolite FOE-thiadone or an “unstable thiadone conjugate” was detected in the brain extracts. The detection was performed by LC-MS based on the mass of 169. Two signals with a mass of 169 were recorded in the time range from 19 to 22 min and one single signal in the first five minutes of the LC-MS analysis.

Small non-GLP experiments were conducted to clarify the chromatographic behaviour of FOE-thiadone in the brain extract and to give an explanation for the two signals with the mass of 169 in the time range from 19 to 22 min.

Due to the observations during the small non-GLP experiments and the scientific knowledge on the appearance of FOE-thiadone in two tautomeric forms, all the observed signals could be assigned to FOE-thiadone. In the small non-GLP experiments FOE-thiadone was stressed with formaldehyde, methanol and hydrochloric acid according to the conditions used during brain sample preparation in the chronic dog study. FOE-thiadone showed a similar chromatographic behaviour in the non-GLP experiments and in the chronic dog study.

**B.6.3.3 Other Routes****B.6.3.3.1 Subacute Dermal Study on Rats**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997)</b>
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<b>Report:</b>	<b>██████████ 1995</b>
<b>Title:</b>	<b>Repeated Dose 2 1-Day Dermal Toxicity Study with Technical Grade FOE 5043 in Rats</b>
<b>Document No:</b>	M-004981-01-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7682 of Sept. 29, 1995
<b>Guidelines:</b>	FIFRA § 82-2; OECD guideline 411; MAFF guideline 59 NohSan No. 4200; EU guideline L383A
<b>GLP</b>	yes

**Material and methods:**

FOE 5043, batch No.: F1036 from July 4, 1991, purity: 97.0 - 98.5% administration: dermal to the shorn skin of Sprague-Dawley rats (Sas:CD(SD)BR) for 21 days (6 hours per day) dosage (a.i.): 0, 20, 150, 1000 mg/kg body weight.

**Findings:**

No compound-related clinical signs. No alterations in food consumption from treated groups.

**Mortality:**

No incidences of mortality.

**Body weight:**

No significant alterations in body weight gain from controls.

**Local skin findings:**

No observed lesions.

**Hematology:**

No biologically-significant alterations from controls.

**Clinical chemistry (blood):**

Compound-dependent effects were limited to alterations in free thyroxine (FT<sub>4</sub>) and thyroxine (T<sub>4</sub>) levels in both sexes. Values from both males and females treated with 1000 mg/kg were significantly decreased from control levels at sacrifice. Additionally, FT<sub>4</sub> levels were significantly reduced from control values, while T<sub>4</sub> levels tended toward a declination in males treated with 150 mg/kg. No declinations from control values were observed from females treated with 150 mg/kg and no alterations from control values were noted from recovery' groups. Decrement in levels of circulating thyroid hormones have been noted previously from animals fed FOE 5043 and have been suspected to be causally related to an increased hepatic mixed function oxidase activity. The induction of hepatic metabolic activity and capacity is considered to be an adaptation of the liver in response to treatment with FOE 5043. In that thyroid hormones are degraded by hepatic oxidases, a decrement in

circulating thyroid hormone levels is anticipated as a passive consequence to induction of this metabolic system. Thus the decrements in FT<sub>4</sub> and T<sub>4</sub> levels noted above are considered as an indirect adaptive response to treatment rather than an adverse effect.

**Table B.6.3.3.1-1 Summary of clinical chemistry (Subacute Dermal Study on Rats)**

Parameter	Dose group (ppm) males				Dose group (ppm) females			
	0	20	150	1000	0	20	150	1000
T4 (µg/dL)	6.3	7.0	5.2	<b>3.2*</b>	4.3	5.1	4.3	<b>2.7*</b>
T3 (ng/mL)	0.7	0.6	0.7	0.5	0.6	0.7	0.6	0.5
TSH (ng/mL)	12.44	14.42	13.07	15.47	17.01	13.82	14.62	14.39
FreeT4 (ng/dL)	3.34	3.53	<b>3.02*</b>	<b>2.61*</b>	3.16	3.66	3.16	<b>2.21*</b>

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

#### **Gross pathology:**

No compound-related lesions noted.

#### **Organ weights:**

From males treated with either 150 or 1000 mg/kg/day both absolute and relative liver weights showed dose-dependent and treatment-related increases above control values. No similar increases were noted from females, and recovery animals showed no alterations in liver weight (either absolute and relative) from control levels. Increases in liver weights are considered to be a consequence of the microsomal induction provoked by FOE 5043, have previously been observed from animals treated orally with FOE 5043, and did not persist through the recovery period. These changes are anticipated as a consequence of induced metabolism and thus are considered as adaptive rather than as adverse responses to treatment.

**Table B.6.3.3.1-2 Organ weight summary data**

	Dose group (ppm) males				Dose group (ppm) females			
	0	20	150	1000	0	20	150	1000
Liver (absolute weight) (g)	10.837	11.165	<b>12.400*</b>	<b>12.911*</b>	7.111	7.265	7.052	7.149
Liver (relative weight) (%)	3.736	3.984	<b>4.357*</b>	<b>4.474*</b>	3.565	3.795	3.660	3.750

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

#### **Histopathology:**

Centrilobular hepatocytomegalia from 1000 mg/kg females. Not observed from males or from recovery animals. Changes characterized by an increase in cytoplasmic smooth endoplasmic reticulum and are considered to be associated with an increase in hepatic metabolism and microsomal induction.

Table B.6.3.3.1-1 Summary results for Subacute Dermal Study on Rats

Flufenacet	Male recovery								Female recovery							
21-d dermal rat	0	20	150	1000	0	1000			0	20	150	1000	0	1000		
<b>Body weight</b>																
BW day 0 [g]	258.2	255.9	255.8	257.8	255.9	255.8			206.9	200.7	200.5	200.4	201.7	203.3		
BW day 7 [g]	283.4	282	282.2	286.8	283.4	283.5			212.7	209.4	207.1	208.9	208.6	206.1		
BW day 14 [g]	307.6	299.1	303.1	309.4	304.5	309.2			219.6	209.8	217.3	214.9	220.1	210.9		
BW day 21 [g]	333.3	319	329.3	336.2	326.2	336.4			230.5	221.1	224.5	223.5	227.5	220.8		
<b>Clinical chemistry</b>																
T4 [µg/dL]	6.3	7	5.2	3.2	*	7.4	6.8		4.3	5.1	4.3	2.7	*	5.3	4.4	
T3 [ng/mL]	0.7	0.6	0.7	0.5		0.7	0.7		0.6	0.7	0.6	0.5		0.8	0.9	
TSH [ng/mL]	12.44	14.42	13.07	15.47		17.73	16.03		17.01	13.82	14.62	15.39		16.93	16.73	
free T4 [µg/dL]	3.34	3.53	3.02	*	2.61	*	2.94	2.99	3.16	3.66	3.16	2.21	*	2.75	2.67	
<b>Hematology</b>																
Cor WBC [ $10^3/\text{mm}^3$ ]	9.5	9.9	8.1	8.8		10.2	9.8		9.9	7.7	*	6.4	*	6.2	*	7.7
RBC [ $10^6/\text{mm}^3$ ]	8.59	8.57	8.41	8.4		8.7	8.66		8.54	8.22		8.1		8.09		8.64
Hb [g/dL]	16.9	17.1	16.9	16.6		16.7	16.4		16.2	15.8		15.7		15.4	*	16.8
HCT [%]	49.4	50.2	49.2	49.2		48.1	47.7		45.8	44.4	*	44.4		44.2	*	47.8
MCV [ $\mu\text{m}^3$ ]	57.6	58.5	59	58.6		55.3	55.2		53.8	54		54.9		54.8		55.5
MCH [pg]	19.6	19.9	20.1	19.7		19.2	19		19	19.2		19.4		19.1		19.5
MCHC [g/dL]	34.1	34.1	34	33.7		34.6	34.4		35.4	35.6		35.4		34.9		35.2
PLTS [ $10^3/\text{mm}^3$ ]	979	916	951	905		896	831		1051	931		958		960		949
<b>Clinical signs</b>																
<b>erythema</b>	4	3	3	3		2	6		2	3		3		2		3
<b>Terminal BW (g)</b>	290	281.1	284.4	288.5		326.9	332.5		199.6	191.8		192.4		190.9		213.3
<b>Organ weight, histopath.</b>																
Liver weight (abs) [g]	10.83	11.165	12.400	*	12.911	*	12.952	12.689	7.111	7.265		7.052		7.149		6.935
Liver weight (rel) [%]	3.736	3.984	4.357	*	4.474	*	3.936	3.788	3.565	3.795		3.660		3.75		3.250

21-d dermal rat	0	20		150		1000		0	1000		0	20		150		1000		0	1000	
centrilobular hepatocytomegaly	0	0		0		0		0	0		0	0		1		4	*	0	0	
Heart weight (rel) [%]	0.381	0.437	*	0.401		0.4		1.349	1.139	*	0.439	0.425		0.44		0.44		0.402	0.41	
Thyroid weight (abs.) [g]	0.021	0.021		0.021		0.022		0.021	0.022		0.017	0.017		0.017		0.02		0.016	0.015	
Thyroid weight (rel.) [%]	0.007	0.007		0.007		0.008		0.006	0.007		0.008	0.009		0.009		0.0104		0.008	0.007	

In the following summary tables statistical significances given as follows:

\* statistically significantly different at  $p < 0.05$

\*\* statistically significantly different at  $p < 0.01$

<b>Conclusion</b>	<p><b>The study is in line with current guidelines. RMS does not object to the NOAEL, supports the proposal of the previous evaluation.</b></p> <p>All observed effects attributed to adaptation to treatment with test material. No adverse effects noted.</p> <p>No-observable-effect levels:</p> <ul style="list-style-type: none"> <li>- 20 mg/kg bw/day for males.</li> <li>- 150 mg/kg bw/day for females</li> </ul> <p>Based on that NOAEL: 1000 mg/kg bw/day</p>
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<b>New studies; not evaluated</b>	A new studies. <b>No EU registration requirement.</b> In the opinion of RMS, presented a pilot study should be regarded as complementary to general information concerning the active substance. Not relevant for reference value derivation.
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<b>Report:</b>	<b>KCA 5.3.3 /02; [REDACTED]; 2008a</b>
<b>Title:</b>	<b>Flufenacet (FOE 5043) - 1-week inhalation pilot study in Wistar rats (exposure 6h/day, 5 days/week)</b>
<b>Document No:</b>	M-300005-01-1
<b>Report No:</b>	AT04505
<b>Guidelines:</b>	OECD 412; Directive 88/302/EEC, Annex V, Method B.29.; US-EPA 712C-98-193, OPPTS 870.3465;
<b>GLP</b>	no

## I. Materials and methods

### A. Materials

#### 1. Test materials:

Name:	FOE 5043
Description:	Whitish to brown flakes
Artikel no. / Development no.:	05125162/0157875
Lot/Batch no:	EDHB001715
Purity:	97%
Stability of test compound:	guaranteed for study duration; expiry date: 2009-05-14

#### 2. Vehicle:

none

#### 3. Test animals:

Species:	Wistar rat
Strain:	Hsd Cpb:WU
Age:	About 2 month
Weight at dosing:	Males: 196 g – 212 g; females: 153 g – 174 g
Source:	[REDACTED]
Acclimatization period:	At least 5-7 days
Diet:	standard fixed-formula diet KLIBA 3883 = NAFAG 9441 pellets maintenance diet for rats and mice (PROVIMI KLIBA SA, Kaiseraugst, Switzerland), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	singly in conventional Makrolon® Type III <sub>H</sub> cages; bedding: Litalabo (S.P.P.S., Frasné, France) and/or Lignocel BK 8-15 (Rettenmaier, Germany)

## B. Study design and methods

### 1. Animal assignment and treatment

Dose:	0-40-200-800 mg/m <sup>3</sup> air
Duration:	5 days, 6h exposure/day
Application route:	Inhalation, nose-only
Group size:	5/sex/group
Observations:	mortality, clinical signs, body weight, rectal temperature, haematology, clinical chemistry, gross necropsy, organ



weight

**2. generation of the test atmosphere / chamber description**

Generation and characterization of chamber atmosphere

	Group 1	Group 2	Group 3	Group 4
Target concentration (mg/m <sup>3</sup> )	control	40	200	800
Nominal concentration (mg/m <sup>3</sup> )	0	51.8	315	1091
Gravimetric concentration (mg/m <sup>3</sup> )*	--	47.9	225	846
Temperature (mean, °C)	20.8	21.7	21.8	21.6
Relative humidity (mean, %)	6.6	9.9	7.4	6.4
MMAD (µm)	--	2.32	2.51	2.43
GSD	--	1.95	1.98	2.06
Aerosol mass < 3 µm (%)	--	64.9	60.4	61.6
Mass recovered (mg/m <sup>3</sup> )	--	43.9	224.6	846.9
MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; -- = not applicable. * = actual concentration of test atmosphere in the vicinity of the breathing zone of the animals				

**II. Results and discussion****A. Mortality**

All exposures were tolerated without test substance-induced mortality.

**B. In life observations**

Animals of groups receiving target concentrations of 40 and 200 mg/m<sup>3</sup> air did not show any signs.

In rats of the 800 mg/m<sup>3</sup> air group the following signs were observed: piloerection, bradypnea, labored and irregular breathing patterns.

**Table 6.3.3/02-1: Summary of sub-acute inhalation toxicity**

Sex	Target concentration (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Mortality
males	0	0	0	5	--	--
	40	0	0	5	--	--
	200	0	0	5	--	--
	800	0	4	5	1d – 5d	--
females	0	0	0	5	--	--
	40	0	0	5	--	--
	200	0	0	5	--	--
	800	0	5	5	1d – 5d	--

\* 1<sup>st</sup> number = number of dead animals; 2<sup>nd</sup> number = number of animals with signs; 3<sup>rd</sup> number = number of animals exposed

0d = day of exposure; -- = not applicable

In comparison to the concurrent air control group, there was no evidence of a conclusive, toxicologically significant effect on body (rectal) temperatures at any exposure concentration. Additionally, the temperature measurements made on control animals demonstrate clearly that the animal restrainer had no apparent effect on the body temperature.

**C. Body weight**

There was no toxicologically consistent effect on body weights up to and including the 800 mg/m<sup>3</sup> group.

**D. Laboratory investigations**Haematology

Haematology revealed in female rats exposed at 800 mg/m<sup>3</sup> significantly decreased red blood cell counts, leukocyte counts, thrombocyte counts, haemoglobin and haematocrit. At this concentration, reticulocytes counts were increased ( $p > 0.05$ ). With regard to the lead changes (haemoglobin and haematocrit) the male rats showed the similar trend.

**Table 6.3.3/02-2: Summary of haematology**

	<b>ERY</b> (10 <sup>12</sup> /L)	<b>HB</b> (g/L)	<b>HCT</b> (L/L)	<b>MCV</b> (fl)	<b>MCH</b> (pg)	<b>MCHC</b> (g/L ERY)
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Males</b>					
0	6.89	132	0.428	62.1	19.2	309
40	6.73	129	0.420	62.3	19.2	308
200	6.74	130	0.414	61.5	19.3	314
800	6.81	126	0.412	60.6	18.6	307
	<b>ERY</b> (10 <sup>12</sup> /L)	<b>HB</b> (g/L)	<b>HCT</b> (L/L)	<b>MCV</b> (fl)	<b>MCH</b> (pg)	<b>MCHC</b> (g/L ERY)
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Females</b>					
0	6.95	136	0.426	61.2	19.6	320
40	6.80	132	0.407	59.8	19.5	326
200	6.91	132	0.406	58.8	19.1	325
800	6.61 <sup>+</sup>	125 <sup>++</sup>	0.398 <sup>+</sup>	60.2	19.0	316
	<b>LEUKO</b> (10 <sup>9</sup> /L)	<b>RETI</b> (‰)	<b>THRO</b> (10 <sup>9</sup> /L)	<b>HEINZ</b> (‰)		
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Males</b>					
0	2.89	27	1190	22		
40	3.67	29	1206	31		
200	3.14	37	1209	36 <sup>+</sup>		
800	4.81	39	1231	38		
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Females</b>					
0	2.48	25	1142	16		
40	2.61	22	1029	28 <sup>+</sup>		
200	3.44	26	995	27		
800	3.01	34	980 <sup>+</sup>	18		

<sup>+</sup> Statistically significant at  $p < 0.05$ , <sup>++</sup> statistically significant at  $p < 0.01$ ,

ERY = Erythrocytes, HB = Hemoglobin, HCT = Hematocrit, MCV = Mean Corpuscular Volume Erythrocytes, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, LEUKO = Leukocytes, RETI = Reticulocytes, THRO = Thrombocytes/Platelets, HEINZ = Heinz bodies

Clinical chemistry

In male rats exposed at 800 mg/m<sup>3</sup> decreased concentrations of T4 and increased concentrations of TSH existed. At 200 mg/m<sup>3</sup> the decrease of T4 gained statistical significance.

**Table 6.3.3/02-3: Summary of clinical chemistry**

Dose (mg/m <sup>3</sup> )	Males			Females		
	T3 (nmol/L)	T4 (nmol/L)	TSH (µg/L)	T3 (nmol/L)	T4 (nmol/L)	TSH (µg/L)
0	1.26	44	6.33	1.15	36	6.18
40	1.10	40	6.93	1.06	31	5.56
200	1.21	36 <sup>+</sup>	6.87	1.14	30	6.50
800	1.28	32	10.21 <sup>+</sup>	1.19	22	7.87

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

T3 Triiodothyronine, T4 Thyroxine, TSH Thyroid stimulating hormone

**F. Organ weight**

Collectively, with regard to the liver this analysis revealed significant changes in organ weights or the organ-to-body weight ratios in male and female rats at 200 mg/m<sup>3</sup> and above. Kidney weights were increased in the female rats exposed at 800 mg/m<sup>3</sup> only.

**Table 6.3.3/02-4: Summary of absolute organ weight data**

Dose (mg/m <sup>3</sup> )	Absolute organ weight (mg)									
	Males					Females				
	Lung	Heart	Liver	Spleen	Kidneys	Lung	Heart	Liver	Spleen	Kidneys
0	957	678	8830	396	1540	853	632	6463	321	1232
40	949	716	8546	385	1508	850	639	6954	403	1313
200	949	675	9371	389	1558	902	616	7905 <sup>++</sup>	369	1309
800	933	663	10140 <sup>++</sup>	382	1534	872	624	9334 <sup>++</sup>	408	1432 <sup>++</sup>

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

**Table 6.3.3/02-5: Summary of relative organ weight data**

Dose (mg/m <sup>3</sup> )	BW (g)	Relative organ weight (mg/100 g) vs. body weight				
		Lung	Heart	Liver	Spleen	Kidneys
	Males					
0	202	473	335	4121	195	762
40	197	481	363	4327	195	764
200	197	487	344	4762 <sup>++</sup>	198	791
800	194	480	341	5211 <sup>++</sup>	196	788
	Females					
0	161	530	393	4015	200	765
40	164	517	388	4228	245	798
200	163	553	377	4837 <sup>++</sup>	226	801
800	166	526	376	5625 <sup>++</sup>	247	863 <sup>+</sup>

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

**G. Gross pathology**

The gross pathological examination of the rats that were sacrificed at the end of the exposure period did not reveal evidence of any treatment-related organ damage.

In some female rats the 800 mg/m<sup>3</sup> air group an enlarged liver was noticed.

<b>Conclusion</b>	<b>Due to the nature of this pilot study, the duration of study period and number of parameters determined does not fully comply with the testing guidelines.</b>  The derived NOAEL based on the actual gravimetric concentration is 48 mg/m <sup>3</sup> (ca. 14 mg/kg bw/day), based on changes in organ weight, hematological and clinical chemistry parameters at the actual gravimetric concentration of 225 mg/m <sup>3</sup> (ca. 66 mg/kg bw/day) and above.
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<b>New studies; not evaluated</b>	<b>A new studies no EU registration requirement.</b> In the opinion of RMS, presented study should be regarded as complementary to general information concerning the active substance. Not relevant for reference value derivation.
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<b>Report:</b>	<b>KCA 5.3.3 /03; [REDACTED]; 2008b</b>
<b>Title:</b>	<b>Flufenacet (FOE 5043) - 4-week subacute inhalation study in Wistar rats (exposure 6h/day, 5 days/week on four consecutive weeks)</b>
<b>Document No:</b>	M-302961-01-2
<b>Report No:</b>	AT04589
<b>Guidelines:</b>	OECD 412, Directive 67/302/EEC, Annex V, Method B.29.; US-EPA 712C-98-193, OPPTS 870.3465;
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test materials:

Name:	FOE 5043
Artikel no. / Development no.:	05125162/0157875
Description:	Whitish to brown flakes
Lot/Batch no:	EDHB001715
Purity:	97%
Stability of test compound:	guaranteed for study duration; expiry date: 2009-05-14

#### 2. Vehicle:

none

#### 3. Test animals:

Species:	Wistar rat
Strain:	Hsd Cpb:WU
Age:	About 2 month
Weight at dosing:	Males: 196 g – 238 g; females: 159 g – 187 g
Source:	[REDACTED]
Acclimatization period:	Approx. 2 weeks
Diet:	standard fixed-formula diet KLIBA 3883 = NAFAG 9441 pellets maintenance diet for rats and mice (PROVIMI KLIBA SA, Kaiseraugst, Switzerland), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	singly in conventional Makrolon® Type IIIh cages; bedding: Lignocel BK 8-15 (Rettenmaier, Germany)

### B. Study design and methods

## 2. Animal assignment and treatment

Dose:	0-20-220-400 mg/m <sup>3</sup> air
Duration:	6h exposure / day, 5 days/week, 4 weeks
Application route:	Inhalation, nose-only
Group size:	10/sex/group
Observations:	mortality, clinical signs, body weight, rectal temperature, ophthalmology, reflex measurement, haematology, clinical chemistry, urinalysis, gross necropsy, organ weight, histopathology

## 2. generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

	Group 1	Group 2	Group 3	Group 4
Target concentration (mg/m <sup>3</sup> )	control	20	220	400
Nominal concentration (mg/m <sup>3</sup> )	0	22	314	513
Gravimetric concentration (mg/m <sup>3</sup> )*	--	19.1	220	409
Temperature (mean, °C)	21.3	22.1	22.4	22.3
Relative humidity (mean, %)	18.9	23.7	21.3	21.7
MMAD (µm)	--	2.23	2.35	2.44
GSD	--	1.90	2.05	2.14
Aerosol mass < 3 µm (%)	--	68.0	63.5	60.8
Mass recovered (mg/m <sup>3</sup> )	--	17.1	228.9	383.7
MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; -- = not applicable. * = actual concentration of test atmosphere in the vicinity of the breathing zone of the animals Recovery: Relative yield gravimetric (actual) concentration to nominal concentration. For details of the dilution of atmospheres see the respective 'Method Section'. Representative exposure period: Target concentrations were defined by the sponsor at the start of study. Accordingly all experimentally verified/calibrated settings had to be changed on the first exposure day with fine-adjustments on the following exposure days. All nominal settings represent the main study period without the adjustment phase.				

## Results and discussion

### A. Mortality

All exposures were tolerated without test substance-induced mortality.

One female rat of the high-level exposure group (400 mg/m<sup>3</sup>) showed clinical signs (flaccidity, high-legged gait, muzzle area with red encrustations) after the second exposure and was sacrificed in a moribund state prior to exposure on the third day in study (day 2). Possibly the rat was injured or injured itself as a result to restraint. The rat was replaced by a new rat from the same batch.

### B. In life observations

An irregular breathing pattern was consistently recorded in one to two female rats of the 400 mg/m<sup>3</sup> group and flaccidity was observed in one male rat of the 220 mg/m<sup>3</sup> group. These signs did not progress over time or occurred in a dose-dependent manner. Tachypnea and piloerection also occurred in single rats at isolated time points. Therefore, the signs recorded in individual rats are concluded to be caused to exposure-related factors (restraint and associated immobilizing stress) of individual animals.

The reflex examination made within the first and last exposure week did not reveal any differences between the groups.

There was no evidence of a conclusive, toxicologically significant effect on body (rectal) temperatures at any exposure concentration in comparison to the concurrent air control group. Additionally, the

temperature measurements made on control animals demonstrate clearly that the animal restrainer had no apparent effect on the body temperature

### C. Body weight

There was no toxicologically consistent effect on body weights up to and including the 400 mg/m<sup>3</sup> group.

Statistical significant changes occurred in all male-rat substance-exposure groups relative to the air control. However, despite this difference to the control, no concentration-dependent changes across exposure groups were apparent. Accordingly, as far as significant changes were observed they are considered to be of no pathodiagnostic relevance.

**Table 6.3.3/03-1: Summary of body weights**

Dose (mg/m <sup>3</sup> )	Day 1	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21	Day 25	Day 28
Mean body weight (g) - males									
0	225.37	221.57	236.94	241.89	258.22	258.76	275.65	275.70	291.51
20	222.08	214.69	228.63	225.63	237.83 <sup>+</sup>	242.70	255.55	259.01	271.80
220	221.08	212.46	224.98	218.89 <sup>++</sup>	230.62 <sup>++</sup>	231.31 <sup>++</sup>	244.91 <sup>++</sup>	246.50 <sup>++</sup>	259.34 <sup>++</sup>
400	223.54	216.24	229.53	223.84 <sup>+</sup>	235.29 <sup>++</sup>	238.06	252.28 <sup>+</sup>	255.00	267.48 <sup>+</sup>
Mean body weight (g) - females									
0	172.64	173.21	181.86	182.14	190.74	191.68	200.30	202.01	208.94
20	171.90	169.98	176.61	173.69 <sup>+</sup>	177.36 <sup>+</sup>	182.45	187.96 <sup>+</sup>	193.94	198.92
220	175.74	177.85	185.58	178.46	183.53	185.90	192.08	197.33	202.16
400	173.61	176.24	181.92	178.55	182.92	186.77	191.51	196.35	199.07

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01,

### D. Ophthalmology

Ophthalmology performed (prior to the start of the study, towards the end of the study) did not reveal any conclusive evidence of test substance-induced changes in the dioptric media or in the fundus.

### E. Laboratory investigations

#### Haematology

Rats exposed to 220 mg/m<sup>3</sup> (most changes significant in males and trend in females) and 400 mg/m<sup>3</sup> (significant in male and female rats) showed evidence of haematological changes indicated by decreased red blood cell counts, decreased haemoglobin and haematocrit, and increased reticulocyte counts and red blood cells with Heinz bodies. Blood differentials revealed that red blood cells were hypochromic (males and females) at 400 mg/m<sup>3</sup>.

**Table 6.3.3/03-2: Summary of haematological examinations**

	<b>ERY</b> (10 <sup>12</sup> /L)	<b>HB</b> (g/L)	<b>HCT</b> (L/L)	<b>MCV</b> (fl)	<b>MCH</b> (pg)	<b>MCHC</b> (g/L ERY)
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Males</b>					
0	7.55	138	0.442	58.6	18.3	312
20	7.65	136	0.432	56.5	17.9	316
220	7.32	129 <sup>++</sup>	0.418 <sup>++</sup>	57.2	17.6	309
400	6.87 <sup>++</sup>	122 <sup>++</sup>	0.407 <sup>++</sup>	59.5	17.8	300 <sup>++</sup>
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Females</b>					
0	7.33	133	0.419	57.1	18.2	318
20	7.49	134	0.411	54.9 <sup>+</sup>	17.9	325 <sup>+</sup>
220	7.09	127	0.399	56.3	17.9	318
400	7.16	131	0.426	59.4 <sup>++</sup>	18.3	308 <sup>++</sup>

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01,  
 ERY = Erythrocytes, HB = Haemoglobin, HCT = Haematocrit, MCH = Mean Corpuscular Haemoglobin,  
 MCHC = Mean Corpuscular Haemoglobin Concentration, MCV = Mean Corpuscular Volume Erythrocytes

**Table 6.3.3/03-3: Summary of haematological examinations**

	<b>LEUKO</b> (10 <sup>9</sup> /L)	<b>RETI</b> (‰)	<b>THRO</b> (10 <sup>9</sup> /L)	<b>HEINZ</b> (‰)	<b>HQUICK</b> (sec)
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Males</b>				
0	3.83	21	1019	0	42.6
20	3.48	19	983	0	43.6
220	4.77	37 <sup>++</sup>	1025	11 <sup>++</sup>	44.0
400	3.74	51 <sup>++</sup>	1066	16 <sup>++</sup>	42.9
<b>Dose (mg/m<sup>3</sup>)</b>	<b>Females</b>				
0	3.29	25	1069	0	38.2
20	3.78	16 <sup>++</sup>	1070	1	36.8
220	3.24	25	1043	4 <sup>++</sup>	37.2
400	3.62	33 <sup>++</sup>	1031	6 <sup>++</sup>	38.2

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01,  
 LEUKO = Leukocytes, RETI = Reticulocytes, THRO = Thrombocytes/Platelets, HEINZ = Heinz bodies,  
 HQUICK = Hepato Quick (prothrombin time)

### Clinical chemistry

Male rats exposed to 220 and 400 mg/m<sup>3</sup> showed decreased serum alkaline phosphatase and triglyceride activities/concentrations. The latter was already significantly decreased at 20 mg/m<sup>3</sup>. T<sub>3</sub> and T<sub>4</sub> were significantly decreased at 400 mg/m<sup>3</sup>. In contrast, in the female rats significant increase in triglycerides occurred; however, without any conclusive dose-dependence. Thyroidal endpoints showed a similar trend as observed in male rats.

Hepatic monooxygenase and cytochrome P450 activities were increased at 20 mg/m<sup>3</sup> and above (changes in cytochrome P450 > O-demethylase > N-demethylase activities).



**Table 6.3.3/03-4: Summary of clinical chemistry determinations in blood**

Dose (mg/m³)	ASAT (U/L)	ALAT (U/L)	ALP (U/L)	GLDH (U/L)	CK (U/L)	LDH (U/L)	Glucose (mol/L)	BILI-t (µmol/L)	PROT (g/L)
	Males								
0	79.9	63.3	201	5.6	278	118	4.76	1.3	57.4
20	82.7	58.6	197	4.7	244	90	5.07	1.2	55.6
220	83.9	56.6	160 <sup>++</sup>	4.4	313	107	5.05	1.3	55.5
400	89.1	56.0	170 <sup>++</sup>	4.8	331	123	5.13	1.2	55.4
	Females								
0	91.5	55.0	128	9.7	343	156	5.10	1.4	58.9
20	106.8	54.6	125	10.4	454	190	5.59	1.0 <sup>+</sup>	56.6 <sup>+</sup>
220	126.6	59.5	131	5.8	525	225	5.21	1.0 <sup>+</sup>	57.6
400	97.5	56.0	135	10.6	380	179	4.90	1.1 <sup>+</sup>	59.3
Dose (mg/m³)	ALB (g/L)	CHOL	TRIGL	Urea	CREA	Na	K	Ca	Cl
	(mmol/L)								
	Males								
0	31.2	1.55	0.59	7.46	60	144	5.4	2.56	99
20	30.7	1.32	0.38 <sup>+</sup>	7.31	56	144	5.5	2.48	99
220	30.5	1.24 <sup>+</sup>	0.34 <sup>++</sup>	7.90	55 <sup>+</sup>	144	5.5	2.52	99
400	30.7	1.31	0.24 <sup>++</sup>	7.87	59	145	6.2	2.51	98
	Females								
0	32.6	1.19	0.37	8.08	57	143	5.3	2.51	98
20	31.7	1.33	0.85 <sup>++</sup>	8.10	56	143	5.1	2.47	99
220	31.6	1.29	0.78 <sup>+</sup>	7.65	55	143	5.0	2.48	102 <sup>++</sup>
400	32.7	1.28	0.61	7.57	61	145	5.5	2.52	99
Dose (mg/m³)	Mg (mmol/L)	P	T3 (nmol/L)	T4	TSH (µg/L)				
	Males								
0	1.35	2.76	1.29	55	7.45				
20	1.27	2.62	1.25	49	9.32				
220	1.27	3.12	1.19	47	8.30				
400	1.40	3.09	1.11 <sup>+</sup>	39 <sup>+</sup>	8.20				
	Females								
0	1.29	2.08	1.14	38	5.67				
20	1.25	2.33	1.16	33	6.71				
220	1.18	2.13	1.07	32	6.31				
400	1.46	2.34	1.00	24 <sup>+</sup>	6.85				

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

ALAT (GPT) = Alanine aminotransferase, ALP = Alkaline phosphatase, ASAT (GOT) = Aspartate aminotransferase, CK = Creatine kinase NAC, GLDH = Glutamate dehydrogenase, LDH = Lactate dehydrogenase, ALB = Albumin, BILI-t = Bilirubin total, CHOL = Cholesterol, CREA = Creatinine, PROT = Protein, TRIGL = Triglycerides, UREA = Urea, T3 = Triiodothyronine, T4 = Thyroxine, TSH = Thyroid stimulating hormone

**Table 6.3.3/03-5: Summary of clinical chemistry determinations in liver tissue**

Dose (mg/m <sup>3</sup> )	males				females			
	N-DEM (mU/g)	O-DEM (mU/g)	P450 (mmol/g)	TRGL (mmol/g)	N-DEM (mU/g)	O-DEM (mU/g)	P450 (mmol/g)	TRGL (mmol/g)
0	127.7	11.7	42.9	5.75	65.0	9.2	36.0	5.61
20	138.1	12.7	55.7 <sup>++</sup>	6.18	77.8	10.3	40.7 <sup>++</sup>	6.18
220	179.3	22.3 <sup>++</sup>	72.6 <sup>++</sup>	6.51	116.6 <sup>++</sup>	17.3 <sup>++</sup>	51.4 <sup>++</sup>	6.47 <sup>+</sup>
400	235.1 <sup>++</sup>	25.5 <sup>++</sup>	73.7 <sup>++</sup>	6.32	131.0 <sup>++</sup>	19.3 <sup>++</sup>	55.2 <sup>++</sup>	6.41

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

N-DEM = Aminopyrine-N-Demethylase, O-DEM = Nitroanisole-0-Demethylase, P450 = Cytochrome P450, TRIGL = Triglycerides

#### Urine analysis

There were no effects considered to be of pathodiagnostic relevance. However, at 400 mg/m<sup>3</sup> there was a tendency of an elevated osmolality of urine. Sediment analysis was unobtrusive.

#### F. Organ weight

In rats of the 220 and 400 mg/m<sup>3</sup> air exposure groups, spleen weights were significantly increased in a concentration-dependent manner. At 400 mg/m<sup>3</sup> liver and kidney weights were increased in addition.

**Table 6.3.3/03-6: Absolute organ weights**

Dose (mg/m <sup>3</sup> )	Brain	Absolute organ weight (mg)							
		Lung	Heart	Liver	Spleen	Kidneys	Adrenals	Testes / Ovaries	Thymus
Males									
0	1813	1185	987	11319	567	2090	51	2968	499
20	1804	1155	915	10087 <sup>+</sup>	550	2038	53	2792	423
220	1737	1118	883 <sup>+</sup>	10095 <sup>+</sup>	635	1993	46	2729	416
400	1776	1174	932	11168	764 <sup>++</sup>	2107	48	2852	406
Females									
0	1764	995	746	8018	455	1532	66	139	381
20	1703	943	724	7741	426	1455	64	124	320
220	1733	1017	768	8523	477	1531	62	129	341
400	1707	1020	730	8658	508	1526	67	136	335

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

**Table 6.3.3/03-7: Relative organ weights versus body weights**

Dose (mg/m <sup>3</sup> )	BW (g)	Brain	Relative organ weight (mg/100g) vs. body weight							
			Lung	Heart	Liver	Spleen	Kidneys	Adre- nals	Testes / Ovaries	Thymus
Males										
0	290	625	409	340	3900	196	721	17	1024	172
20	271	667	426	337	3722	203	752	20	1031	256
220	257 <sup>++</sup>	684	438	343	2916	249 <sup>++</sup>	777	18	1064	160
400	268	667	439	349	4186	288 <sup>++</sup>	789 <sup>+</sup>	18	1068	150
Females										
0	207	854	481	361	3871	220	741	32	67	184
20	195 <sup>+</sup>	872	483	370	3957	218	746	33	64	164
220	198	877	514	388	4295 <sup>++</sup>	241	775	31	65	172
400	199	857	512	366	4339 <sup>++</sup>	255 <sup>++</sup>	766	34	68	168

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

**Table 6.3.3/03-8: Relative organ weights versus brain weights**

Dose (mg/m <sup>3</sup> )	Brain	Relative organ weight (mg/100 g) vs. brain weight							
		Lung	Heart	Liver	Spleen	Kidneys	Adre- nals	Testes / Ovaries	Thymus
Males									
0	1813	65606	54570	627728	31478	115750	2806	164046	27649
20	1804	64120	50785	560832	30514	113018	2931	154632	23418
220	1737	64617	50886	583968	36686 <sup>+</sup>	115146	2641	157454	24028
400	1776	66123	52438	628809	42142 <sup>++</sup>	118642	2671	160397	22806
Females									
0	1764	56438	42339	454730	25787	86960	3739	7876	21645
20	1703	55406	42511	454003	25001	85584	3779	7293	18774
220	1733	58730	44271	492504	27534	88371	3576	7418	19719
400	1707	59775	42769	507192 <sup>++</sup>	29784 <sup>+</sup>	89408	3913	7933	19606

<sup>+</sup> Statistically significant at p<0.05, <sup>++</sup> statistically significant at p<0.01

### G. Gross pathology

The gross pathological examination of the rats that were sacrificed at the end of the exposure period did not reveal evidence of any treatment-related organ damage.

### H. Micropathology

At the end of the exposure period, histopathological evaluation revealed goblet cell hyperplasia in the proximal nasal cavity at all exposure levels. In the more posterior levels, goblet cell hyperplasia together with eosinophilic globules and focal inflammatory infiltrates in the olfactory epithelium occurred at 220 and 400 mg/m<sup>3</sup>. Beginning epithelial alterations, including atrophy or degenerative changes, occurred at the 400 mg/m<sup>3</sup> exposure level only. In the larynx, epithelial alteration and concomitant increased inflammatory infiltrates epithelial metaplasia occurred at 220 and 400 mg/m<sup>3</sup>.

Minimal epithelial effects were already observed at 20 mg/m<sup>3</sup>; however, without conclusive influx of inflammatory cells. No changes were observed in the trachea or lung.

Focal tubular atrophy and/or degeneration of the testes, spermatid debris in the testes and epididymides and oligospermia occurred at all exposure levels. Retinal atrophy and/or degeneration occurred at 20 mg/m<sup>3</sup> and above in a concentration-dependent manner. However, based on the histopathological findings observed in the upper respiratory tract some non-specific irritant stress might have caused these effects and may have superimposed immobilization related distress. Based on these thoughts these changes appear to be associated with non-specific effects.

In the liver, cytoplasmic change and/or hypertrophy occurred in males, beginning in at 20 mg/m<sup>3</sup>. Prussian Blue stained slides revealed a minimal pigmentation; however, expressed in a concentration dependent manner. This type of pigmentation was more pronounced in female as compared to males.

In the spleen, an increased hematopoietic activity existed at 220 mg/m<sup>3</sup> and above, in some rats associated with increased blood congestion. Prussian Blue stained sections revealed a concentration-dependent increase at 220 mg/m<sup>3</sup> and above.

In the thyroid, follicular cell hypertrophy occurred at 220 mg/m<sup>3</sup> and above in males and at 400 mg/m<sup>3</sup> in females.

The findings listed above are assessed to be related to the exposure to the test compound. All other findings seen during histopathological evaluation are assessed to be of spontaneous nature. **Due to the absence of evidence of adversity the no-observed-adverse effect level is considered to be 20 mg/m<sup>3</sup>.**

<b>Conclusion</b>	<b>The study is in line with current guidelines.</b> The derived <b>NOAEC</b> based on the actual gravimetric concentration is 19 mg/m <sup>3</sup> (ca. 7 mg/kg bw/day), based on changes in organ weight, hematological and clinical chemistry parameters, histopathological changes the nasal cavity and larynx, spleen, testes, thyroid at 220 mg/m <sup>3</sup> (ca. 81 mg/kg bw/day) and above.
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## Summary of toxicity studies

**Table 1: Summary of acute toxicity studies\***

Route/Study	Species	Sex	Results	Reference
Oral	Mouse	M F	LD <sub>50</sub> : 1331 mg/kg bw 1756 mg/kg bw	██████████, 1991 M-004850-01-1
Oral <sup>2)</sup>	Rat	M	LD <sub>50</sub> : 683 mg/kg bw	██████████, 1992a M-004864-01-1
Oral <sup>1)</sup>	Rat	M F	LD <sub>50</sub> : 1617 mg/kg bw 589 mg/kg bw	██████████, 1993 M-004865-02-1
Dermal	Rat	M F	LD <sub>50</sub> : >2000 mg/kg bw >2000 mg/kg bw	██████████, 1992 M-004843-01-1
Inhalation (aerosol, 4h)	Rat	M F	LC <sub>50</sub> : >3740 mg/m <sup>3</sup> >3740 mg/m <sup>3</sup>	██████████, 1990 M-004844-01-1
Skin irritation	Rabbit	M	Not irritating	██████████, 1992 M-004846-01-1
Eye irritation	Rabbit	M	Not irritating	██████████, 1992 M-004847-01-1
Skin sensitisation Buehler method	Guinea pig	M	Not sensitizing	██████████, 1992 M-004845-01-1
Skin sensitisation M&K method	Guinea pig	M	Sensitizing	██████████, 1994 M-004637-01-1
<b>Skin sensitisation M&amp;K method</b>	<b>Guinea pig</b>	<b>F</b>	<b>Sensitizing</b>	<b>██████████, 1995 M-004677-01-1</b>
<b>Skin sensitization Local lymph node assay</b>	<b>Mouse</b>	<b>F</b>	<b>Not sensitizing</b>	<b>██████████, 2004 M-090513-01-1</b>
<b><i>In vitro</i> 3T3 NRU phototoxicity test</b>	<b>BALB/c 3T3 cells</b>		<b>Not phototoxic</b>	<b>Heppenheimer, 2013 M-464615-01-1</b>

\* New studies, i.e. studies previously not submitted, are written in bold M = male, F= female; <sup>1)</sup> animals were fasted (overnight); <sup>2)</sup> animals were non-fasted

### Comprehensive summary of acute toxicity, primary irritation and sensitization studies:

FOE 5043 has a low to moderate order of acute toxicity by the oral route, and a low order of acute toxicity by the dermal and inhalation routes of exposure. It is not irritating to the skin, and essentially no irritating to the eyes. The results of the dermal sensitization studies revealed equivocal evidence of allergenic potential. The Maximization test was positive, the more practice relevant Buehler test was negative

**Table 2: Summary of short-term toxicity studies**

Study	Sex	NOAEL mg/kg bw/day	LOAEL	Main findings seen at LOAEL	Reference
Rat 21-day dermal	M F	1000 1000	-- --	No adverse effects noted. T4 ↓, liver findings considered adaptive response to treatment.	██████████, 1995 M-004981-01-1
Rat 1-week inhalation	M, F	~14 48 mg/m <sup>3</sup>	~66 225 mg/m <sup>3</sup>	T4 ↓ Liver: rel. weight ↑	██████████, 2008 M-300005-01-1
Rat 4-week inhalation	M, F	~7 19 mg/m <sup>3</sup>	~81 220 mg/m <sup>3</sup>	HB ↓, HCT ↓, RETI ↑, HEINZ ↑, AP ↓, TG ↓, Liver: enzymes ↑, rel. weight ↑, spleen: weight ↑, histopathological changes in nasal cavity and larynx, spleen, testes, thyroid, liver	██████████, 2008 M-302961-01-1
Rat 90-day feeding	M F	-- <sup>a)</sup> 7.2	6.0 29	HB ↓, T4 ↓, GLUC ↓, Liver: weight ↑, hepatocellular swelling, cell degeneration or necrosis; spleen: brown granular pigment accumulation within red pulp; kidney: mild renal proximal tubule injury	██████████, 1995 M-004999-01-1
Mouse 90-day feeding	M F	18 25	64 91	T4 ↓ Liver: rel. weight ↑	██████████, 1995 M-004985-01-1
Dog 90-day feeding	M F	1.7 1.7	7.2 6.9	ALAT ↓, LDH ↑, albumin ↓, globulin ↑, T4 ↓, GLUC ↓, Spleen: pigment, kidney: rel. weight ↑	██████████, 1995 M-004977-02-1
Dog 1-year feeding	M F	1.3 1.1	28 27	Hb ↓, Hct ↓, MCV ↓, MCH ↓, MCHC ↓, CHOL ↑, GLUC ↓, T4/T3 ↓, ALAT ↓, AP ↑, albumin ↓, Liver, heart, kidney: abs. + rel. weight ↑	██████████, 1995, 1997 M-005001-02-2

a) The subchronic NOEL for males was established on the basis of the toxicity profile which emerged through the first year of the 2-year rat study. M = male, F = female, ↑ = increase, ↓ = decrease

### Comprehensive summary of short-term toxicity:

In subchronic oral studies in rats, mice, and dogs, the main target organs affected by exposure to FOE 5043 were brain, thyroid, liver, kidney, and spleen as indicated by changes in clinical chemistries, organ weights and/or histopathological findings. The comparative species differences in toxicological profile, find the rat and the mice similar in primary and secondary target organs, but a sensitivity of certain cell types was observed in the dog as evidenced by histopathological lesions of vacuoles in the brain. Alterations in circulating serum thyroid hormones (thyroxine-T4 and triiodothyronine-T3) were observed in each species and were considered indicative of hepatic interference. Primary hematological parameters affected by treatment in each species included changes in erythrocytes, platelets, hemoglobin, and hematocrit concentrations. Histopathological findings generally correlated with alterations in organ weights. A decrease in body weight gain was observed in rats and mice

### B.6.4 Genotoxicity testing

#### Summary of genotoxicity testing

Mutagenicity studies with flufenacet were consistently negative. Point mutation assays in bacteria and mammalian cells revealed no evidence of mutagenic potential. In vitro and in vivo cytogenetic studies revealed no evidence of clastogenicity, and an unscheduled DNA synthesis assay using primary rat hepatocytes revealed no evidence of genotoxic activity. Thus, flufenacet is not mutagenic, clastogenic or genotoxic.

Furthermore, the conduct of an *in vivo* study in germ cells was not regarded necessary as there is no evidence of an effect on germ cells in other toxicological studies.

#### **Photomutagenicity**

According to the new data requirements (Commission regulation (EU) N° 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013), the conduct of a photomutagenicity study should be considered if the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is greater than  $1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , and if the structure of the molecule indicates a potential for photomutagenicity.

For flufenacet there is no evidence of a photoreactivity potential and the Ultraviolet/visible molar extinction/absorption coefficient is smaller than  $1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ .

**Therefore photomutagenicity testing is not required.**

**Table 6.4-1: Summary of genotoxicity testing\***

Study	Test system	Results		Reference
		activation	non-activation	
<i>In-vitro</i>				
Bacterial reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	negative	negative	Herbold, 1995 M-004696-01-1
<b>Bacterial reverse mutation assay</b>	<b><i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537</b>	<b>negative</b>	<b>negative</b>	<b>Sokolowski, 2010 M-395211-01-1</b>
Mammalian cell gene mutation test (HGPRT)	Chinese hamster lung fibroblasts V79	negative	negative	Brendler-Schwaab, 1994 M-004634-01-1
Mammalian chromosome aberration test	Chinese hamster ovary cells CHO	negative	negative	Gahlmann, 1995 M-004692-01-1
Unscheduled DNA synthesis (UDS) assay	Primary rat hepatocytes	negative	negative	Brendler-Schwaab, 1992 M-004577-01-1
<i>In-vivo</i>				
Micronucleus test	Mouse bone marrow	negative		██████, 1993 M-004588-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

**B.6.4 Genotoxicity testing**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997); Unscheduled DNA synthesis in rat liver primary cell cultures in vitro studies was presented and evaluated during the EU process for Annex I listing.</b>
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**B.6.4.1 In vitro studies**

<b>Report:</b>	<b>KCA 5.4.1 /01; S. Brendler-Schwaab; 1992</b>
<b>Title:</b>	<b>FOE 5043 - Test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro.</b>
<b>Document No:</b>	M-004577-01-1
<b>Report No:</b>	Bayer AG, unpublished report no. 21885 of Dec. 03, 1992
<b>Guidelines:</b>	EEC Directive 87/302/EEC; OECD guideline no. 482; New and Revised Health Effects Test Guidelines October 1984 (U.S.): EPA HG - DNA - Unshed Syn., October 1983
<b>GLP</b>	Yes

**Material and methods:**

FOE 5043, batch no.: 17001/90, purity: 92.6%, dissolved in DMSO positive control: 2-acetylaminofluorene (2-AAF): 0.5 µg/mL duration: single administration.

**Findings:**

The results of the UDS assay are summarized in the tables.

**Cytotoxicity assay**

Cells seeded per dish:  $7.5 \times 10^5$  viable cells

Time of treatment: 23 hours



Test condition		Dish no. 1	Dish no. 2	Average number	Viable Cells	Relative survival
		(cells x10 <sup>6</sup> )	(cells x10 <sup>6</sup> )	of cells x10 <sup>6</sup>	(%)	(%) <sup>a</sup>
vehicle control <sup>b</sup>		0.49	0.82	0.66	77.4	100.0
FOE 5043						
3.9 µg/ml		0.47	0.59	0.53	78.8	101.8
7.8 µg/ml		0.47	0.85	0.66	58.2	75.2
15.6 µg/ml		0.52	0.38	0.45	23.0	29.7
31.25 µg/ml		0.61	0.42	0.52	18.5	23.9
62.5 µg/ml		0.22	0.18	0.20	26.7	34.5
125.0 µg/ml		0.23	0.63	0.43	0	0
250.0 µg/ml		0.13	0.15	0.14	0	0
500.0 µg/ml		I	I			

a relative to vehicle control

b 1% vehicle in medium

I insoluble material present: if both dishes are affected, no more values are available for that concentration

## Summary of data of the rat hepatocyte UDS assay

Test condition		Net grains per nucleus <sup>a</sup>	Mean cytoplasmic grain counts <sup>b</sup>	Average <sup>c</sup> % cells in repair	Survival <sup>d</sup> (%)
		± S.D.	± S.D.		
vehicle control (DMSO; 1%)		-1.49 ± 0.43	3.93 ± 0.53	0	100.0
FOE 5043					
2.5	µg/ml	-0.97 ± 0.31	2.58 ± 0.93	0	99.9
5.0		-1.33 ± 0.09	3.58 ± 0.52	0	76.8
10.0	X	-0.17 ± 0.06	0.99 ± 0.06	0	64.5
20.0		-0.34 ± 0.27	5.07 ± 0.42	0.7	64.6
40.0	X	-0.56 ± 0.04	2.06 ± 0.52	0	42.2
60.0		no slides evaluable			11.4
80.0		no slides evaluable			6.4
positive control 2-AAF					
0.25	µg/ml	9.32 ± 0.74	3.75 ± 0.82	88.0*	71.2

a average of net nuclear grain counts on duplicate or triplicate coverslips

b average values for duplicate or triplicate coverslips

c average values of percentages of cells with 5 or more net nuclear grains on duplicate or triplicate coverslips

d number of viable cells relative to vehicle control

\* significant increase (significance level ≤ 0.05)

X one slide not evaluable

<b>Conclusion</b>	<b>The study is in line with current guidelines.</b> The test article is considered negative in the <i>in vitro</i> Rat Primary Hepatocyte UDS Assay.
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Previous evaluation	In DAR for original approval (1997); <b>Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay <i>in vitro</i> was presented and evaluated during the EU process for Annex I listing.</b>
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Report:	KCA 5.4.1 /02; S. Brendler-Schwaab; 1994
Title:	<b>FOE 5043 - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay <i>in utero</i>.</b>
Document No:	M-004634-01-1
Report No:	Bayer AG, unpublished report no. 23538 of Dec. 09. 1994
Guidelines:	EEC Directive 87/302/EEC; OECD guideline no. 475, Xcw and Revised Health Effects Guidelines October 1984 (U.S.); EPA HG - Gene Muta - Somatic cells, October 1984
GLP	Yes

## I. Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043
Synonym:	flufenacet
Lot/Batch no:	Fl. 036 of July 04, 1991
Purity:	97.1-97.5%
Stability of test compound:	approved for study duration (until January 12, 1994)

#### 2. Control Materials:

Negative control:	untreated
Vehicle control:	DMSO 1% (v/v)
Positive controls:	<u>without activation:</u> Ethylmethansulfonate (EMS): 0.9 mg/mL <u>with activation:</u> Dimethylbenzanthracene (DMBA): 20 µg/mL

#### 3. Test system:

Cell culture maintenance:	V79 cell line  Laboratory cultures were maintained in plastic tissue culture vessels at 37°C in a humidified atmosphere containing approximately 5% CO <sub>2</sub> . Exponential growth of cell cultures was maintained by subculturing at least twice a week. Cells were maintained in hypoxanthine-free Eagle's Minimal Essential Medium (MEM, Earle) supplemented with nonessential aminoacids, L-glutamine (2 mM), MEM-vitamins, NaHCO <sub>3</sub> , penicillin (100 units/mL), streptomycin (100 µg/mL) and heat-inactivated fetal calf serum (f.c.: 10%).  During treatment with FOE 5043, the serum content was reduced to 2%. For selection of mutants, a hypoxanthine-free culture medium was used, containing 10 µg/mL of 6-thioguanine (6-TG) .
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Metabolic activation:	S9 derived from male Wistar rats (Aroclor 1254 induced rat
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liver)

## B. Study design and methods

Test concentrations:

Without S9 mix:

0-7.8-15.6-31.3-62.5-125-250-500 µg/mL

With S9 mix::

0-7.8-15.6-31.3-62.5.125-180-250-500 µg/mL

Cell treatment:

Cells were exposed to test compound, solvent or positive controls for 5 hours (non-activated and activated); after washing, cells were cultured for 7 days prior to cell selection; following expression,  $3 \times 10^5$  cells/dish (total of 8 dishes) were cultured for 6 to 7 days in selection medium to determine numbers of mutants and 200 cells/plate (3 plates) were cultured for 6 to 7 days without selection medium to determine cloning efficiency (CE).

Evaluation criteria:

An assay will be considered positive if a dose-dependent, significant and in parallel cultures reproducible increase in mutant frequency is observed. It is desirable to obtain this dose-relationship for at least 3 doses. To be significant, the mutagenic response to the substance should be a least two to three times that of the highest negative or vehicle control value observed in that trial. If this result can be reproduced in a second assay, the test article is considered to be mutagenic.

## II. Results and discussion

### A. Solubility and Cytotoxicity

There was precipitation of the test article after addition of the test article-vehicle solution to the medium starting to be evident at stock solution concentrations of 30 mg/mL. Therefore, the mutagenicity assay was conducted with a maximum concentration of 500 µg/mL. Under both treatment conditions, none or only very slight cytotoxic effects were induced.

The absolute cloning efficiencies for the vehicle controls in the valid mutation assays varied from 87.5% to 110.8% without activation and from 71.0% to 102.7% with activation demonstrating good cloning conditions for these experiments.

### B. Mutation Assay

The vehicle control mutant frequencies of the trials used for assessment were all in the normal range of background frequencies for the assay. In contrast, the positive controls EMS and DMBA induced a distinct mutagenic effect in mutant frequency, which was significantly increased over the negative controls demonstrating the sensitivity of the test system and the ability to detect known mutagens.

No biologically relevant and reproducible increase of the mutation frequency over the concurrent vehicle controls was evident, both with and without metabolic activation.

**Table B.6.4.1-1 Summary of results for mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay *in utero*.**

	concentration	S9 mix	Survival to treatment		Relative population growth	Total mutant colonies	absolute cloning efficiency		Mutant frequency mutant colonies/10 <sup>6</sup> cells
	µg/mL		Mean colony number	SD	% vehicle control		%	SD	
<b>Experiment I / 5 hr treatment</b>									
Negative control		–	157.7	17.4	130.03	7	90.3	15.8	3.2
Negative control		–			119.4	3	103.8	4.2	1.2
Vehicle control		–	138.0	8.9	100.0	5	101.3	8.1	2.4
Vehicle control		–			100.0	4	110.8	1.3	1.4
Positive control EMS	900	–	48.3	7.6	28.6	680	85.8	7.1	330.2
Positive control EMS	900	–			31.2	1157	70.0	8.3	688.7
FOE 5043	7.8	–	165.7	19.6	236.9	7	72.8	8.5	4.0
	7.8	–			179.9	2	77.3	2.4	1.1
	15.6	–	138.7	6.1	111.8	14	99.3	4.6	5.9
	15.6	–			154.1	7	89.2	6.0	3.3
	31.3	–	83.3	5.7	145.5	7	88.0	8.0	3.3
	31.3	–			141.1	3	90.0	4.4	1.4
	62.5	–	62.0	12.0	131.6	9	82.5	9.8	4.5
	62.5	–			120.4	8	93.5	2.6	3.6
	125.0	–	94.5	2.1	150.6	4	84.2	1.2	2.0
	125.0	–			150.9	2	86.3	8.0	1.0
	250.0	–	6.0	7.2	60.1	4	70.0	6.8	2.4
	250.0	–			19.3	4	93.7	5.5	2.0

EMS = Ethylmethansulfonate

	concentration	S9 mix	Survival to treatment		Relative population growth	Total mutant colonies	absolute cloning efficiency		Mutant frequency mutant colonies/10 <sup>6</sup> cells
	µg/mL		Mean colony number	SD	% vehicle control		%	SD	
<b>Experiment II / 5 hr treatment</b>									
Negative control		–	160.3	6.0	108.9	13	109.7	0.8	4.9
Negative control		–			129.7	12	90.5	6.7	5.5
Vehicle control		–	154.0	11.5	100.0	10	104.3	10.7	4.0
Vehicle control		–			100.0	9	87.5	11.8	4.3
Positive control EMS	900	–	99.0	21.2	51.3	1378	79.9	2.1	720.4
Positive control EMS	900	–			73.3	1038	58.5	4.3	739.3
FOE 5043	7.8	–	153.3	18.1	186.0	8	72.7	5.3	4.6
	7.8	–			155.0	1	83.7	7.8	0.5
	15.6	–	160.7	20.8	162.7	9	72.3	5.0	5.2
	15.6	–			169.7	7	78-8	7.5	3.7
	31.3	–	162.7	26.5	147.9	11	94.0	5.1	4.9
	31.3	–			144.4	1	91.7	8.8	0.5
	62.5	–	144.7	2.9	174.9	10	68.8	7.4	6.1
	62.5	–			188.9	1	74.5	6.1	0.6
	125.0	–	122.7	15.4	204.3	28	56.8	3.2	20.5
	125.0	–			163.8	8	49.0	7.0	6.8
	250.0	–	6.7	1.5	52.2	2	55.5	8.4	1.5
	250.0	–			97.4	6	50.3	7.5	5.0
	500.0	–	0.0	0.0	--	--	--	--	--
	500.0	–			--	--	--	--	--

EMS = Ethylmethansulfonate

	concentration	S9 mix	Survival to treatment	Relative population growth	Total mutant colonies	absolute cloning efficiency	Mutant frequency mutant colonies/10 <sup>6</sup> cells
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	µg/mL		Mean colony number	SD	% vehicle control		%	SD	
<b>Experiment I / 5 hr treatment</b>									
Negative control		+	179.0		121.4	8	86.2	5.2	3.9
Negative control		+			120.7	2	100.3	20.1	0.8
Vehicle control		+	183.3		100.0	19	102.7	12.6	7.7
Vehicle control		+			100.0	10	102.2	11.0	4.1
Positive control DMBA	20	+	128.7		70.5	164	78.2	9.4	87.4
Positive control DMBA	20	+			73.5	198	73.0	5.9	113.0
FOE 5043	7.8	+	212.7	8.3	81.9	18	104.3	12.1	7.2
	7.8	+			87.6	29	112.3	9.0	10.8
	15.6	+	207.0	11.3	105.3	7	84.7	1.1	3.4
	15.6	+			95.9	10	104.0	2.2	4.0
	31.3	+	160.7	23.7	120.3	8	81.5	6.6	4.7
	31.3	+			110.7	12	95.3	7.6	5.2
	62.5	+	143.0	11.5	136.9	4	76.3	8.1	2.2
	62.5	+			117.5	14	86.0	0.0	6.8
	125.0	+	148.3	14.2	118.4	18	77.8	8.3	9.6
	125.0	+			92.9	16	103.0	3.5	6.5
	180.0	+	120.3	16.2	111.8	6	84.2	5.1	3.0
	180.0	+			71.4	5	89.0	5.7	2.3
	250.0	+	88.3	9.0	82.7	12	89.5	4.5	5.6
	250.0	+			106.9	14	78.2	4.6	7.5

DMBA = Dimethylbenzanthracene

	concentration	S9 mix	Survival to treatment	Relative population growth	Total mutant colonies	absolute cloning efficiency	Mutant frequency mutant colonies/10 <sup>6</sup> cells
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	µg/mL		Mean colony number	SD	% vehicle control		%	SD	
<b>Experiment I / 5 hr treatment</b>									
Negative control		+	152.7		182.4	4	84.5	16.8	2.0
Negative control		+			100.1	3	85.5	3.3	1.5
Vehicle control		+	159.7		100.0	6	86.2	4.5	2.9
Vehicle control		+			100.0	13	71.0	3.1	7.6
Positive control DMBA	20	+	82.7		87.1	102	56.3	8.9	75.5
Positive control DMBA	20	+			78.5	110	61.7	12.2	74.3
FOE 5043	15.6	+	161.0	8.5	193.5	17	54.3	5.1	13.0
	15.6	+			152.2	5	63.7	3.5	3.3
	31.3	+	163.7	6.1	165.8	5	47.3	7.5	4.4
	31.3	+			158.3	5	39.3	3.0	5.3
	62.5	+	173.3	25.1	153.2	4	51.8	7.7	3.2
	62.5	+			139.4	9	83.0	7.7	4.5
	125.0	+	109.0	17.1	185.8	8	63.8	7.8	5.2
	125.0	+			141.2	3	64.7	13.5	1.9
	180.0	+	128.7	6.8	144.1	6	66.0	5.0	3.8
	180.0	+			105.1	11	86.0	8.9	5.3
	250.0	+	123.7	5.5	116.8	8	48.7	7.6	6.8
	250.0	+			115.3	12	53.0	5.6	9.4
	500.0	+	72.3	10.0	82.4	7	47.5	1.3	6.1
	500.0	+			66.0	16	54.5	12.3	12.2

DMBA = Dimethylbenzanthracene

### Findings:

The test material, FOE 5043, was assayed for mutagenic activity at the HGPRT locus in V79 cells from 7.8 µg/mL to 500 µg/mL with and without activation. Under no activation conditions, FOE 5043 induced slight cytotoxic effects as seen by decreases in survival to treatment, relative population growth and cloning efficiency. Under activation conditions, no significant cytotoxicity was observed:



therefore, FOE 5043 was tested up to its limits of solubility under culture conditions. There was no significant dose-related or reproducible increase in mutant frequency above that of the negative controls. In contrast, the positive controls ethylmethanesulfonate (without S9 mix) and dimethylbenzanthracene (with S9 mix) produced a clearly mutagenic effect in the assay.

<b>Conclusion</b>	<p>The test material, FOE 5043, was assayed for mutagenic activity at the HGPRT locus in V79 cells from 7.8 µg/ml to 500 µg/mL, both with and without activation. Under both treatment conditions, none or only very slight cytotoxic effects were induced. The vehicle control mutant frequencies of the trials used for assessment were all in the normal range of background frequencies for the assay. In contrast, the positive controls EMS and DMBA induced a distinct mutagenic effect in mutant frequency, which was significantly increased over the negative controls demonstrating the sensitivity of the test system and the ability to detect known mutagens.</p> <p><b>FOE 5043 is considered to be non mutagenic in the V79-HGPRT forward mutation assay, both with and without metabolic activation.</b></p>
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Previous evaluation	In DAR for original approval (1997); In vitro mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells was presented and evaluated during the EU process for Annex I listing.
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Report:	KCA 5.4.1 /03; R. Gahlmann; 1995
Title:	FOE 5043 - In vitro mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells
Document No:	M-004692-01-1
Report No:	Bayer AG. unpublished report no. 24340 of Oct. 04, 1995
Guidelines:	EEC Directive 79/83 I/EEC; OECD Guideline no. 473. HP A Title 40, Subpart F-Genetic Toxicology: In vitro mammalian cytogenetics
GLP	Yes

## I. Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043
Synonym:	flufenacet
Lot/Batch no:	Fl. 036 of July 04, 1991
Purity:	97.5%
Stability of test compound:	approved for study duration (until January 30, 1993)

#### 2. Control Materials:

Negative control:	untreated
Vehicle control:	<u>Test substance:</u> ethanol
	<u>Positive controls:</u> Hanks' balanced salt solution
Positive controls:	<u>without activation:</u>
	Mitomycin C: 2 µg/mL
	<u>with activation:</u>
	Cyclophosphamid: 10 µg/mL

#### 3. Test system:

Cell culture maintenance:	Chinese hamster ovary (CHO) cells were grown in 20 ml of medium per 75 cm <sup>2</sup> flasks. Incubation of the cells was always performed at 37°C in a CO <sub>2</sub> -incubator (air to CO <sub>2</sub> ratio 95:5). Cells were grown in medium containing 5 or 10% of fetal calf serum.
Metabolic activation:	S9 derived from Wistar rats (Aroclor 1254 induced rat liver)

### B. Study design and methods

Test concentrations:	With and without S9 mix: 0, 8, 40 and 200 µg/mL
Cell treatment:	Approximately 1 x 10 <sup>6</sup> cells were seeded in 20 mL of medium per 75 cm <sup>2</sup> flasks and exposed to test compound, solvent or positive control for 4 h with and without metabolic activation. Cells exposed to test material, solvent or positive control were harvested 8 h, 24 h or 30 h after the beginning of treatment with

and without S9 mix. For spindle inhibition 0.2 mL colcemid (40 µg/mL water) was administered 2 h before cell harvest.

Cells were treated with hypotonic solution and fixed with a solution of ethanol/acetic acid (3:1). Slides were prepared by dropping the harvested cultures on clean slides. The slides were stained with 5 % Giemsa solution. At least two slides were generated from the cells of each flask.

Slides were coded prior to analysis. The mitotic index was determined by counting 1000 cells per culture. 100 cells were examined per replicate culture (200 per dose) and were scored for structural aberrations and for numerical aberrations (polyploidy).

**Evaluation criteria:**

A test was considered to be positive if a dose-dependent and statistically significant increase of aberration frequencies was observed which was outside the range of the historical solvent controls.

An increased incidence of gaps of both types without concomitant increase of other aberration types was not considered as indication of a clastogenic effect.

A test was considered equivocal if there was an increase which was statistically significant but not concentration-dependent, or if a concentration-dependent increase occurred which was not statistically significant.

**Findings:**

Chinese hamster ovary cells were treated with FOE 5043 at the concentrations of 8, 40 and 200 µg/mL medium without and with S-9 mix FOE 5043 induced cytotoxic effects in cells with and without metabolic activation. The mitotic indices were markedly reduced after treatment of cells with the highest concentration of the test substance of 200 µg/mL without S9 mix for all three harvest times (8, 24, 30 hours after the beginning of the treatment). Cytotoxic effects were less predominant with S9 mix. At the highest concentration, the strongest reduction of the mitotic index was hereby detected for the 8 hour harvest time (38.7%). No statistically significant or biological relevant increases of numbers of metaphases with aberrations were detected 8, 24 or 30 hours after beginning of the four hour treatment with the test substance with and without S9 mix. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

**Results:**

The clastogenic potential of FOE 5043 was evaluated in the cytogenetics assay in vitro with Chinese hamster ovary cells. Cells were exposed to concentrations of 8, 40 and 200 µg/mL with and without S9 mix.

**Mitotic Index:**

The mitotic indices determined in the main study are listed in Table 1 below. Mitotic indices after treatment with FOE 5043 without S9 mix: The mitotic indices for cells treated with the highest

concentration of 200 µg/mL were markedly reduced to 9.7, 62.2 and 66.4% as compared to solvent controls for the harvest times of 8, 24, and 30 hours, respectively. Mitotic indices after treatment with FOE 5043 with S9 mix: The mitotic index for cells treated with the highest concentration of 200 µg/mL was markedly reduced to 38.7% relative to the concomitant solvent control at the harvest time of 8 hours. Moderate effects were noticed for the 30 hour harvest time (78.8%) and no effect for the 24 hour harvest time.

**TABLE 1**  
**Mitotic Index**

Experimental group	Concentration (µg/ml)	Time of harvest (h)	Mitotic nuclei in 1000 cells	
			absolute <sup>4</sup>	solvent control (%)
<b>without metabolic activation</b>				
Solvent Control <sup>1</sup>	0	8	46.5	100.0
FOE 5043	200	8	4.5	9.7
Solvent Control <sup>1</sup>	0	24	49.0	100.0
Untreated Control	0	24	60.0	122.4
FOE 5043	8	24	45.5	92.9
FOE 5043	40	24	40.5	82.7
FOE 5043	200	24	30.5	62.2
Positive Control <sup>2</sup>	2	24	44.5	90.8
Solvent Control <sup>1</sup>	0	30	55.0	100.0
FOE 5043	200	30	36.5	66.4
<b>with metabolic activation</b>				
Solvent Control <sup>1</sup>	0	8	37.5	100.0
FOE 5043	200	8	14.5	38.7
Solvent Control <sup>1</sup>	0	24	47.0	100.0
Untreated Control	0	24	37.0	78.7
FOE 5043	8	24	43.5	92.6
FOE 5043	40	24	42.0	89.4
FOE 5043	200	24	48.0	102.1
Positive Control <sup>3</sup>	10	24	41.0	87.2
Solvent Control <sup>1</sup>	0	30	49.5	100.0
FOE 5043	200	30	39.0	78.8

1 = Ethanol

2 = Mitomycin C

3 = Endoxan

4 = average of duplicate cultures

Chinese hamster ovary cells were treated with FOE 5043 at the concentrations of 8, 40, 200 µg/mL medium with and without S9 mix. FOE 5043 induced cytotoxic effects in cells with and without metabolic activation. The mitotic indices were markedly reduced after treatment of cells with the highest concentration of the test substance of 200 µg/ml without S9 mix for all three harvest times (8, 24 and 30 hours after the beginning of the treatment). Cytotoxic effects were less predominant with S9 mix. At the highest concentration, the strongest reduction of the mitotic index was hereby detected for the 8 hour harvest time (38.7%). No statistically significant or biologically relevant increases of numbers of metaphases with aberrations were detected 8, 24 or 30 hours after the beginning of the four hour treatment with the test substance with and without S9 mix. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

**Table B.6.4.1-2 Summary of cells with structural aberrations in *in vitro* mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells**

Substance Dose (µg/mL)	+/- S9	Cells scored	Metaphases with aberrations (%)		Mitotic Index (%)
			Including gaps	Excluding gaps	
Experiment 1 (4 hour treatment + 8 hour harvest, +/- S9)					
Solvent (Ethanol)	—	200	2.5	2.0	100.0
FOE 5043 200	—	200	0.5	0.5	9.7
Solvent (Ethanol)	+	200	1.0	0.5	100.0
FOE 5043 200	+	200	4.0	3.0	38.7

<sup>a</sup> statistical significant at  $p \leq 0.01$

**Summary of cells with structural aberrations**

Substance Dose (µg/mL)	+/- S9	Cells scored	Metaphases with aberrations (%)		Mitotic Index (%)
			Including gaps	Excluding gaps	
Experiment 2 (4 hour treatment + 24 hour harvest, +/- S9)					
Solvent (Ethanol)	—	200	0.5	0.5	100.0
Untreated control	—	200	3.0	3.0	122.4
FOE 5043 8	—	200	1.5	1.0	92.9
40	—	200	0.5	0.5	82.7
200	—	200	2.5	2.5	62.2
Mitomycin C 2	—	200	49.5**	49.5**	90.8
Solvent (Ethanol)	+	200	1.5	1.5	100.0
Un treated control	+	200	1.5	1.3	78.7
FOE 5043 200	+	200	0.5	0.5	92.6
1200	+	200	1.0	1.0	89.4
2400	+	200	2.0	2.0	102.1
Cyclophosphamide 2	+	200	19.0**	18.5**	87.2
Experiment 3 (4 hour treatment + 30 hour harvest, +/- S9)					
Solvent (Ethanol)	—	200	1.0	0.5	100.0
FOE 5043 200	—	200	1.0	1.0	66.4
Solvent (Ethanol)	+	200	1.0	1.0	100.0
FOE 5043 200	+	200	1.0	1.0	78.8

<sup>a</sup> statistically significant at  $p \leq 0.01$

Based on this test, FOE 5043 is considered to be non clastogenic for mammalian cells with and without metabolic activation *in vitro*.

<b>Conclusion</b>	<b>FOE 5043 is considered to be non clastogenic for mammalian cells with and without metabolic activation <i>in vitro</i>.</b>
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Previous evaluation	<b>In DAR for original approval (1997); In vitro mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells was presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>KCA 5.4.1 /03; B. Herbold; 1995</b>
Title:	<b>FOE 5043 - Salmonella/microsome test plate incorporation and preincubation method</b>
Document No:	M-004696-01-1
Report No:	Bayer AG, unpublished report no 23948 of April 24, 1995
Guidelines:	EEC Directive 92/69/EEC B. 14; OECD Guideline no. 471; New and Revised Health Effects Test Guidelines October 1984. (U.S.); EPA HG - Gene Muta - S. typhimurium, October 1984
GLP	Yes

#### Material and methods:

FOE 5043, batch number: 898313105, purity: 96.8% dissolved in DMSO single exposure to *Salmonella typhimurium* TA1535, TA100, TA1537, TA98. Dosage: FOE 5043: 16 - 5000 µg/plate; repeat test: 16 - 5000 ug. plate (preincubation method) positive controls:

- sodium azide (Na-azid): 10 µg /plate (TA1535)
- nitrofurantoin (NF): 0.2 µg /plate (TA100)
- 4-nitro-1,2-phcnylenc diamine (4-NPDA): 10 µg /plate ( 1 A1537)
- 4-nitro-1,2-phenylene diamine (4-NPDA): 0.5 µg /plate (TA98)
- 2-aminoanthracene (2-AA): 3 µg /plate

application volume: 0.1 mL/plate, 0.1 mL/tube (preincubation method)



**Findings:**

Summary of mean values without S9 mix (initial and repeat test)

Dosage	Strain			
µg/plate	TA1535	TA100	TA1537	TA98
0	9	92	9	39
16	9	83	10	30
50	9	100	8	34
158	8	92	10	28
500	10	83	8	33
1581	10	84	9	29
5000	5	80	8	33
Na-azide	725			
NF		278		
4-NPDA			136	199
µg/tube				
0	15	103	8	22
16	11	105	9	26
50	14	108	9	17
158	14	123	9	24
500	10	90	10	19
1581	7	82	6	21
5000	5	83	4	20
Na-azide	898			
NF		545		
4-NPDA			154	136

Summary of mean values with S9 mix (initial and repeat test)

Dosage	Strain			
µg/plate	TA1535	TA100	TA1537	TA98
0	14	106	10	45
16	10	107	14	51
50	9	125	12	44
158	13	101	10	44
500	12	104	10	44
1581	11	82	10	37
5000	9	94	9	45
2-AA	129	1605	264	1248
µg/tube				
0	13	116	9	25
16	11	125	7	29
50	12	122	8	32
158	12	140	10	31
500	12	134	11	30
1581	10	113	11	26
5000	9	114	10	22
2-AA	219	1982	576	937

<b>Conclusion</b>	FOE 5043 produced bacteriotoxic effects from 500 µg/plate and 500 µg /tube onwards. FOE 5043 has to be regarded as non-mutagenic.
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<b>New studies; not evaluated</b>	<b>A new supporting studies.</b> <i>Salmonella typhimurium</i> reverse mutation assay with flufenacet technical; <b>Non EU authorities</b> [SANCO/10597/20 03- Rev 10.1]
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**In 2010 for registration of flufenacet in Japan, a bacterial reverse mutation assay was conducted. This new study showed no evidence of a mutagenic potential and thus, confirmed that flufenacet is not mutagenic.**

<b>Report:</b>	<b>KCA 5.4.1 /05; Sokolowski, A.; 2010; M-395211-01</b>
<b>Title:</b>	<b>Salmonella typhimurium reverse mutation assay with flufenacet technical</b>
<b>Document No:</b>	M-395211-01-1
<b>Report No:</b>	Bayer AG, unpublished report no 23948 of April 24, 1995
<b>Guidelines:</b>	OECD 471; Commission Regulation (EC) No. 440/2008, Method B13/14; US-EPA 712-C-98-247; <b>Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

<b>Test material:</b>	FOE 5043 (flufenacet techn.)
Description:	Beige solid
Lot/Batch no:	NK61AX0177
Purity:	96.8%
Stability of test compound:	guaranteed for study duration; expiry date: 2012-09-03
<b>Vehicle and/or positive control:</b>	DMSO
	Sodium azide (Na-azide), 4-nitro-o-phenylene diamine (4-NOPD), methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)
<b>Test system:</b>	<i>Salmonella typhimurium</i> strains TA1535, TA1537, TA100, TA98, TA102
<b>Metabolic activation:</b>	S9 mix
<b>Study design and methods</b>	
<b>Dose:</b>	0-3-10-33-100-333-1000-2500-5000 µg/plate
	positive controls:
	Na-azide: 10 µg/plate
	4-NODD: 10 µg/plate
	MMS: 3.0 µg/plate
	2-AA: 2.5, 10.0 µg/plate
<b>Application volume:</b>	0.1 mL
<b>Incubation time /temperature:</b>	Pre-incubation: 60 minutes, 37 °C
	48 hours, 37 °C

## Results and discussion

The potential of flufenacet to induce gene mutations was investigated according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) in two independent experiments both with and without liver microsomal activation (S9 mix).

The plates incubated with the test item showed normal background growth up to the highest concentration in all strains used.

In experiment I, toxic effects, evident as a reduction in the number of revertants were observed at 2500 µg/plate in strain TA1535 without S9 mix and in strain TA1537 with S9 mix.

In experiment II, toxic effects were observed at 5000 µg/plate in strain TA102 without S9 mix and in strains TA1537 and TA98 with S9 mix, and from 1000 - 5000 µg/plate in strain TA102 with S9 mix. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with flufenacet at any dose level, neither in the presence nor 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.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

**Table 5.4.1/05-1: Summary of results**

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA1535	TA1537	TA98	TA100	TA102
Pre-Experiment and Experiment I							
Without Activation	DMSO		16 ± 3	8 ± 2	42 ± 2	203 ± 14	380 ± 47
	Untreated		13 ± 2	9 ± 2	45 ± 4	199 ± 8	414 ± 22
	Flufenacet techn.	3	14 ± 1	8 ± 1	46 ± 6	222 ± 15	373 ± 9
		10	14 ± 1	7 ± 2	38 ± 4	184 ± 15	384 ± 19
		33	13 ± 3	7 ± 2	46 ± 7	197 ± 18	353 ± 5
		100	11 ± 2	8 ± 0	41 ± 2	196 ± 14	334 ± 31
		333	11 ± 5	6 ± 1	46 ± 12	194 ± 20	345 ± 13
		1000	15 ± 5 <sup>P</sup>	8 ± 1 <sup>P</sup>	35 ± 1 <sup>P</sup>	199 ± 15 <sup>P</sup>	355 ± 31 <sup>P</sup>
		2500	6 ± 2 <sup>P</sup>	8 ± 3 <sup>P</sup>	38 ± 9 <sup>P</sup>	198 ± 12 <sup>P</sup>	300 ± 34 <sup>P</sup>
		5000	8 ± 3 <sup>P</sup>	4 ± 2 <sup>P</sup>	40 ± 2 <sup>P</sup>	203 ± 12 <sup>P</sup>	318 ± 49 <sup>P</sup>
	NaN3	10	1675 ± 199			1632 ± 187	
	4-NOPD	10			306 ± 21		
	4-NOPD	50		71 ± 1			
	MMS	3.0					3021 ± 785
With Activation	DMSO		20 ± 4	13 ± 4	41 ± 5	199 ± 4	475 ± 54
	Untreated		14 ± 1	11 ± 4	42 ± 7	206 ± 8	490 ± 51
	Flufenacet techn.	3	20 ± 6	14 ± 4	45 ± 4	197 ± 17	488 ± 77
		10	18 ± 5	12 ± 3	45 ± 5	197 ± 10	405 ± 6
		33	16 ± 5	10 ± 3	44 ± 8	193 ± 11	495 ± 100
		100	16 ± 1	13 ± 5	43 ± 1	207 ± 5	497 ± 113
		333	13 ± 4	11 ± 3	48 ± 7	188 ± 5	429 ± 55
		1000	12 ± 3 <sup>P</sup>	9 ± 0 <sup>P</sup>	48 ± 7 <sup>P</sup>	173 ± 8 <sup>P</sup>	420 ± 104 <sup>P</sup>
		2500	12 ± 3 <sup>P</sup>	6 ± 3 <sup>P</sup>	37 ± 4 <sup>P</sup>	176 ± 16 <sup>P</sup>	451 ± 26 <sup>P</sup>
		5000	12 ± 3 <sup>PM</sup>	6 ± 3 <sup>P</sup>	45 ± 9 <sup>P</sup>	129 ± 16 <sup>P</sup>	221 ± 7 <sup>P</sup>
	2-AA	2.5	379 ± 6	300 ± 22	1773 ± 300	2793 ± 30	
	2-AA	10.0					1592 ± 469

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA1535	TA1537	TA98	TA100	TA102
Experiment II							
Without Activation	DMSO		12 ± 2	9 ± 5	21 ± 1	119 ± 1	327 ± 39
	Untreated		13 ± 4	8 ± 2	31 ± 5	157 ± 8	357 ± 11
	Flufenacet techn.	10	9 ± 3	13 ± 1	23 ± 4	117 ± 16	281 ± 11
		33	13 ± 5	8 ± 3	18 ± 4	111 ± 11	279 ± 23
		100	13 ± 3	10 ± 4	23 ± 3	122 ± 15	324 ± 5
		333	15 ± 2	10 ± 2	23 ± 1	122 ± 5	302 ± 47
		1000	9 ± 1 <sup>P</sup>	11 ± 3 <sup>P</sup>	24 ± 5 <sup>P</sup>	117 ± 12 <sup>P</sup>	229 ± 27 <sup>P</sup>
		2500	8 ± 1 <sup>P</sup>	9 ± 4 <sup>P</sup>	24 ± 3 <sup>P</sup>	86 ± 22 <sup>P</sup>	210 ± 16 <sup>P</sup>
		5000	6 ± 1 <sup>P</sup>	8 ± 3 <sup>P</sup>	14 ± 5 <sup>P</sup>	65 ± 8 <sup>PM</sup>	135 ± 3 <sup>P</sup>
	NaN3	10	1639 ± 236			1739 ± 203	
	4-NOPD	10			371 ± 43		
	4-NOPD	50		77 ± 12			
	MMS	3.0					1718 ± 109
With Activation	DMSO		18 ± 5	11 ± 4	34 ± 4	111 ± 9	419 ± 41
	Untreated		16 ± 7	14 ± 4	37 ± 1	264 ± 90	513 ± 34
	Flufenacet techn.	10	15 ± 4	12 ± 2	37 ± 4	107 ± 12	351 ± 23
		33	17 ± 6	12 ± 2	34 ± 8	116 ± 14	317 ± 6
		100	17 ± 2	9 ± 2	27 ± 3	139 ± 8	335 ± 38
		333	17 ± 6	10 ± 3	30 ± 11	139 ± 10	338 ± 23
		1000	15 ± 3 <sup>P</sup>	12 ± 3 <sup>P</sup>	37 ± 4 <sup>P</sup>	99 ± 15 <sup>P</sup>	170 ± 16 <sup>P</sup>
		2500	19 ± 3 <sup>P</sup>	10 ± 4 <sup>P</sup>	30 ± 3 <sup>P</sup>	93 ± 1 <sup>P</sup>	169 ± 27 <sup>P</sup>
		5000	10 ± 5 <sup>PM</sup>	5 ± 1 <sup>PM</sup>	12 ± 1 <sup>PM</sup>	62 ± 9 <sup>PM</sup>	77 ± 11 <sup>PM</sup>
	2-AA	2.5	347 ± 29	207 ± 28	1742 ± 49	1737 ± 118	
	2-AA	10.0					2434 ± 485

NaN<sub>3</sub> = sodium azide; 2-AA = 2-aminoanthracene, MMS = methyl methane sulfonate, 4-NOPD = 4-nitro-o-phenylene-diamine; P = Precipitate, M = Manual count

<b>Conclusion</b>	Flufenacet is considered to be non-mutagenic in <i>Salmonella typhimurium</i> reverse mutation assay.
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**B.6.4.2 In vivo studies in somatic cells**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  FOE 5043 - Micronucleus test on the mouse studies were presented and evaluated during the EU process for Annex I listing.
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<b>Report:</b>	<b>KCA 5.4.2 /01; ████████; 1993</b>
<b>Title:</b>	<b>FOE 5043 - Micronucleus test on the mouse</b>
<b>Document No:</b>	M-004588-01-1
<b>Report No:</b>	Bayer AG, unpublished report no. 22384 of July 14, 1993
<b>Guidelines:</b>	EEC Directive 84/449/EEC B.12; OECD guideline no. 474, New and Revised Health Effects Test Guidelines October 1984; EPA HG - Chromo - Micronucleus
<b>GLP</b>	Yes

**Material and methods:**

FOE 5043, mixed batch Fl. 036 of July 4. 1991. purity: 97.5%. dissolved in corn oil; single intraperitoneal application to mice (BorNMRI/SPF Han) dosage: 250 mg/kg bw (test substance); 20 mg/kg bw (positive control cyclophosphamide) application volume: 5 mL/kg bw (test substance, negative control); 10 mL/kg bw (positive control cyclophosphamide. 250 mg/kg FOE 5043 was chosen as MTD for this test. Measurements: 16, 24 and 48 hours resp. after administration, the animals were sacrificed and the femoral marrow was prepared. To produce the smears the method used was according to W. Schmid, 1975.

**Findings:**

Results of micronucleus test on the mouse with FOE 5043 Comparison of group means.

	hours after treatment	number of evaluated polychromatic erythrocytes	number of normochromatic erythrocytes per 1000 polychromatic erythrocytes	micronucleated cells per 1000	
				normochromatic erythrocytes	polychromatic erythrocytes
negative control	24	10000	1155 ± 315	1.4 ± 1.1	1.5 = 1.0
FOE 5043 250 mg/kg	16	10000	1069 ± 304	1.1 ± 0.9	1.2 = 0.9
FOE 5043 250 mg/kg	24	10000	1172 ± 429	1.6 ± 1.3	1.8 = 1.5
FOE 5043 250 mg/kg	48	10000	1219 ± 266	1.9 ± 0.9	1.9 = 2.0
positive control cyclophosphamide 20 mg/kg	24	10000	915 ± 334	1.7 ± 1.5	16.2* = 7.1

\* p < 0.01 in non-parametric Wilcoxon ranking test

<b>Conclusion</b>	The micronucleus test on the mouse, i.e. a somatic mutagenicity test system in vivo, provided no indication of a clastogenic effect of FOE 5043 at the tested dose of 250 mg/kg bw intraperitoneal.
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#### **B.6.4.3      *In vivo* studies in germ cells**

<b>Conclusion</b>	<b>Overall it is concluded that flufenacet did not show a genotoxic potential and no evidence of an effect on germ cells was seen in other toxicological studies. <u>RMS supports that an <i>in vivo</i> study in germ cells is not regarded necessary.</u></b>
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### Summary of genotoxicity testing

Mutagenicity studies with FOE 5043 were consistently negative. Point mutation assays in bacteria and mammalian cells revealed no evidence of mutagenic potential. *In vitro* and *in vivo* cytogenetic studies revealed no evidence of clastogenicity, and an unscheduled DNA synthesis assay using primary rat hepatocytes revealed no evidence of genotoxic activity. Thus, FOE 5043 is not mutagenic, clastogenic or genotoxic.

Study	Test system	Results		Reference
		activation	non-activation	
In-vitro				
Bacterial reverse mutation assay	S. typhimurium TA98, TA100, TA1535, TA1537	negative	negative	Herbold, 1995 M-004696-01-1
Bacterial reverse mutation assay	S. typhimurium TA98, TA100, TA102, TA1535, TA1537	negative	negative	Sokolowski, 2010 M-395211-01-1
Mammalian cell gene mutation test (HGPRT)	Chinese hamster lung fibroblasts V79	negative	negative	Brendler-Schwaab, 1994 M-004634-01-1
Mammalian chromosome aberration test	Chinese hamster ovary cells CHO	negative	negative	Gahlmann, 1995 M-004692-01-1
Unscheduled DNA synthesis (UDS) assay	Primary rat hepatocytes	negative	negative	Brendler-Schwaab, 1992 M-004577-01-1
In-vivo				
Micronucleus test	Mouse bone marrow	negative		██████, 1993 M-004588-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold



### B.6.5 Long-term toxicity and carcinogenicity

Evidence of toxicity from exposure to flufenacet was observed in chronic feeding studies on mice and rats. In the oncogenicity mouse study, findings included increased blood methemoglobin content and ocular cataracts.

For rats, the toxicological response could be broadly characterized as involving structural and/or functional alterations in liver-, kidney-, hematologic/spleen-, and thyroid-related endpoints. The liver was considered the primary target organ with increases in organ weight, cell size and number, and/or associated hepatic parameters. Hepatocytomegaly was exhibited species exposed to higher doses of flufenacet. The flufenacet -induced liver changes would appear to be fundamentally adaptive in nature as the organism's principal metabolic organ responds to physiological need to clear, biotransform, and excrete a xenobiotic.

The haematological profile of the rats indicated a mild anaemia for animals at higher dose levels.

Thyroid involvement was noted by an increase in thyroid organ weights. The lower levels of exposure used in the chronic rat study, as compared to the sub-chronic bioassay, suggested a dose >800 ppm (highest dose tested) was necessary for a broader and more significant toxicological response in this tissue. The thyroid organ changes resulting from exposure to flufenacet are likely to be a secondary effect in response to hepatic induction.

Ophthalmological findings noted in the rat included cataracts and ocular scleral mineralization.

Renal pelvic epithelial hyperplasia was observed in the kidneys of rats.

No evidence of an oncogenic potential of flufenacet was found in the long-term feeding studies in rats and mice.

**Table 5.5-1: Summary of long-term studies**

Study	Sex	NOAEL mg/kg bw/day	LOAEL	Main findings seen at LOAEL	Reference
Rat 2-year feeding	M F	1.2* 1.5	19 24	BW gain ↓, structural and/or functional alterations in liver-, kidney-, haematopoietic-, and thyroid-related endpoints.	██████████, 1995c, M-005062-02-1
Mouse 20-month feeding	M F	7.4 9.4	30 77	MetHB ↑ Ocular cataracts ↑	██████████, 1995d M-005060-02-1

M = male, F = female, BW = body weight, MetHB = Methemoglobin

\* It has to be noted that during the first review of flufenacet the NOEL (1.2/1.5 mg/kg bw/day males/females) of the 2-year toxicity study in rats as stated in the monograph and baseline dossier KCA 5.5, was changed to a LOEL (1.2 mg/kg bw/day) during the ECCO meeting(s) as stated in the end point list of Annex 2 of Report of ECCO 73 and as presented in the Review Report for flufenacet (7469/VI/98-Final – 3<sup>rd</sup> July 2003). This endpoint (LOEL of 1.2 mg/kg bw/day) was solely based on a background change (minimal-slight renal pelvic mineralization) which is commonly observed in ageing rats. In the study considered here this finding was observed at higher incidences compared to concurrent controls after 2-year exposure to flufenacet in all dose groups in males (25, 400, and 800 ppm) and in the mid and high dose group in females (400 and 800 ppm). Due to the high frequency of this background lesion in controls and the absence of a clear dose response regarding the severity of this finding, the slight (though significant) increases in the treated groups were not considered an adverse change.

**B.6.5.1 Rat**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>[REDACTED]; 1995c</b>
<b>Title:</b>	Technical Grade FOE 5043: A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat.
<b>Document No:</b>	M-005062-02-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7798 of Oct. 03. 1995
<b>Guidelines:</b>	FIFRA Guideline No. 83-5; TSCA guideline no. 798.3320; OECD guideline no. 453; MAFF guideline 59 NohSan No. 4200; EU-guidelmc 87/
<b>GLP</b>	Yes

**Material and methods:**

FOE 5043, batch no.: Fl. 036 from July 04. 1991, purity 95.2% - 99.0% Corn oil at 1% by weight of the diet; acetone/corn oil mixture used to dissolve the test article prior to mixing with the dietary carrier. The control diet was prepared the same way, excluding only the test substance. administration: Oral by feeding (dietary admixture with rodent chow in "etts" form) for 2 years to Fischer 344 rats (CDF|F-344|BR) doses (AI): 0 - 25 - 400 - 800 ppm, corresponding to: 0 - 1.2 - 19.3 - 39.0 mg/kg bw/day in males, 0 - 1.5 - 24.4 - 49.8 mg/kg bw/day in females.

**Findings - General observations:**

No differences with respect to physical appearance, activity, behavior, condition of coat, appetite or thirst between dosed and control animals were seen.

**Body weight:**

Body weight gam (BWG) was evaluated by comparing the mean final body weight of a chemically-treated group to that of the untreated control group, both of which were recorded just prior to necropsy. Based on this criteria, BWG remained unaffected in 25 ppm males and females and 400 ppm males. An 8% decline in BWG was noted in 400 ppm females while 10 and 17% declines in BWG were measured in 800 ppm males and females, respectively.

**Table B.6.5.1-1 Intergroup comparison of body weights / gain (g) - selected time points (2-year sacrifice group)**

	<b>Male</b>			
<b>Dose level (ppm)</b>	<b>0</b>	<b>25</b>	<b>400</b>	<b>800</b>

Week 1	121.4	122.4	126.2	<b>131.7*</b>
Week 13	287.5	288.1	295.1	295.8
Week 50	369.6	374.3	373.2	370.2
Week 86	378.9	391.0	377.0	368.1
Week 102	373.5	375.1	367.8	<b>338.0*</b>
Body weight gain (week 1-13) [% change vs control]	166.1 --	165.7 [0]	168.9 [+2]	164.1 [-1]
Body weight gain (weeks 1 - 50) [% change vs control]	248.2 --	251.9 [+1]	247.0 [0]	238.5 [-4]
Body weight gain (weeks 1 - 102) [% change vs control]	252.1 --	252.7 [0]	241.6 [-4]	<b>206.3*</b> [-18]
<b>Female</b>				
Week 1	99.2	99.0	101.2	<b>102.4*</b>
Week 13	167.5	166.2	165.5	<b>162.3*</b>
Week 50	204.2	200.8	<b>196.4*</b>	<b>188.5*</b>
Week 86	247.1	246.1	<b>229.4*</b>	<b>213.3*</b>
Week 102	259.1	254.0	<b>236.6*</b>	<b>217.4*</b>
Body weight gain (weeks 1 - 13) [% change vs control]	68.3 --	67.2 [-2]	64.3 [-6]	<b>59.9*</b> [-12]
Body weight gain (weeks 1 - 50) [% change vs control]	105 --	101.8 [-3]	<b>95.2*</b> [-9]	<b>86.1*</b> [-18]
Body weight gain (weeks 1 - 102) [% change vs control]	159.9 --	155 [-3]	<b>135.4*</b> [-15]	<b>115*</b> [-28]

\*statistically significant difference from control  $p \leq 0.05$  (Anova + Dunnetts test two-sided)

### Mortality:

FOE 5043 had no effect on animal survival.

### Feed intake:

Food consumption, assessed in terms of both g consumed/animal/day and g consumed/kg body weight/day. remained unaffected in both sexes at all doses tested.

### Ophthalmology:

No evidence of treatment-related changes

### Hematology:

Hematology considerations following exposure to FOE 5043 included increases in the following:

- methemoglobin content for both sexes at 400 ppm and greater;
- platelet count for males at 800 ppm;

c) leukocyte count for males at 400 ppm and for both sexes at 800 ppm.

**Table B.6.5.1-2 Summary of hematology – selected parameters**

Parameter		Male			
Dose level (ppm)		0	25	400	800
Cor-WBC x 10 <sup>3</sup> /mm <sup>3</sup>	12 month	5.8	6.8	<b>9.3*</b>	<b>8.6*</b>
	18 month	5.5	6.7	<b>8.2*</b>	<b>8.5*</b>
	24 month	7.3	6.8	7.5	7.2
PLTS 10 <sup>3</sup> /mm <sup>3</sup>	12 month	769	773	767	<b>852*</b>
	18 month	765	805	827	<b>853*</b>
	24 month	752	758	834	817
METHb (%)	12 month	0.6	0.6	<b>1.7*</b>	<b>2.7*</b>
	18 month	0.6	<b>0.8*</b>	<b>1.6*</b>	<b>2.5*</b>
	24 month	0.7	1.1	<b>1.4*</b>	<b>1.9*</b>
		Female			
Cor-WBC x 10 <sup>3</sup> /mm <sup>3</sup>	12 month	3.7	3.6	<b>4.4*</b>	<b>4.6*</b>
	18 month	3.6	3.6	<b>4.5*</b>	<b>4.7*</b>
	24 month	5.3	5.8	4.4	6.4
PLTS 10 <sup>3</sup> /mm <sup>3</sup>	12 month	737	711	706	748
	18 month	686	665	723	724
	24 month	622	602	684	692
METHb (%)	12 month	0.7	0.6	<b>1.2*</b>	<b>1.9*</b>
	18 month	0.6	<b>0.8*</b>	<b>1.3*</b>	<b>2.1*</b>
	24 month	1.3	1.2	<b>2.1*</b>	<b>2.4*</b>

\* p ≤ 0.05 Anova + Dunnetts tests (two-sided)

#### Clinical chemistry:

Clinical chemistry considerations following exposure to FOE 5043 included increases for the following concentrations:

- a) serum cholesterol in females at 400 ppm and greater;
- b) total serum protein and globulin in females at 800 ppm;
- c) serum calcium in both sexes at 800 ppm;
- d) γ-glutamyltransferase in males at 400 ppm and greater.

Also, there was a decrease in serum triglyceride concentration in males at 400 ppm and greater.

**Table B.6.5.1-3 Summary of clinical chemistry – selected parameters**

Parameter		Male			
Dose level (ppm)		0	25	400	800
Chol (mg/dL)	6 month	45	45	43	42
	12 month	67	72	64	63
	24 month	155	185	124	134

T-Prot (g/dL)	6 month	7.5	7.6	<b>7.8*</b>	<b>8.0*</b>
	12 month	7.1	7.3	7.3	<b>7.5*</b>
	24 month	7.0	7.1	6.8	6.8
Glob (g/dL)	6 month	3.9	3.7	<b>3.3*</b>	4.0
	12 month	3.6	<b>3.8*</b>	<b>3.8*</b>	<b>4.1*</b>
	24 month	3.9	4.1	3.8	3.8
Trig (mg/dL)	6 month	102	100	<b>67*</b>	<b>51*</b>
	12 month	166	171	<b>103*</b>	<b>70*</b>
	24 month	204	319	181	141
Calc (mg/dL)	6 month	11.6	<b>11.9*</b>	<b>12.0*</b>	<b>12.2*</b>
	12 month	11.9	<b>12.1*</b>	<b>12.2*</b>	<b>12.3*</b>
	24 month	11.0	11.2	<b>11.3*</b>	<b>11.4*</b>
GGT (U/L)	6 month	1	1	1	2
	12 month	2	3	<b>3*</b>	<b>6*</b>
	24 month	6	8	12	<b>16*</b>
<b>Female</b>					
Chol (mg/dL)	6 month	72	75	<b>82*</b>	<b>91*</b>
	12 month	94	103	<b>117*</b>	<b>126*</b>
	24 month	147	143	145	149
T-Prot (g/dL)	6 month	7.4	7.5	7.6	<b>7.9*</b>
	12 month	7.7	7.8	<b>8.1*</b>	<b>8.1*</b>
	24 month	7.6	7.6	7.5	7.8
Glob (g/dL)	6 month	3.8	3.9	<b>4.0*</b>	<b>4.2*</b>
	12 month	3.7	3.9	<b>4.0*</b>	<b>4.2*</b>
	24 month	3.9	3.8	3.8	4.0
Trig (mg/dL)	6 month	62	63	60	60
	12 month	78	88	90	86
	24 month	216	238	134	101
Calc (mg/dL)	6 month	11.7	11.8	11.8	12.0
	12 month	12.2	12.3	<b>12.4*</b>	<b>12.4*</b>
	24 month	11.4	<b>11.7*</b>	11.6	11.7
GGT (U/L)	6 month	2	3	3	4
	12 month	3	3	3	3
	24 month	5	4	4	7

\*  $p \leq 0.05$  Anova + Dunnetts tests (two-sided)

### Urinalysis:

Urinary considerations following exposure to FOE 5043 included:

- increased pH for both sexes at 400 ppm and greater;
- increased nitrite content in males at 400 ppm and greater;
- decreased specific gravity for both sexes at 400 ppm and greater;
- decreased ketone concentration for both sexes at 800 ppm;
- decreased urobilinogen and protein concentration in females at 800 ppm.

**Table B.6.5.4-1 Summary of urinalysis – selected parameters**

Parameter		Male			
Dose level (ppm)		0	25	400	800
pH	6 month	7.5	7.6	<b>7.8*</b>	<b>8.0*</b>
	12 month	7.6	7.9	<b>8.0*</b>	<b>8.0*</b>
	24 month	7.7	7.7	7.8	7.9
Nit	6 month	0	0	0	<b>0*</b>
	12 month	0	0	0	<b>1<sup>\$</sup></b>
	24 month	1	1	1	1
Sp. Gr.	6 month	1.068	1.066	<b>1.052*</b>	<b>1.039*</b>
	12 month	1.054	1.049	<b>1.036*</b>	<b>1.030*</b>
	24 month	1.037	1.038	1.031	<b>1.027*</b>
Ket (mg/dL)	6 month	10	13	10	8
	12 month	5	6	5	5
	24 month	1	1	0	0
Uro (Eu/dL)	6 month	0.4	0.5	0.2	0.2
	12 month	0.9	0.8	<b>0.5*</b>	<b>0.3*</b>
	24 month	0.4	0.4	0.2	<b>0.2*</b>
Pro (mg/dL)	6 month	240	210	207	<b>110*</b>
	12 month	260	260	232	<b>180*</b>
	24 month	300	300	300	300
		Female			
pH	6 month	7.5	<b>7.2*</b>	7.6	<b>7.8*</b>
	12 month	7.2	7.3	<b>7.6*</b>	<b>7.8*</b>
	24 month	7.4	7.3	7.6	<b>7.8*</b>
Nit	6 month	0	0	0	0
	12 month	0	0	0	0
	24 month	0	0	0	0
Sp. Gr.	6 month	1.046	<b>1.060<sup>\$</sup></b>	1.043	<b>1.033<sup>\$</sup></b>
	12 month	1.044	1.043	<b>1.033<sup>\$</sup></b>	<b>1.027<sup>\$</sup></b>
	24 month	1.034	1.035	<b>1.025*</b>	<b>1.021*</b>
Ket (mg/dL)	6 month	3	5	3	<b>1*</b>
	12 month	1	2	0	0
	24 month	1	1	0	0
Uro (Eu/dL)	6 month	0.5	0.7	0.4	<b>0.2*</b>
	12 month	0.8	0.9	0.6	<b>0.3*</b>
	24 month	0.2	<b>0.3*</b>	0.2	0.2
Pro (mg/dL)	6 month	54	<b>86<sup>\$</sup></b>	29	<b>16<sup>\$</sup></b>
	12 month	22	38	17	<b>2<sup>\$</sup></b>
	24 month	257	280	204	<b>136*</b>

\* p ≤ 0.05 Anova + Dunnetts tests (two-sided)

<sup>\$</sup> p ≤ 0.05 Kruskal-Wallis Anova + Mann-Whitney u-tests (two-sided)**Gross necropsy:**

Incidence of uterine cysts in 2-year 800 ppm females

**Organ weights:**

Organ weight considerations following exposure to FOE 5043 included weight changes in the heart, liver, spleen, and thyroid of 1-year 400 and 800 ppm males and females; and weight changes in the brain, heart, kidney, liver, lung, ovary, testes, spleen, and thyroid of 2-year 400 and/or 800 ppm males and females.

**Table B.6.5.1-5 Organ weight summary data – selected organs**

Dose level (ppm)			0	25	400	800
			Male			
Brain (g)	12 month	abs	1.926	1.939	1.886	1.922
		rel	0.523	0.520	0.499	0.519
	24 month	abs	1.980	1.968	1.934*	1.906*
		rel	0.561	0.556	0.560	0.604 <sup>\$</sup>
Heart (g)	12 month	abs	1.058	1.073	1.110	1.116
		rel	0.286	0.287	0.293	0.301*
	24 month	abs	1.263	1.250	1.264	1.228
		rel	0.357	0.351	0.365	0.387*
Kidney (g)	12 month	abs	3.112	3.188	3.088	3.169
		rel	0.840	0.850	0.813	0.854
	24 month	abs	3.394	3.484	3.338	3.367
		rel	0.954	0.982	0.961	1.063 <sup>\$</sup>
Liver (g)	12 month	abs	13.896	14.564	15.785*	16.641*
		rel	3.748	3.885	4.158*	4.483*
	24 month	abs	15.252	15.762	15.359	14.826
		rel	4.283	4.440	4.414	4.599
Lung (g)	12 month	abs	1.529	1.598	1.564	1.569
		rel	0.414	0.427	0.412	0.422
	24 month	abs	2.069	2.015	1.926	1.914
		rel	0.594	0.570	0.556	0.607
Spleen (g)	12 month	abs	0.650	0.659	0.646	0.720*
		rel	0.176	0.176	0.171	0.194*
	24 month	abs	1.860	2.008	1.136	0.968 <sup>\$</sup>
		rel	0.535	0.590 <sup>\$</sup>	0.327	0.296 <sup>\$</sup>
Testes (g)	12 month	abs	3.145	3.225	3.165	3.239
		rel	0.853	0.863	0.838	0.875
	24 month	abs	5.262	5.862	6.487	5.713
		rel	1.465	1.630	1.872	1.792
Thyroid (g)	12 month	abs	0.024	0.023	0.027	0.027*
		rel	0.0064	0.0061	0.0071	0.0074*
	24 month	abs	0.028	0.031	0.033	0.034
		rel	0.0080	0.0086	0.0094	0.0110 <sup>\$</sup>
			Female			
Brain (g)	12 month	abs	1.755	1.770	1.714	1.687*
		rel	0.887	0.866	0.866	0.909

Dose level (ppm)			0	25	400	800
	24 month	abs	1.798	1.786	<b>1.753*</b>	<b>1.738*</b>
		rel	0.713	0.717	<b>0.761*</b>	<b>0.838*</b>
Heart (g)	12 month	abs	0.695	0.710	0.710	0.705
		rel	0.351	0.346	0.359	<b>0.379*</b>
	24 month	abs	0.966	0.927	0.928	<b>0.918*</b>
		rel	0.383	0.372	0.402	<b>0.442*</b>
Kidney (g)	12 month	abs	1.764	1.776	1.788	1.716
		rel	0.890	0.863	0.903	0.923
	24 month	abs	2.204	2.277	<b>2.149*</b>	<b>2.105*</b>
		rel	0.912	0.912	0.929	<b>1.012<sup>\$</sup></b>
Liver (g)	12 month	abs	7.866	8.291	<b>8.604*</b>	<b>8.654*</b>
		rel	3.956	4.018	<b>4.345*</b>	<b>4.653*</b>
	24 month	abs	10.249	10.256	10.105	9.835
		rel	4.055	4.099	<b>4.360*</b>	<b>4.724*</b>
Lung (g)	12 month	abs	1.046	1.066	1.017	1.016
		rel	0.528	0.517	0.514	0.547
	24 month	abs	1.398	1.324	<b>1.296*</b>	<b>1.308*</b>
		rel	0.554	0.532	0.562	<b>0.629*</b>
Spleen (g)	12 month	abs	0.443	0.467	0.453	0.483
		rel	0.224	0.227	0.229	<b>0.260*</b>
	24 month	abs	1.102	1.258	0.792	0.754
		rel	0.452	0.507	0.350	0.363
Ovary (g)	12 month	abs	0.163	0.121	0.189	0.118
		rel	0.084	0.061	0.097	0.064
	24 month	abs	0.154	0.132	0.285	<b>0.223<sup>\$</sup></b>
		rel	0.061	0.071	0.124	<b>0.107<sup>\$</sup></b>
Thyroid (g)	12 month	abs	0.017	0.020	0.017	0.019
		rel	0.0083	0.0097	0.0087	<b>0.0100*</b>
	24 month	abs	0.022	0.021	0.024	0.029
		rel	0.0088	0.0083	<b>0.0105<sup>\$</sup></b>	<b>0.0138<sup>\$</sup></b>

\* p ≤ 0.05 Anova + Dunnetts tests (two-sided)

\$ p ≤ 0.05 Kruskal-Wallis Anova + Mann-Whitney u-tests (two-sided)

abs: absolute organ weight

rel: relative organ weight as % of body weight

### Histopathology:

Histopathological considerations following exposure to FOE 5043 included increased incidences of:

- hepatocytomegaly in 1- and 2-year 400 and 800 ppm males and females;
- hepatic individual cell necrosis in 1-year 800 ppm males and females and 2- year 800 ppm males;
- hepatic biliary hyperplasia/fibrosis in 2-year 400 and 800 ppm males;
- cataracts in 2-year 800 ppm females;
- splenic pigmentation in 1-year 400-ppm females and 1-year 800 ppm males and females
- uterine cystic endometrial hyperplasia in 2-year 400 and 800 ppm females;



g) granulomatous pneumonia in 2-year 400 ppm males and 2-year 800 ppm males and females; and h) suppurative inflammation of the skull (nasal turbinates, nasolacrimal duct, and/or middle ear) in 2-year 800 ppm males.

**Table B.6.5.1-6 Selected histopathology findings (males 24 month group)**

Dose level (ppm)		0	25	400	800
		Male			
Number examined		50	50	50	50
Kidney	pelvic mineralisation	32 (1.6)	<b>45*</b> <b>(1.6)*</b>	<b>46*</b> <b>(2.1)*</b>	<b>45*</b> <b>(2.2)*</b>
	pelvic epithelial hyperplasia	29 (1.7)	36 (1.7)	<b>45*</b> <b>(2.2)*</b>	<b>41*</b> <b>(2.4)*</b>
Liver	hepatocytomegaly	0	0	<b>26*</b> <b>(1.3)*</b>	<b>41*</b> <b>(1.7)*</b>
	hepatic individual cell necrosis	2 (1.5)	2 (1.0)	6 (2.0)	<b>17*</b> <b>(1.9)*</b>
	Hepatic biliary hyperplasia/fibrosis	31 (1.7)	30 (1.9)	<b>40*</b> <b>(2.2)*</b>	<b>42*</b> <b>(2.3)*</b>
	Hepatic tigroid basophilic foci/areas of alteration	17 (1.1)	15 (1.6)	11 (1.8)	5 (2.0)
Spleen	splenic pigmentation	10 (1.2)	13 (1.8)	17 <b>(2.0)*</b>	22 <b>(2.5)*</b>
Eye	ocular scleral mineralization	29 (1.6)	30 (1.7)	<b>41*</b> <b>(1.9)*</b>	<b>44*</b> <b>(2.1)*</b>
	cataract	12 (3.4)	10 (3.2)	7 (3.6)	12 (2.8)
Harderian gland	lymphocytic inflammation	0	1 (3.0)	3 (1.7)	5 (1.4)
Lung	granulomatous inflammation	0	4 <b>(1.8)*</b>	<b>6*</b> <b>(2.0)*</b>	<b>11*</b> <b>(2.6)*</b>
Skull	suppurative inflammation of the nasal turbinates, nasolacrimal ducts, and/or middle ear	12 (1.9)	18 (2.1)	18 <b>(2.2)*</b>	<b>26*</b> <b>(2.7)*</b>

\* Indicates a statistically significant difference from control;  $p \leq 0.05$

() Average severity of animals with lesions: 1 (minimal) to 5 (severe)

**Table B.6.5.1-7 Selected histopathology findings (females 24 month group)**

Parameter		Female			
Dose level (ppm)		0	25	400	800
Number examined		50	50	50	50
Kidney	pelvic mineralisation	27 (1.6)	35 (1.4)	<b>37*</b> <b>(1.6)</b>	<b>42*</b> <b>(1.8)*</b>
	pelvic epithelial hyperplasia	29 (1.6)	32 (1.4)	34 (1.5)	39 <b>(1.8)*</b>
Liver	hepatocytomegaly	2 (1.0)	1 (1.0)	<b>32*</b> <b>(1.4)*</b>	<b>31*</b> <b>(2.2)</b>
	hepatic individual cell necrosis	4 (2.3)	2 (2.0)	4 (2.0)	9 (2.3)
	Hepatic biliary hyperplasia/fibrosis	7 (1.4)	2 (1.0)	5 (1.2)	5 (1.8)
	Hepatic tigroid basophilic foci/areas of alteration	26 (2.0)	27 (1.9)	11 <b>(1.4)*</b>	3 <b>(1.3)*</b>
Spleen	splenic pigmentation	26 (1.5)	28 (1.7)	24 (1.9)	33 <b>(2.3)*</b>
Eye	scleral mineralization	27 (1.4)	33 (1.4)	<b>36*</b> <b>(2.1)*</b>	<b>40*</b> <b>(2.5)*</b>
	cataract	12 (2.8)	15 (2.9)	18 (2.5)	<b>28*</b> <b>(2.0)*</b>
Uterus	cystic endometrial hyperplasia	13 (1.7)	15 (2.0)	<b>24*</b> <b>(2.0)*</b>	<b>24*</b> <b>(2.3)*</b>
Harderian gland	lymphocytic inflammation	13 (1.2)	11 (1.5)	21 (1.5)	<b>22*</b> <b>(1.9)*</b>
Lung	granulomatous inflammation	2 (2.0)	0	2 (2.0)	8* <b>(2.3)*</b>
Skull	suppurative inflammation of the nasal turbinates, nasolacrimal ducts, and/or middle ear	18 (1.9)	17 (2.2)	17 (1.9)	20 (2.5)

\* Indicates a statistically significant difference from control;  $p \leq 0.05$

() Average severity of animals with lesions: 1 (minimal) to 5 (severe)

**Comparative Overview of the Tumors found by Site, Type, Number, and whether Benign or Malignant**

M = male; F = female

b = benign; m = malignant

DOSAGE:	Control		25 ppm		400 ppm		800 ppm	
	male	female	male	female	male	female	male	female
<b>Adrenals</b>								
Pheochromocytoma(b)	8	2	11	4	7	0	5	2
Pheochr., Malig. (m)	1	1	0	0	1	0	0	0
Adenoma, Cortical(b)	0	1	0	1	1	1	0	0
<b>Bone, sternum</b>								
Osteosarcoma(m)	1	0	0	0	0	0	0	0
<b>Heart</b>								
Neurilemmoma(b)	0	0	0	0	0	1	1	0
<b>Kidneys</b>								
Nephroblastoma(b)	1	0	0	0	0	1	0	0
<b>Liver</b>								
Adenoma, Hepatocellular(b)	0	0	2	0	2	0	1	0
Carc., Hepatocell. (m)	0	0	0	1	0	1	0	0
Cystadenoma(b)	1	0	0	0	0	0	0	0
<b>Lymph node(s), other</b>								
Malig. Fib. Histio. (m)	0	0	1	0	0	0	0	0
<b>Mammary gland</b>								
Fibroadenoma(b)	0	5	2	7	1	5	0	1
Adenocarcinoma(m)	0	1	0	1	0	2	0	0
Adenoma(b)	0	1	0	2	0	0	0	0
Cystadenoma(b)	0	0	0	2	0	0	0	0
Fibroma(b)	0	1	0	0	0	0	0	0
<b>Multicentric</b>								
Leuk., Mononuc. Cell(m)	18	11	23	23	2	11	2	5
Mesothelioma(m)	0	0	2	0	2	0	0	0
Sarcoma, Histiocytic	0	0	0	0	1	1	0	0
<b>Muscle, other</b>								
Fibrosarcoma(m)	0	0	1	0	0	0	0	0
Malig. Fib. Histio. (m)	0	0	1	0	0	0	0	0
<b>Ovaries</b>								
Carcinoma(m)	0	0	0	0	0	0	0	1

continued

**Comparative Overview of the Tumors found by Site, Type, Number, and whether Benign or Malignant**  
(continued)

M = male; F = female

b = benign; m = malignant

DOSAGE:	Control		25 ppm		400 ppm		800 ppm	
	male	female	male	female	male	female	male	female
<b>Pancreas</b>								
Adenoma, Islet Cell(b)	0	0	0	0	1	0	2	0
Carcinoma, Islet Cell(m)	2	0	2	0	0	0	0	0
<b>Parathyroids</b>								
Adenoma(b)	2	0	0	0	0	0	2	0
<b>Pituitary</b>								
Adenoma(b)	17	14	20	22	10	9	7	10
Carcinoma(m)	1	0	2	0	0	1	1	0
Cystadenoma(b)	2	0	0	0	0	0	0	0
<b>Skin, forelimb</b>								
Fibroma(b)	2	0	0	0	0	0	0	0
<b>Skin, hindlimb</b>								
Fibrosarcoma(m)	0	0	0	0	0	0	0	1
Basal Cell Tumor(b)	0	0	0	0	1	0	0	0
<b>Skin, protocol</b>								
Fibroma(b)	0	0	0	0	0	1	0	0
<b>Skin, other</b>								
Adenoma(b)	1	0	0	0	1	0	2	0
Fibroadenoma(b)	1	0	0	0	0	0	0	0
Fibroma(b)	2	1	1	0	1	0	2	0
Fibrosarcoma(m)	1	0	1	1	0	0	0	1
Keratoacanthoma(b)	1	0	0	0	1	0	3	0
Papilloma(b)	1	0	1	0	0	0	0	0
Trichoepithelioma(b)	0	0	0	0	0	0	2	0
Melanoma(b)	0	0	0	0	0	1	0	0
<b>Skin, ear</b>								
Papilloma(b)	1	0	0	0	0	0	0	0
<b>Spleen</b>								
Hemangioma(b)	1	0	0	0	0	1	0	0
Hemangiosarcoma(m)	0	0	1	0	1	0	0	0
<b>Testes</b>								
Interst. Cell Tumor(b)	42	0	47	0	45	0	47	0
<b>Thymus</b>								
Sarcoma(m)	0	0	0	0	0	0	0	1

continued

**Comparative Overview of the Tumors found by Site, Type, Number, and whether Benign or Malignant**  
(continued)

M = male, F = female

b = benign; m = malignant

DOSAGE:	Control		25 ppm		400 ppm		800 ppm	
	male	female	male	female	male	female	male	female
<b>Thyroids</b>								
Adenoma,C-Cell(b)	4	3	3	0	5	1	3	5
Adenoma,Follicular(b)	0	0	1	0	0	0	0	1
Carcinoma,C-Cell(m)	4	1	4	0	8	2	3	1
Carcinoma,Follicular(m)	0	0	0	0	0	0	0	1
<b>Urinary bladder</b>								
Sarcoma(m)	0	0	0	0	0	0	1	0
Carcinoma,Trans'l cell(m)	0	1	0	0	0	0	0	0
<b>Uterus</b>								
Adenocarcinoma(m)	1	0	0	0	0	2	0	2
Adenoma(b)	0	0	0	1	0	0	0	0
Carcinoma(m)	0	0	0	0	0	1	0	0
Neurofibrosarcoma(m)	0	0	0	0	0	1	0	0
Polyp,Endometr.Stromal(b)	0	16	0	18	0	12	0	14
Sarcoma,Endo.Str.(m)	0	0	0	0	0	1	0	0
Number of Rats Examined	50	50	50	50	50	50	50	50
Total Number of Tumors	116	59	127	83	91	56	84	47
Number of Malignant Tumors	29	15	38	25	16	21	6	13

<b>Conclusion</b>	<p><b>The study is in line with current guidelines. RMS supports the proposal of the previous evaluation.</b></p> <p>Chronic NOAEL: 25 ppm (equal to 1.2 mg/kg bw/day [males] and 1.5 mg/kg bw/day [females]).</p> <p>No evidence of an oncogenic potential of FOE 5043 to rats.</p>
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**B.6.5.2 Mouse**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>Technical Grade FOE 5043: An Oncogenicity Toxicity Testing Study in the Mouse studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>██████████ (1995d)</b>
<b>Title:</b>	Technical Grade FOE 5043: An Oncogenicity Toxicity Testing Study in the Mouse
<b>Document No:</b>	M-005060-02-1
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7795 of Oct. 03, 1995
<b>Guidelines:</b>	FIFRA § 83-2; TSCA guideline No. 798.3300; OECD guideline No. 451; MAFF guideline 59 NohSan No. 4200; EU-guideline 87/302 Part B
<b>GLP</b>	Yes

**Material and methods:**

FOE 5043, batch No.: Fl 036 from July 04, 1995, purity : 95.2% - 98.5% Corn oil at 1% by weight of the diet; acetone/corn oil mixture used to dissolve the test article prior to mixing with the dietary carrier. The control diet was prepared the same way., excluding only the test substance. administration: dietary admixture with rodent chow in "etts" form, oral by feeding to mice (CD-1 [ICR]/BR) approx. 20 months doses: 0 - 50 - 200 - 400 ppm corresponding to: 0 - 7.4 - 30.4 - 62.2 mg/kg body weight/day in males; 0 - 9.4 - 38.4 - 77.2 mg/kg bw/day in females.

**Findings - General observations:**

Increasing incidence general opacity of eye in 400 ppm males and females; increase pale appearance of eye in 200 and 400 ppm males and females

**Body weight:**

Body weight gain remained unaffected relative to untreated controls in both sexes at all doses tested.

**Mortality:**

FOE 5043 had no effect on animal survival.

**Feed intake:**

Food consumption, assessed in terms of both g consumed/animal/day and g consumed/kg body weight/day, remained unaffected in both sexes at all doses tested.

**Hematology:**

Increasing methemoglobin content in 200 and 400 ppm males and females.

**Gross pathology:**

Increasing incidence of ocular opacity in 400 ppm males and females. The increases in the overall numbers were statistically significant in males in all dose groups and in females at 200 and 400 ppm. When the distribution of cataracts (bilateral versus unilateral) was analysed statistically, the incidence of cataracts in the 50 ppm males appeared more equivocal when compared to controls. In addition, when compared to historical control data collected from oncogenicity studies in CD-1 mouse from the same testing facility the overall number of cataracts observed at the low dose are well within the historical control range of 3-17 and 5-22 for males and females, respectively. Thus, the increased incidence in the overall number of cataracts at the low dose is considered to be a variation of the background range of this spontaneous and age-related lesion, and not a treatment-related effect.

Most of the cataracts observed were minimal to slight in severity and were characterized as a granular alteration confined to the lens fibers along the posterior perimeter of the lens. Occasionally in the more severely affected cases, a band of granular or clumped lens fibers extended from the posterior pole to involve the anterior lens. One mechanism of drug-induced cataractogenesis, which would appear to be particularly relevant to the toxicological profile of flufenacet being described in this report, has been postulated to involve interference with the maintenance of adequate lenticular levels of reduced glutathione (GSH). This is supported by various literature reports that documented that inadequate levels of reduced glutathione levels in the lens is one mechanism by which drug-induced cataractogenesis can occur. Flufenacet may instill deficiencies in the lenticular levels of reduced glutathione either directly or indirectly through increases in blood methemoglobin content, which would essentially create a general oxidative environment/stress during the lifetime of the animal followed by organ damage in the more sensitive tissues (e.g. eye).

**Table B.6.5.2-1 Detailed summary of observed eye cataracts**

Observations	HCD#	Dose group (ppm) males				HCD#	Dose group (ppm) females			
		0	50	200	400		0	50	200	400
Overall no of cataracts	3-17	7	16*	32*	38*	5-22	5	12	33*	42*
Average severity##	1.0-2.9	1.1	1.4*	1.6*	2.1*	1.0-3.1	1.0	1.1	1.2*	2.0*
Unilateral cataracts	--	3	6	7	7	--	4	7	10	6
Bilateral cataracts	--	4	10	25*	31*	--	1	5	23*	36*

\* statistically significant different from control ( $p \leq 0.05$ )

# Historical control data of CD-1 mouse collected from oncogenicity studies conducted at the same testing facility within  $\pm 3$  years of the conduct of this study.

## Average severity of observed lesions: 1 (minimal) to 5 (severe)

**Organ weights:**

No evidence of a treatment-related effect.

**Histopathology:**

Increasing incidence of ocular cataracts in 50, 200, and 400 ppm males and females.



**Comparative Overview of the Tumors found by Site, Type, Number, and whether Benign or Malignant**

M = male; F = female

b = benign; m = malignant

DOSAGE:	Control		50 ppm		200 ppm		400 ppm	
	male	female	male	female	male	female	male	female
<b>Adrenals</b>								
Phochromocytoma(b)	0	0	0	1	0	0	0	0
Adenoma,Cortical(b)	1	0	2	0	0	0	2	0
Adenoma,Spindle Cell(b)	0	0	0	0	0	1	0	1
<b>Bone, rib/costochondral</b>								
<b>Junction</b>								
Neoplasm,Metastatic(m)	1	0	0	0	0	0	0	0
<b>Bone, other</b>								
Hemangiosarcoma(m)	0	0	0	0	0	1	0	0
Osteosarcoma(m)	0	0	0	0	0	0	0	1
<b>Heart</b>								
Neoplasm,Metastatic(m)	1	0	0	0	0	0	0	0
<b>Kidneys</b>								
Adenoma(b)	0	0	0	0	1	0	1	0
Hemangiosarcoma(m)	1	0	0	0	0	0	0	0
<b>Liver</b>								
Adenoma,Hepatocellular(b)	2	1	2	0	2	1	3	0
Carc.,Hepatocell.(m)	1	1	2	0	0	0	1	0
Hemangiosarcoma(m)	3	0	1	1	1	1	0	2
<b>Lymph node(s).other</b>								
Hemangiosarcoma(m)	1	0	0	0	0	0	0	0
<b>Lymph node, mesenteric</b>								
Hemangiosarcoma(m)	0	0	1	0	0	0	0	0
<b>Mammary gland</b>								
Adenoacanthoma(b)	0	0	0	1	0	0	0	2
<b>Multicentric</b>								
Leukemia,Granulocytic(m)	0	0	0	0	0	1	0	0
Malignant Lymphoma(m)	3	10	1	8	1	6	1	8
Mast Cell Tumor,Malig.(m)	1	0	0	0	0	1	0	0
Sarcoma,Histiocytic(m)	0	3	0	2	0	1	0	1
<b>Muscle,other</b>								
Hemangiosarcoma(m)	0	1	0	0	0	1	0	0
<b>Ovaries</b>								
Adenoma(b)	0	0	0	0	0	0	0	1
Cystadenoma(b)	0	1	0	4	0	1	0	0
Granulosa Cell Tumor(b)	0	0	0	0	0	0	0	2
Luteoma(b)	0	0	0	0	0	0	0	1

continued

**Comparative Overview of the Tumors found by Site, Type, Number, and whether Benign or Malignant**  
(continued)

M = male; F = female

b = benign; m = malignant

DOSAGE:	Control		50 ppm		200 ppm		400 ppm	
	male	female	male	female	male	female	male	female
<b>Pancreas</b>								
Neoplasm, Metastatic(m)	1	0	0	0	0	0	0	0
<b>Parathyroids</b>								
Adenoma(b)	0	1	0	0	0	0	0	0
<b>Pituitary</b>								
Adenoma(b)	1	3	0	2	0	0	0	2
<b>Skin, ear</b>								
Papilloma(b)	0	0	1	0	0	0	0	0
<b>Skin, forelimb</b>								
Hemangioma(b)	0	0	0	0	0	1	0	0
<b>Spleen</b>								
Hemangiosarcoma(m)	0	0	2	0	0	1	0	0
<b>Testes</b>								
Interst. Cell Tumor(b)	2	0	0	0	0	0	0	0
I.S. Cell Tum., Mal. (m)	0	0	0	0	1	0	0	0
<b>Thymus</b>								
Neoplasm, Metastatic(m)	1	0	0	0	0	0	0	0
<b>Thyroids</b>								
Adenoma, C-Cell(b)	0	0	0	1	0	0	0	0
Adenoma, Follicular(b)	0	0	0	1	0	0	0	0
<b>Uterus</b>								
Hemangioma(b)	0	0	0	1	0	0	0	0
Hemangiosarcoma(m)	0	1	0	0	0	1	0	0
Leiomyoma(b)	0	1	0	0	0	0	0	0
Leiomyosarcoma(m)	0	0	0	1	0	1	0	0
Polyp, Endometr. Stromal(b)	0	0	0	2	0	3	0	1
Number of Rats Examined	50	50	50	50	50	50	50	49
Total Number of Tumors	20	23	12	25	6	22	8	22
Number of Malignant Tumors	14	16	7	12	3	15	2	12

<b>Conclusion</b>	<p><b>The study is in line with current guidelines. RMS does not object to the NOAEL, supports the proposal of the previous evaluation.</b></p> <p>NOAEL: 50 ppm (equal to 7.4 mg/kg bw/day [males] and 9.4 mg/kg bw/day [females]).</p> <p>No evidence in the mouse of an oncogenic response to FOE 5043.</p>
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### Summary of long-term studies

Study	Sex	NOAEL mg/kg bw/day	LOAEL	Main findings seen at top dose	Reference
Rat 2-year feeding	M F	1.2 1.5	19 24	BW gain ↓, structural and/or functional alterations in liver-, kidney-, haematopoietic-, and thyroid-related endpoints.	██████████, 1995c, M-005062-02-1
Mouse 20-month feeding	M F	7.4 9.4	30 77	MethHB ↑ Ocular cataracts ↑	██████████, 1995d M-005060-02-1

M = male, F = female, BW = body weight, MethHB = Methemoglobin

- For rats and dogs, the toxicological response could be broadly characterized as involving structural and/or functional alterations in liver-, kidney-, hematologic/spleen-, and thyroid-related endpoints. The liver was considered the primary target organ with increases in organ weight, cell size and number, and/or associated hepatic parameters. Hepatocytomegaly was exhibited in both species exposed to higher doses of FOE 5043.
  - The FOE 5043-induced liver changes would appear to be fundamentally adaptive in nature as the organism's principal metabolic organ responds to physiological need to clear, biotransformation, and excrete a xenobiotic.
  - The hematological profile of the rats and dogs indicated a mild anemia for animals at higher dose levels. Thyroid involvement was noted in both species by an increase in thyroid organ weights, and for dogs, a decrease in thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) levels. The lower levels of exposure used in the chronic rat study, as compared to the subchronic bioassay, suggested a dose >800 ppm (highest dose tested) was necessary for a broader and more significant toxicological response in this tissue. The thyroid organ changes resulting from exposure to FOE 5043 are likely to be a secondary effect in response to hepatic induction.
  - Ophthalmological findings noted in the rat included cataracts and ocular scleral mineralization. For dogs, eye effects included minimal to moderate vacuolization of the ciliary body epithelium and cystic vacuolization of the peripheral optic retina.
  - In dogs, specialized testing such as computerized electrocardiograms, clinical neurological examinations, and quantitative electroencephalography revealed a number of compound-related effects.
  - Renal pelvic epithelial hyperplasia was observed in the kidneys of rats and dogs. A minimal to moderate axonopathy was noted in the brain, spinal cord and sciatic nerve of dogs.
- No evidence of an oncogenic potential of FOE 5043 was found in the long-term feeding studies in rats and mice.

### B.6.6 Reproductive toxicity and carcinogenicity

#### Summary of reproductive and developmental toxicity studies

The reproductive toxicity of flufenacet was studied in a generational studies in rats and developmental toxicity studies in rats and rabbits.

Dietary levels up to and including 500 ppm (**premating: 37/41 mg/kg bw/day in males/females**), the highest dose tested, had no effect on reproduction when fed to rats over a period of 2 generations. In parental animals, there was a compound-related reduction in body weights for P generation females during the pre-mating phase. Other effects occurring in the P and F generation adults included increased absolute and relative liver weights and histopathological changes in the liver. The NOAELs obtained for overall and reproductive toxicity were **100** and **500 ppm**, respectively.

In an oral developmental toxicity study in rats, developmental effects were observed at 125 mg/kg bw/day (highest dose tested) as demonstrated by decreased foetal body weights, and increased incidences of delayed ossification and skeletal variation. These effects were correlated with a reduction in body weight and food consumption in dams at 125 mg/kg bw/day. The NAOEL for both maternal and developmental toxicity in the rat via oral administration was 25 mg/kg bw/day.

In an oral rabbit developmental toxicity study, developmental effects occurred at doses of 125 and 200 mg/kg bw/day. Effects included reduced foetal weights, and increased incidences of delayed ossification and skeletal variation. Maternal toxicity was characterized by clinical signs, reduced body weight gain during treatment, and an increase incidence of histopathological changes in the liver. The NOELs established in the rabbit for maternal and developmental toxicity by oral administration were 5 and 25 mg/kg bw/day, respectively.

Overall, it can be concluded that flufenacet is not a reproductive or developmental toxicant. The developmental effects observed were restricted to the higher dose levels which produced overt maternal toxicity.

**Table 6.6-1: Summary of reproductive and developmental toxicity studies**

Study	Sex	NOAEL (mg/kg bw/d)	LOAEL	Main effects seen at LOAEL	Reference
Rat 2-generation feeding	M F	7.4 8.2	37 41	BW ↓ in P females during pre-mating  No reproductive effects.	██████████, 1995 M-004984-03-1
Rat oral (gavage) developmental	Dam Fetal	25 25	125 125	Maternal: BW ↓, food consumption ↓ Fetal: BW ↓, delayed ossification and/or skeletal variation ↑ in some skeletal elements	██████████, 1995 M-004976-02-1
Rabbit oral (gavage) developmental	Dam Fetal	5 25	25 125	Maternal: soft stool, BW gain ↓ during treatment, histopathological liver changes Fetal: skeletal variation ↑	██████████, 1995 M-004979-01-1

M = male, F = female, D = dam, Fet = fetus, BW = body weight

↓ = decrease, ↑ = increase

**B.6.6.1 Multi-Generation Study on Rats**

Previous evaluation	In DAR for original approval (1997);  A two-generation dietary reproduction study in rats using technical grade FOE 5043 studies were presented and evaluated during the EU process for Annex I listing.
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Report:	████████████████████ (1995)
Title:	A two-generation dietary reproduction study in rats using technical grade FOE 5043
Document No:	M-004984-03-1
Report No:	Bayer Corporation, unpublished report no. 7695 of June 19, 1995
Guidelines:	US-EPA-FIFRA, § 83-4 (1984), US-EPA-TSCA, 40 CFR Section 798.4700, OECD Guideline 416 (1983), JMAFF guideline 59 NohSan No. 4200 (1985)
GLP	Yes

**Material and methods:****A. Materials****1. Test material:**

	FOE 5043
Synonym(s):	flufenacet
Specification no.:	n.a.
Description:	cream powder
Lot/Batch no:	Fl. 036 from 7/4/91
Purity:	95.2-99.0%
Stability of test compound:	n.a.

**2. Test animals**

Species:	rat
Strain:	CD Sprague-Dawley
Sex:	Male and female (nulliparous and non-pregnant)
Source:	████████████████████
Acclimatisation period:	Two weeks
Diet:	Purina Mills Rodent Lab Chow 5001-4 in "etts" form, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually (except mating period) housed in suspended stainless steel wire-mesh cages containing deotized cage board in the bedding tray. During the gestation and lactation phases females were housed individually in polycarbonate cages with Bed-O-Cobs bedding.

Environmental conditions: Temperature: 18 – 26°C  
 Humidity: 40-70%  
 Air changes: not reported  
 Photoperiod: 12 hr light/dark cycle

## B. Study design and methods

### 1. Animal assignment and treatment:

Route of administration: Oral (diet)  
 Exposure: The P adults were bred to produce the F1 litters after receiving the test compound for ten weeks. The F1 pups selected to be parents for the F2 generation were placed on treated feed following weaning. Approximately two weeks following the weaning of the last litter, these animals received treated feed for an additional ten weeks prior to mating.

Group size: 30/sex/dose/generation

Dose levels: Dose in mg/kg bw/day<sup>1</sup>:

Level	Pre-mating (male/female)	Gestation	Lactation <sup>2</sup>
20 ppm	1.4/1.5	1.3	2.4
100 ppm	7.4/8.2	6.9	13.3
500 ppm	37.4/41.4	36.2	68.7

<sup>1</sup> average of the mean doses for the P and F1 animals

<sup>2</sup> The values are the average dose for the first two weeks of the lactation phase, as pups begin eating food during the third week of lactation.

Dose selection rationale: These doses were selected based principally upon the data generated from a pilot study in rats with FOE 5043 in the diet.

Mating procedure: Breeding was accomplished by co-housing one female with one male. Each morning, vaginal smears were examined microscopically for sperm. When sperm were observed, the female was placed in a polycarbonate cage for the gestation and lactation periods.

Animals were co-housed for up to 21 consecutive days. Unmated females were then co-housed for up to seven days during a fourth week of breeding with a proven male from the same dose group.

### 2. Dose preparation and analysis:

**Dose preparation:**

An acetone/corn oil mixture was used as a vehicle to dissolve the test substance prior to mixing with the dietary carrier (final vehicle concentration: 1% corn oil and 1% acetone).

The control diet was prepared the same as the treated diet (including the acetone/corn oil mixture), excluding only the test chemical.

**Analysis:**

The FOE 5043 concentration in the ration was determined by liquid chromatographic analysis.

The distribution of FOE 5043 mixed with rodent ration was determined at 10 and 1000 ppm, which bracketed the low and high test concentrations.

The stability of FOE 5043 mixed in rodent ration at 10 and 1000 ppm, which bracketed the low and high test concentrations, was assessed under freezer conditions (approximately -23°C) for 51 days and at room temperature (approximately 22°C) for 14 days

**3. Observations:****Mortality and clinical signs:**

Twice daily observations (once daily on weekends and holidays) were conducted for moribundity, mortality, and clinical signs by observing the animals in their cages. Detailed observations were made once a week by removing the animals from their cages. All clinical signs were recorded when initially observed, and once a week thereafter during the weekly detailed observation.

**Body Weight and food consumption determinations:**

During the ten-week period prior to the P and F1 matings, body weight and food consumption were measured once a week for each female. Body weight and food consumption were taken for dams during gestation and lactation as follows: body weight - days 0, 6, 13, and 20 during gestation and days 0, 4, 7, 14, and 21 during lactation; food consumption - once a week during gestation, twice a week during week 1 of lactation and once a week during weeks 2 and 3 of lactation.

Body weight was measured once a week for each male during the study. Food consumption was measured once a week, except during the co-housing period, for each male during the study.

**Pup Data:**

The number of live and stillborn pups was recorded for each litter. Litter counts were performed daily from days 0 to 21. Individual pup weights were recorded as soon as possible after completion of delivery (day 0), and on days 4, 7, 14, and 21. Each litter was culled on day 4 to yield eight pups, including, if possible, four males and four females per litter. Adjustments were made by the random selection of pups from each litter. Culled pups were sacrificed and subjected to a gross necropsy examination. No adjustment was made in litters of eight or fewer pups.

**Sacrifice and pathology:**

Adult rats and weanlings were sacrificed by carbon dioxide asphyxiation. The pups culled on day 4 were sacrificed by intracranial injection of an approved euthanasia solution. Adult P and F1 males were sacrificed after the last F1 and F2 litters, respectively, were delivered and nondelivering dams had gone past day 24 of gestation. Adult P and F1 females were sacrificed after each dam's pups were weaned/died or when day 24 of gestation was reached.

All adults and pups were necropsied. Terminal body weights, liver weights, and gonad weights were collected from all P and F1 adults. Relative organ weights were calculated for all organs weighed (organ weight/body weight X 100). A wide range of tissues were collected from the P and F1 adults and fixed in 10% buffered formalin. Tissues were processed routinely and stained with hematoxylin and eosin (H&E). Recuts and special stains were requested as deemed necessary.

**4. Statistical Analysis and reproductive indices:**

Statistical analysis: Continuous data that were examined statistically were evaluated initially for equality or homogeneity of variance using Bartlett's test. Group means were further analyzed by a one-way variance analysis (ANOVA) followed by Dunnett's test. In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test for between-group comparisons.

Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the chi-square, Fisher exact, or chi-square and Fisher exact tests.

For the Bartlett test, a probability (p) value  $\leq 0.001$  was considered significant; for all other statistical tests, differences with p values  $\leq 0.05$  were considered statistically significant. Software from

DATATOX was used to analyze the gross pathologic, body weight and food consumption data, for all other data



software from SAS was used for the analysis.

Reproductive indices: mating index, fertility index, gestation index, birth index, live birth index, viability index

## II. Results and discussion

### 1. Parental animals

#### A. Mortality

There were no treatment-related deaths during the study.

#### B. Clinical signs of toxicity

There were no compound-related clinical signs.

#### C. Body weight

For body weight, there was no compound-related effect for P and F1 males, or F1 females. However, for P females there was a compound-related decrease in body weight in the high-dose group during the pre-mating phase of the study. The mean body weight for the P females in the high-dose group began diverging from the other dose groups on day 32, and on days 63 and 70 the body weight for the high-dose group was statistically significantly lower than the control group (7% and 6%, respectively) which was continued throughout the gestation and lactation phases. When evaluated in regards to body weight gain, a statistically significant 17% decrease in weight gain was observed during premating. However there was no statistically significant difference in the weight gain for the high-dose group during the P gestation or lactation period.

Sporadic statistically significant differences observed in all dose groups are considered incidental.

**Table B.6.6.1-1 Intergroup comparison of mean body weights (g) - selected time points**

	Pre-mating/mating							
	P adults (First generation)							
Dose level (ppm)	Male				Female			
	0	20	100	500	0	20	100	500
Day 0	249.5	247.7	254.1	253.3	183.5	182.9	186.9	185.3
Day 7	298.3	294.3	310.1*	305.0	208.4	208.0	215.5	210.3
Day 14	339.4	337.6	352.6	343.9	228.3	228.3	232.6	227.6
Day 70	520.9	520.0	533.8	524.2	310.9	314.1	309.3	292.0*
Day 105	574.1	569.2	579.4	566.7	--	--	--	--
	F <sub>1</sub> adults (Second generation)							
Day 0	286.4	299.8	299.3	279.3	202.2	209.0	204.3	198.9
Day 7	351.0	360.9	360.5	346.6	224.2	233.3	235.2	226.2
Day 14	399.5	413.2	402.4	389.7	246.6	257.8	255.3	246.5
Day 70	575.1	602.9	600.9	566.3	313.2	332.1*	325.6	308.6
Day 119	645.3	681.6	671.8	641.1	--	--	--	--

	Gestation							
	First generation dams				Second generation dams			
Day 0	314	319	316	295*	308	333*	328*	309
Day 6	341	346	342	321*	325	346	342	329
Day 13	372	376	372	351*	359	383*	375	358
Day 20	463	469	461	433*	442	459	464*	439
Gain	149	150	145	138	133	126	136	131
	Lactation							
	First generation dams				Second generation dams			
Day 0	353	359	352	335*	350	363	360	349
Day 4	358	360	356	337*	352	375*	371*	345
Day 7	361	365	367	345	356	373	371	352
Day 14	393	386	381	364*	369	386	382	375
Day 21	390	387	387	379	357	387*	385*	375*

\*  $p \leq 0.05$  Anova + Dunnetts tests (two-sided)

#### D. Food consumption

There was no compound-related effect on food consumption for males or females during pre-mating, and gestation or lactation for P and F1 dams.

Sporadic statistically significant differences observed in all dose groups are considered incidental.

#### E. Reproductive performance

There were no compound-related effects on adult reproductive parameters (estrous cyclicity, insemination length, mating index, fertility index, gestation index and length, implantation sites and birth index).

**Table B.6.6.1-2 Summary of reproductive parameters for P-generation**

Dose level (ppm)	0	20	100	500
<b>P-generation dams (F1-pups)</b>				
Number cohoused	29	30	30	30
Number sperm positive	29	30		
Mating Index	100	100	100	100
number of animals delivered	25	22	23	26
number of animals with implants	27	22	24	26
Number of animals with live born pups	25	22	23	26
Fertility index	93	73	80	87
Gestation index	93	100	96	100
Insemination length (days)	2.7	3.8	3.1	2.2
Gestation length (days)	21.5	21.5	21.7	21.7
Birth index (%)	93	94	86	90
No of litters	25	22	23	26
Litter size	16	15	15	14
Still born	0	0	0	0
unknown	0	0	0	0

Dose level (ppm)	0	20	100	500
<b>F1-pups</b>				
Viability index LD 4	99	99.0	95	96
Viability index LD 7	100	100	96	100
Viability index LD 14	99	99	96	99
Viability index LD 21	99	99	96	99
Live birth index	99	99	96	98
Sex ratio (% males)	53	54	53	51
Pup weight (LD0)	6.6	6.8	7.0	6.6
Pup weight (LD4)	9.6	10	10.3	9.8
Pup weight (LD7)	15.5	16.6	17.3* (+12%)	15.7
Pup weight (LD14)	31.7	32.9	33.7* (+6%)	30.7
Pup weight (LD21)	48.6	50.7	52.6* (+8%)	48.3

**Table B.6.6.1-3 Summary of reproductive parameters for F1-generation**

Dose level (ppm)	0	20	100	500
<b>F1-generation dams</b>				
Number cohoused	30	30	30	30
Number sperm positive	30	28	30	30
Mating Index	100	100	100	100
number of animals delivered	23	20	27	27
number of animals with implants	23	20	27	27
Number of animals with live born pups	23	20	27	27
Fertility index	77	71	90	90
Gestation index	100	100	100	100
Insemination length (days)	3.9	3.8	3.1	2.9
Gestation length (days)	21.9	21.8	21.8	21.9
Birth index (%)	90	90	93	89
No of litters				
Litter size	14	16	16*	14
Still born	0	0	0	0
unknown	0	0	0	0
<b>F2-pups</b>				
Viability index LD 4 (pre-culling)	96	98	99	94
Viability index LD 7	100	100	100	100
Viability index LD 14	100	99	100	96
Viability index LD 21	100	99	100	96
Live birth index	99	98	99	96
Sex ratio (% males)	47	46	47	49
Pup weight (LD0)	6.7	6.6	6.7	6.8
Pup weight (LD4)	10.7	10.2	10.2	9.9
Pup weight (LD7)	17.5	17.4	16.7	16.1
Pup weight (LD14)	34.5	35.5	33.7	31.4* (-9%)
Pup weight (LD21)	51.8	53.7	52.2	48.6

## F. Organ weight

There was no compound-related effect on, ovary weights, testicle weights, or male liver weights. Absolute and relative hepatic weights were increased in P and F1 high-dose group females and in F1 mid-dose group females. There was a statistically significant lower relative liver weight (8%) in the P mid-dose group males which is considered incidental.

Ovary weights, both absolute and relative, were statistically significantly decreased in P high-dose group females (absolute ovary weights were 18% below the control group). This change did not occur in the F1 females in the high-dose group, therefore, it is not considered a compound-related effect. Absolute and relative ovary weights were significantly increased over control values in F1 mid-dose group rats (17% above the control group). This was considered a chance variation.

**Table B.6.6.1-4 Summary of selected mean adult organ weights**

Parameter			P adults (first generation)			
Dose level (ppm)			0	20	100	500
Body weight (g)	male		585.8	581.7	600.5	586.0
	female					
Liver (g)	male	a	27.173	25.430	25.403	28.743
		r (%)	4.628	4.353	<b>4.243*</b>	4.904
	female	a	20.078	20.496	20.129	<b>23.803*</b>
		r (%)	5.431	5.665	5.693	<b>6.761*</b>
Ovary (g)	male	a	--	--	--	--
		r (%)	--	--	--	--
	female	a	0.184	0.170	0.171	<b>0.150*</b>
		r (%)	0.050	0.047	0.049	<b>0.043*</b>
			F1 adults (second generation)			
Body weight (g)	male		656.3	693.4	679.8	647.3
	female					
Liver (g)	male	a	30.645	30.678	31.148	30.635
		r (%)	4.675	4.407	4.590	4.735
	female	a	18.403	19.399	<b>21.853*</b>	<b>22.430*</b>
		r (%)	5.368	5.357	<b>6.071*</b>	<b>6.543*</b>
Ovary (g)	male	a	--	--	--	--
		r (%)	--	--	--	--
	female	a	0.155	0.169	<b>0.181*</b>	0.160
		r (%)	0.045	0.047	<b>0.051<sup>§</sup></b>	0.047

\* significantly different from control, Anova + Dunnetts tests (two-sided)  $p \leq 0.05$

<sup>§</sup> significantly different from control, Kruskal Wallis + Mann-Whitney U tests (two-sided)  $p \leq 0.05$

a: absolute organ weight (g)

r: relative organ weights as % of body weight

## G. Gross necropsy

There were no compound-related gross lesions in P and F1 males and females. All gross lesions noted in adult rats were considered incidental.

## H. Histopathology

In the liver, increased weight and morphologic evidence of hepatocellular hypertrophy were observed. Absolute and relative hepatic weights were increased in P and F1 high- dose group females and in F: mid-dose group females. Morphologic evidence of hepatocellular hypertrophy was noted in the P and F1, high-dose group males, in the F1, high-dose group females, and in the F1, mid-dose group males. These compound-induced changes are considered an adaptive change due to an induction of metabolic enzymes brought on by treatment with FOE 5043.

**Table B.6.6.1-5 Summary of histopathology findings**

Parameter			P adults (first generation)			
Dose level (ppm)			0	20	100	500
Number examined			30	30	30	30
Liver	Hepatocytomegaly	male	0	0	<b>0</b>	<b>14*</b> <b>(2.1)*</b>
		female	0	0	0	3 (1.3)
			F1 adults (second generation)			
Number examined			30	30	30	30
Liver	Hepatocytomegaly	male	0	2 (2.0)	<b>9*</b> <b>(2.1)*</b>	<b>14*</b> <b>(2.3)*</b>
		female	0	--	2 (2.0)	<b>17*</b> <b>(2.2)*</b>

\* Indicates a statistically significant difference from control;  $p \leq 0.05$

() Average severity of animals with lesions: 1 (minimal) to 5 (severe)

## 2. Offspring

### A. Clinical signs of toxicity

There were no compound-related clinical signs for pups.

### B. Litter parameters

There was no compound-related effect on pup gender, litter size and viability indices.

### C. Body weight

There was no compound-related effect on the pup weight.

An incidental finding was statistically significant higher pup weights observed in the F1 mid-dose group on days 0, 7, 14, and 21 (6-12% greater than the control group).

During the F2 generation the high-dose group pup body weights were lower than the control group on days 4, 7, 14, and 21 (6-9% lower than the control group) with statistical significance occurring on day 14. However, the lower body weights were not considered compound-related for the following reasons:

1. There was no effect on pup body weight in the high-dose group during the F1 breeding.
2. The F1 mid-dose group pups had the same magnitude of weight difference from the control group, but in the opposite direction. This indicates that the lower body weight in the F2 high-dose group pups was within the normal variation for pup weights in this study.
3. On day 21, the F2 high-dose group pups weighed the same as the F2 control group.
4. The F2 high-dose group pup weights for days 4, 7, 14, and 21 were within the range of the historical controls for each time period.

**Table B.6.6.1-6 Intergroup comparison of mean body weights (g) - selected time points**

	Pup weight							
	F <sub>1</sub> pups (♂+♀)				F <sub>2</sub> pups (♂+♀)			
Dose level (ppm)	0	20	100	500	0	20	100	500
Day 4 (postcull)	9.6	10.0	10.3	9.8	10.7	10.2	10.0	9.9
Day 7	15.5	16.6	17.3*	15.7	17.5	17.4	16.7	16.1
Day 14	31.7	32.9	33.7*	30.7	34.5	35.5	33.7	31.4*
Day 21	48.6	50.7	52.6*	48.3	51.8	53.7	52.2	48.6

\* Indicates a statistically significant difference from control;  $p \leq 0.05$

#### **D. Gross necropsy**

There were no compound-related gross observations in 0- to 3-day found dead, 4-day culls, 21-day weanlings, or in other pups in either the F1 or F2 litters.

### **III. Conclusion**

Based on decreased body weights and hepatic effects in the high dose group the parental NOAEL is 100 ppm (7.4 / 8.2 mg/kg bw/day (m/f)). However, as the changes in the liver are considered an adaptive change .

FOE 5043 did not cause reproductive toxicity at maternally and paternally toxic doses. Thus, the offspring and reproductive NOAEL is 500 ppm (37.4 / 41.4 mg/kg bw/day (m/f)).

<b>Conclusion</b>	<p><b>The study is in line with current guidelines. RMS does not object to the NOAEL supports the proposal of the previous evaluation.</b></p> <p>The parental (overall) NOAEL is 100 ppm (7.4 / 8.2 mg/kg bw/day (m/f)) based on decreased body weights and hepatic effects in the high dose group.</p> <p>The offspring and reproductive NOAEL is 500 ppm (37.4 / 41.4 mg/kg bw/day (m/f)).</p>
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**B.6.6.2 Developmental Toxicity Studies**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>A developmental toxicity study with orally administered FOE 5043 technical in the rat studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report:</b>	<b>[REDACTED]; 1995</b>
<b>Title:</b>	A developmental toxicity study with orally administered FOE 5043 technical in the rat
<b>Document No:</b>	M-004976-02-1
<b>Report No:</b>	Miles Inc., unpublished report no. 7471 of 10 January 1995
<b>Guidelines:</b>	FIFRA § 83-3; TSCA, 40 CFR Section 798.4900; OECD Guideline 414; MAFF guideline 59 NohSan No. 4200; EU-Guideline 87/302 Part B
<b>GLP</b>	Yes

**Material and methods:**

FOE 5043, batch number: FL.036 from 04 July 1991 purity: 97.2% formulated in Aqueous carboxymethylcellulose 0.5%, with polyoxyethelene sorbitan monooleate (Tween 80), 0.4% oral administration by gastric intubation on gestation days 6-15 to Sprague-Dawley rats:

Dosage (a.i.): 0, 5, 25, 125 mg/kg bw/day

**Findings:****Maternal data**

<b>Dose mg/kg</b>	<b>Deaths</b>	<b>Clinical signs</b>	<b>necrospy</b>	<b>Reduced food consumption</b>	<b>Decreased body weight gain</b>
0	0	no	no	0	0
5	no	no	no	no	no
25	no	no	no	no	no
125	no	yes	no	yes	yes

**Reproduction parameters**

<b>Dose mg/kg</b>	<b>Post implantation loss</b>	<b>Embryo lethality</b>	<b>Teratogenicity</b>	<b>Fetotoxicity (body weight) (g)</b>
0	no influence	387/420 7.9%	no	3.5
5	no influence	405/447 9.4%	no	3.6
25	no influence	405/447 9.4%	no	3.6
125	no influence	373/401 7.0%	no	3.3**

**Fetal data:**

Reduced fetal body weight at the high dose. No adverse effects on external appearance, viscera (including the head), skeletons, sex ratio, or the number of live fetuses.

**Table B.6.6.2-1 Summary of a developmental toxicity study with orally administered FOE 5043 technical in the rat**

<b>Flufenacet</b>								97.2% purity
<b>Dev Tox Rat Treatment GD 6-15</b>	<b>Female (Charles River crl:CD (SD) BR)</b>							
<i>Dose. (mg/kg)</i>	<b>0</b>	<b>5</b>		<b>25</b>		<b>125</b>		
No of females inseminated	30	30		30		30		
No of females pregnant	28	29		28		27		
Killed or found dead before termination	0	0		0		0		
No of females with resorptions only	0	0		0		0		
No of females with live fetuses	28	29		28		27		
Food consumption d 1 [g]	20.3	21		21.3		21.5		
Food consumption d 6 [g]	22.6	23.5		23.2		23.0		
Food consumption d 7 [g]	21.6	22.9		20.6		12.9	**	
Food consumption d 12 [g]	24.9	25.3		24.8		22.6	*	
Food consumption d 16 [g]	26.9	26.7		26.5		28.4		
Food consumption d 20 [g]	27.2	27.3		27.1		28.6		
BW GD 0 [g]	248.1	253.4		252.1		255.5	*	
BW GD 6 [g]	269.8	278.2	*	273.7		277.9	*	
BW GD 8 [g]	275.3	284.4	*	279.3		264.7	**	
BW GD 9 [g]	280.5	288.7	*	282.4		269.9	**	
BW GD 10 [g]	285.6	293.6		288.1		276.1	*	
BW GD 11 [g]	291.5	300.3		294.9		281.9	*	
BW GD 12 [g]	295.1	304.1		298.6		286.4		
BW GD 13 [g]	300.5	308.8		302.8		292.1		
BW GD 15 [g]	313.2	232.2		316.4		306.9		
BW GD 16 [g]	324	333.4		327.3		318.1		
BW GD 20 [g]	383.5	393.5		388.2		374		
adjusted body weight [g]	304.3	313.5		307.8		298.4		
BW gain d 6-20 [g]	135.5	140.1		136		118.5	**	
BW gain d 6-16 [g]	54.2	55.2		53		40.2	**	
<i>Dose (mg/kg)</i>	<b>0</b>	<b>5</b>		<b>25</b>		<b>125</b>		
BW actual gain [g]	56.2	60		55.6		42.9	**	
N females examined	28	29		28		27		
Placental weight (mg)	0.55	0.54		0.57		0.55		
Mean N corpora lutea	15.0	16.0		16.0		16.0		
Mean N implants	15	15.4		15		14.9		
No of litters	28	29		28		27		
Litter size (mean)	13.8	14		13.9		13.8		
Mean % preimplant loss	5.5	4.1		7.8		5.3		
Mean postimplantation loss	8.1	9.8		6.9		7.1		
No of dead fetuses	0	0		0		1		
No of viable fetuses	387	405		390		373.0		
Mean N live fetuses	13.8	14		13.9		13.8		



Median % male fetuses	50	50		50		45.5			
Mean N resorptions	1.2	1.5		1.1		1			
Fetal weight of males [g]	3.6	3.7		3.7		3.4	**		
Fetal weight of females [g]	3.4	3.5		3.6		3.2	**		
fetal incidence skeletal variations									
<b>extra ribs (%)</b>	1	1.9		1.5		9.2	**		
<b>wavy or curved ribs (%)</b>	1	2.4		2		3.2			
<b>vertebrae: thoracic-centra incompl. Ossified (%)</b>	73.4	71		72.2		84.9	*		
<b>vertebrae: caudal arches unossified (%)</b>	1.5	4.3		4.5		13.5	**		
Litter incidence skeletal variations									
<b>extra ribs (%)</b>	3.6	6.9		7.4		42.3	**		
<b>wavy or curved ribs (%)</b>	7.1	17.2		11.1		15.4			
<b>vertebrae: thoracic-centra incompl. Ossified (%)</b>	100	100		96.3		100			
<b>vertebrae: caudal arches unossified (%)</b>	10.7	17.2		25.9		46.2	*		

**HCD from study report**

extra ribs (%)	HCD (1990-1992): 0.5-15.8
wavy or curved ribs (%)	HCD (1990-1992): 1.0-4.9
vertebrae: thoracic-centra incompl. Ossified (%)	HCD (1990-1992): 58.1-90.9
vertebrae: caudal arches unossified (%)	HCD (1990-1992): 0.5-2.4
<b>Litter incidence skeletal variations</b>	
extra ribs (%)	HCD (1990-1992): 3.4-45.8
wavy or curved ribs (%)	HCD (1990-1992): 4.0-17.9
vertebrae: thoracic-centra incompl. Ossified (%)	HCD (1990-1992): 96.2-100
vertebrae: caudal arches unossified (%)	HCD (1990-1992): 1.0-9.1

<b>Conclusion</b>	<p><b>The study is in line with current guidelines.</b></p> <p>No observable adverse effect level: 25 mg/kg bw/day (maternal and reproduction parameters) developmental effects were observed at 125 mg/kg bw/day (highest dose tested) as demonstrated by decreased fetal body weights, and increased incidences of delayed ossification and skeletal variation. These effects were correlated with a reduction in body weight and food consumption in dams at 125 mg/kg bw/day. The NOAEL for both maternal and developmental toxicity in the rat via oral administration was 25 mg/kg bw/day.</p>
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**B.6.6.3 Oral Study on Rabbits**

Previous evaluation	In DAR for original approval (1997);  A developmental toxicity study with orally administered FOE 5043 technical in the rabbit. studies were presented and evaluated during the EU process for Annex I listing.
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Report:	██; 1995
Title:	A developmental toxicity study with orally administered FOE 5043 technical in the rabbit
Document No:	M-004979-01-1
Report No:	Miles Inc., unpublished report no. 7661 of 22 May 1995
Guidelines:	FIFRA § 83-3; TSCA 40 CFR Section 798.4900, OECD guideline 414: MAFF guideline 59 NohSan No. 4200; EU-guideline 87/302 Part B
GLP	Yes

**Material and methods:****A. Materials****1. Test material:**

FOE 5043
Synonym(s): flufenacet
Specification no.: n.a.
Description: cream-colored powder
Lot/Batch no: Fl. 036 from 7/4/91
Purity: 98.5%
Stability of test compound: stable during use in this study

**2. Vehicle**

CMC (0.5% carboxymethylcellulose sodium and 0.4% polyoxyethylene sorbitan mono oleate (Tween 80 NF) in distilled water)

**3. Test animals**

Species:	rabbit
Strain:	New Zealand White
Age at the time of breeding:	Male: > 1 year Female: > 30 weeks
Weight at the time of breeding:	Male: 3.76-4.62 kg Female: 2.91-4.22 kg
Source:	██
Acclimatisation period:	at least 26 days
Diet:	130 g of Purina Certified Rabbit Chow #5322 (PMI Feeds, Inc., St. Louis, MO) daily
Water:	Tap water, <i>ad libitum</i>  All rabbits were housed individually in stainless steel

cages.

Environmental conditions:

Temperature: 18 – 21°C

Humidity: 40-70%

Air changes: not reported

Photoperiod: 12 hr light/dark cycle

## **B. Study design and methods**

### **1. Animal assignment and treatment:**

Route of administration:

Oral (gavage)

Exposure:

All does were dosed once daily from days 6 through 18 of gestation for a total of 13 consecutive days at a volume of 5 mL/kg.

Group size:

20 artificially inseminated female rabbits/dose

Dose levels:

5, 25, 125 and 200 mg/kg bw + 2 control groups<sup>a</sup>

Dose selection rationale:

Doses were selected based on a range finding study conducted prior to this study.

Mating procedure:

Breeding was accomplished by means of artificial insemination employed over 4 days for the primary groups and 2 days for the supplemental groups.

Semen was collected from proven bucks, evaluated, diluted in 0.9% sterile saline, and administered intravaginally by pipette to randomly selected does. Prior to insemination, does were primed by intravenous HCG (human chorionic gonadotropin) injection (approximately 50 USP units) and they received a second intravenous injection of HCG (approximately 100 USP units) concomitant with the pipetted semen.

### **2. Dose preparation and analysis:**

Dose preparation:

The test article was prepared as either a 0.0% [(0 mg/mL) - on separate occasions for primary and supplemental groups], 0.1% (1 mg/mL), 0.5% (5 mg/mL), 2.5% (25 mg/L), or 4.0% [(40 mg/kg) - supplemental group] suspension in CMC.

Analysis:

The different test article concentrations in CMC vehicle were analyzed regarding FOE 5043 concentration and homogeneity prior administration. Stability testing demonstrated that test article suspensions in concentrations ranging from 0.25 to 50 mg/mL in the CMC vehicle, remained stable for at least 36 days under conditions mimicking those used in the study.

### **3. Observations:**

Maternal observations:

All does were observed 14 twice daily for overt changes in appearance and behavior. Doe body weights were obtained on days 0, 6-19, 21, and 29 of gestation. Food consumption

was monitored (days 1, 6, 7, 12, 15, 19, 23, and 29 of gestation).

On the twenty-ninth day of gestation, all does were sacrificed by barbiturate overdose. Pregnancy was confirmed, the abdomen was opened, ovaries excised, and the corpora lutea graviditatis counted and recorded. The uterine horns were transected at the cervix, trimmed along the antimesometrial margin, removed, and weighed. Each uterine horn was opened longitudinally and the amniotic sacs displaced to one side to facilitate inspection of the uterine walls for the presence of resorptions. All fetuses and resorptions were removed and each implant was noted. The abdominal and thoracic viscera from the females were scrutinized, livers and thyroid glands were removed and weighed, and gross pathological changes were recorded. A number of maternal reproductive parameters (e.g., pregnancy rates, litter size) were measured.

#### Fetal evaluations:

Each fetus was removed from its amniotic membranes, the umbilical cord was severed close to its attachment to the fetus, and viability of the fetus was determined.

Each fetus was blotted dry, removing blood and amniotic fluid, sexed and weighed. Individual placentas, corresponding to each fetus, were trimmed of extraneous tissue, blotted dry, and weighed. A complete external examination was conducted on each fetus. All fetuses were sacrificed by intracranial injection of 15% w/v aqueous sodium pentobarbital.

A complete internal examination was performed (with the assistance of a stereoscope) examining the abdominal and thoracic viscera on approximately one-half of the fetuses.

Following visceral examination fetuses were placed into Bouin's fixative, stored, and later free-hand razor blade sections were made transversely through the mouth to the back of the head, then frontally through the nasal septum, eyes and cerebrum.

Following external and internal examination all fetuses were fixed *in toto* in 70% ethanol and stored. These fetuses were later eviscerated and processed using a refinement of the KOH Alizarin Red-S method for clearing tissue and staining fetal bone, and then evaluated for general skeletal development.

#### 4. Statistical Analysis:

Statistical analysis of the data consisted of application of one or more of the following tests: Dunnett's, Dunnett's test on rank data, Fisher's exact, and the two sample t-test.

#### Findings:

#### Maternal data

Dose mg/kg	Deaths	Clinical signs	necrospy	Reduced food consumption	Decreased body weight gain
0	no	no	no	no	no
5	no	no	no	no	no
25	no	no	Yes*	no	no
125	no	yes	Yes*	no	decreased bodyweight gain
200	no	yes	Yes*	no	body weight loss

\* histopathological liver changes

### Reproduction parameters

Dose mg/kg	Post implantation loss	Embryoletality	Teratogenicity	Fetotoxicity (body weight) (g)
0	no influence	95/116 18%	no	47.6
5	no influence	111/112 1%	no	45.9
25	no influence	101/104 3%	no	49.0
125	no influence	111/80 3.8%	no	46.1
200	no influence	74/ 79 6.3%	no	44.2 **

\*\* significantly different from control at the 0.01 level

**Table: B.6.6.3-1 A developmental toxicity study with orally administered FOE 5043 technical in the rabbit**

<b>Flufenacet</b>			Exp. Start:	26.07.1993						97.2% purity
<i>Dose (mg/kg)</i>	<b>0</b>	<b>5</b>	<b>25</b>		<b>125</b>		<b>0</b>	<b>200</b>		
No of females inseminated	20	20		20		20		20	20	
No of females pregnant	15	17		17		15				
Killed or found dead bef termin	0	0		0		1		0	1	
No of females w resorptions only	1	0		0		0		0	0	
No of females aborted	0	0		1		0			0	
Food consumption d 1 [g]	126	129.4		129.1		129.2		129.6	127.4	
Food consumption d 6 [g]	130	130		129.1	*	129.4		129.8	129.6	
Food consumption d 7 [g]	130	130		129.5		129.5		129.7	128.5	
Food consumption d 12 [g]	130	130		129.7		129.5	**	129.2	129.6	
Food consumption d 15 [g]	124.1	130		129.4		120.4		129.4	130.0	*
Food consumption d 19 [g]	129.6	129.6		129.1		121.4		129.6	127.9	
BW GD 0 [kg]	3.64	3.54		3.57		3.65		3.47	3.44	
BW GD 6 [kg]	3.68	3.63		3.64		3.7		3.63	3.61	
BW GD 8 [kg]	3.68	3.64		3.65		3.69		3.58	3.47	
BW GD 9 [kg]	3.67	3.63		3.64		3.67		3.59	3.45	
BW GD 10 [kg]	3.68	3.62		3.63		3.66		3.61	3.48	
BW GD 11 [kg]	3.69	3.63		3.66		3.67		3.6	3.47	
BW GD 12 [kg]	3.7	3.64		3.65		3.66		3.66	3.5	*
BW GD 13 [kg]	3.77	3.72		3.72		3.7		3.72	3.55	*
BW GD 15 [kg]	3.82	3.78		3.78		3.79		3.8	3.65	
BW GD 19 [kg]	3.83	3.79		3.82		3.79		3.75	3.61	
BW GD 29 [kg]	3.99	3.86		3.94		3.95		3.88	3.78	
adjusted body weight [kg]	3.53	3.45		3.52		3.6		3.51	3.44	
<i>Dose (mg/kg)</i>	<b>0</b>	<b>5</b>	<b>25</b>		<b>125</b>		<b>0</b>	<b>200</b>		
BW gain d 6-29 [kg]	0.35	0.32		0.36		0.3		0.41	0.34	
BW gain d 6-19 [kg]	0.15	0.15		0.18		0.09		0.12	0	**
BW actual gain [kg]	-0.11	-0.09		-0.05		-0.05		0.05	0	
Placental weight [g]	6	6		6		6		6	6.1	
Mean N corpora lutea	7.9	7.2		6.1		6.9		7.2	6.9	

Mean N implants	7.7.	6.6		6.5		5.7		5.9	5.6	
No of litters	14	17		16		14		18	14	
Litter size (mean)	6.3	6.5		6.3		5.5		5.3	5.3	
Total no of fetuses	95	111		101		77		96	74	
No of dead fetuses	0	5		3		3		3	2	
No of viable fetuses	95	106		98		74.0		93	72	
Fetal weight of males [g]	47.5	46		48.4		45.4		49.9	45.1	**
Fetal weight of females [g]	47	45.9		48.8		45.4		49	44	**
fetal incidence skeletal variations										
<b>supernumerary ribs (%)</b>	58.9	61.3		60.2		83.8	**	64	96	**
<b>extra lumbar vertebrae (i.e. add. Presacral vertebrae)</b>	10.5	21.7		16.3		44.6	**	28.3	47.2	**
<b>delayed ossification</b>										
<b>enlarged frontanelles of skull bones (%)</b>	18.9	14.2		20.4		28.4		23.9	55.6	**
Litter incidence skeletal variations										
<b>extra lumbar vertebrae (i.e. add. Presacral vertebrae)</b>	28.6	41.2		37.5		85.7	*	50	85.7	

\* significantly different from control at the 0.05 level

\*\* significantly different from control at the 0.01 level

### Organ weights

In the main study, although not statistically significant, there was an increase in the mean liver weights and the liver-to-body weight ratios in the 25 and 125 mg/kg groups of rabbits. The increase is probably attributable to the higher incidence of hypertrophy of the hepatocytes in these 2 groups.

In the supplemental study, there was a slight increase in the mean thyroid weights and the thyroid-to-body weight ratio in the 200 mg/kg group when compared to the control group. However, no specific microscopic alterations were seen to account for this mild weight variation

**Table B.6.6.3-2 Summary of mean organ weights**

Dose level (mg/kg bw/day)	0	5	25	125	0	200
actual liver weight (g)	96.6	95.4	102 (+6%)	105.8 (+10%)	113	108 (-4%)
relative liver weight (g/kg bw)	27.3	27.9	29	29.5	32.3	31.6

			(+6%)	(+8%)		(-2%)
actual thyroid weight (g)	0.27	0.26	0.27	0.27	0.27	0.31 (+15%)
relative thyroid weight (g/kg bw)	0.08	0.08	0.08	0.08	0.08	0.09 (+13%)

### Histopathology

Although gross examination of tissues at necropsy revealed no evidence of treatment-related changes, a vacuolar change in the hepatocytes characterized by a foamy appearance of the cytoplasm was observed in 2 of 20 does from the 25 mg/kg group and 12/20 and 11/20 of the 125 and 200 mg/kg groups, respectively. In most cases, there were associated changes of hypertrophy and a ground-glass appearance of the hepatocytic cytoplasm indicative of increased quantities of smooth endoplasmic reticulum.

**Table B.6.6.3-3 Summary of non-neoplastic microscopic findings in liver and thyroid**

Parameter		Dose (mg/kg bw/day)					
		0	5	25	125	0	200
	Animals examined	20	20	20	20	20	20
Liver	Inflammation chronic portal triad	3 (15%)	6 (30%)	4 (20%)	4 (20%)	4 (20%)	1 (5%)
	vacuolar change, hepatocyte	1 (5%)	0 (0%)	2 (10%)	12 (60%)	1 (5%)	11 (55%)
	vacuolar change, glycogenic	3 (15%)	3 (15%)	2 (10%)	1 (5%)	6 (30%)	3 (15%)
	vacuolar change, fatty	4 (20%)	4 (20%)	1 (5%)	0 (0%)	2 (10%)	2 (10%)
	Hypertrophy, hepatocyte	3 (15%)	2 (10%)	6 (30%)	12 (60%)	5 (25%)	19 (95%)
	hematopoiesis, minimal	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
	Hepato. ground glass appearance	1 (5%)	0 (0%)	4 (20%)	8 (40%)	2 (10%)	11 (55%)
	Single hepatocyte necrosis	3 (15%)	0 (0%)	0 (0%)	5 (25%)	2 (10%)	1 (5%)

**Table B.6.6.3-3 Severity of microscopic liver findings– main study**

Parameter		Dose (mg/kg bw/day)			
		0	5	25	125
Liver	vacuolar change, hepatocyte multifocal, minimal	--	--	1	2
	vacuolar change, hepatocyte multifocal, mild	1	--	1	6
	vacuolar change, hepatocyte multifocal, moderate	--	--	--	3
	vacuolar change, glycogenic multifocal, minimal	--	1	--	--
	vacuolar change, glycogenic multifocal, mild	3	2	1	1



Parameter		Dose (mg/kg bw/day)			
		0	5	25	125
	vacuolar change, glycogenic multifocal, moderate	--	--	1	--
	vacuolar change, fatty multifocal, minimal	--	1	--	--
	vacuolar change, fatty multifocal, mild	3	1	--	--
	vacuolar change, fatty multifocal, moderate	1	2	--	--
	Hypertrophy, hepatocyte Multifocal, minimal	--	2	1	4
	Hypertrophy, hepatocyte Multifocal, mild	3	--	3	5
	Hypertrophy, hepatocyte Multifocal, moderate	--	--	--	3
	hematopoiesis, minimal	--	1	1	--
	Hepato. ground glass appearance multifocal, minimal	--	--	1	--
	Hepato. ground glass appearance multifocal, mild	1	--	2	5
	Hepato. ground glass appearance multifocal, moderate	--	--	1	3
	Single hepatocyte necrosis focal/unilateral, minimal	--	--	--	1
	Single hepatocyte necrosis focal/unilateral, mild	2	--	--	2
	Single hepatocyte necrosis Multifocal, minimal	--	--	--	1
	Single hepatocyte necrosis Multifocal, mild	1	--	--	1

\* significantly different from respective control  $p \leq 0.05$

\*\* significantly different from respective control  $p \leq 0.01$

**Table Incidence of non-neoplastic microscopic findings – selected parameter – supplemental study**

Parameter		Dose (mg/kg bw/day)	
		0	200
Liver	vacuolar change, hepatocyte multifocal, mild	1	2
	vacuolar change, hepatocyte multifocal, minimal	--	3
	vacuolar change, hepatocyte multifocal, moderate	--	5
	vacuolar change, glycogenic multifocal, mild	3	3
	vacuolar change, glycogenic multifocal, moderate	1	--
	vacuolar change, glycogenic	2	--

Parameter	Dose (mg/kg bw/day)	
	0	200
multifocal, marked		
vacuolar change, fatty multifocal, mild	1	1
vacuolar change, fatty multifocal, moderate	1	1
Hypertrophy, hepatocyte Multifocal, mild	3	15
Hypertrophy, hepatocyte Multifocal, minimal	1	13
Hypertrophy, hepatocyte Multifocal, moderate	1	--
Hepato. ground glass appearance multifocal, mild	2	6
Hepato. ground glass appearance multifocal, minimal	--	2
Hepato. ground glass appearance multifocal, moderate	--	3
Single hepatocyte necrosis focal/unilateral, mild	2	--
Single hepatocyte necrosis focal/unilateral, minimal	--	1
Distended follicle(s), focal/unilateral, mild	1	1
Distended follicle(s), focal/unilateral, moderate	4	3
Distended follicle(s), bilateral/mild	--	1
Distended follicle(s), bilateral, moderate	1	--

\* significantly different from respective control  $p \leq 0.05$

\*\* significantly different from respective control  $p \leq 0.01$

## 2. Offspring

### A. Viability and sex ratio

There were 11 nonviable fetuses in the primary phase of the study: 0 in the control group, 5 in the 5 mg/kg group, 3 in the 25 mg/kg group, and 3 in the 125 mg/kg group. In the supplemental phase, there were 5 nonviable fetuses: 3 in the control group and 2 in the 200 mg/kg group.

Fetal sex ratios, based on a percentage of male progeny, were not significantly different from the control for any treatment group.

### B. Fetal and placental weight

Fetal weights were significantly ( $p \leq 0.01$ ) reduced for male, female and combined fetuses from the 200 mg/kg group. Fetal weights were unaffected for all other groups. Placental weights were comparable between the treatment groups and their respective control group.

**Table B.6.6.3-4 Mean Weight of viable fetuses (g)**

Parameter	Dose (mg/kg bw/day)					
	0	5	25	125	0	200

Parameter	Dose (mg/kg bw/day)					
	0	5	25	125	0	200
Male	47.5	46.0	48.4	45.6	49.9	45.1**
Female	47.0	44.7	48.8	45.4	48.7	43.8**
Combined	47.6	45.9	49.0	46.1	49.1	44.2**

\* significantly different from respective control  $p \leq 0.05$

\*\* significantly different from respective control  $p \leq 0.01$

### C. External and visceral examination

The test article, administered at doses up to and including 200 mg/kg, produced no statistically significant and/or lexicologically meaningful increase in either the fetal or litter incidence of soft tissue variations and/or malformations. A few scattered changes of a common variety, which were considered to be of spontaneous origin, were observed in both the treatment and the control groups.

The test article did not promote gross pathological changes in the placentas.

### D. Skeletal Examination

There appeared to be a treatment-related effect on skeletal variations for the 125 and 200 mg/kg group and delayed ossification of the skull for the 200 mg/kg group. The only finding that was statistically significant ( $p \leq 0.05$ ) on a litter basis was an increase in additional presacral vertebrae (extra lumbar vertebrae) for the 125 mg/kg group. On a litter basis this finding was outside the historical control range (HCR) for both the 125 and 200 mg/kg groups (85.7% for both groups - HCR 44.4-77.8%).

On an individual basis there was a statistically significant ( $p \leq 0.05$  or  $0.01$ ) increase in supernumerary ribs (83.8% and 95.8% for the 125 and 200 mg/kg groups, respectively - HCR 52.1-79.9%) and additional presacral vertebrae (44.6% and 47.2% for the 125 and 200 mg/kg groups, respectively - HCR 11.8-30.6%).

Corresponding to the reduction in fetal weight at 200 mg/kg, there was a statistically significant ( $p \leq 0.01$ ) increase in the individual incidence of delayed ossification (55.6% - HCR 18.7-47.0%) and enlarged fontanelles (55.6% - HCR 17.9-45.5%) of skull bones.

A significant ( $p \leq 0.05$  or  $0.01$ ) increase in the unossified 5th sternal segment for the 5 mg/kg group and a decrease in lagging of ossification of the 5th sternal segment for the 200 mg/kg group were considered to be inconsequential as they were not dose-dependent or were an improvement over the corresponding control groups, respectively. There was no increase in skeletal malformations for any treatment group, and there was no evidence of delayed ossification or increase in skeletal variations at doses levels up to and including 25 mg/kg.

**Table B.6.6.3-5 Fetal incidence of skeletal findings – selected parameter**

Parameter		Dose (mg/kg bw/day)					
		0	5	25	125	0	200
Number examined		95/92	106	98	74	92	72
Skull	Fontanelle enlarged	18 (18.9)	15 (14.2%)	20 (20.4%)	21 (28.4%)	22 (23.9%)	40 (55.6%)**
	Bones incomplete ossified	22/ (23.9/)	16 (15.1%)	20 (20.4%)	21 (28.4%)	22 (23.9%)	40 (55.6%)**
Ribs	Extra	56 (58.9%/)	65 (61.3%)	59 (60.2%)	62 (83.8)**	59 (64.1%)	69 (95.8)**
Vertebrae	Lumbar-arches extra	10 (10.5)	23 (21.7%)	16 (16.3%)	33 (44.6%)**	26 (28.3%)	34 (47.2%)*
	Lumbar-	10/26	23	16	33	26	34

Parameter		Dose (mg/kg bw/day)					
		0	5	25	125	0	200
	centra extra	(10.5)	(21.7%)	(16.3%)	(44.6%)**	(28.3%)	(47.2%)*
Sternebrae	5 <sup>th</sup> unossified	2 (2.1%)	12 (11.3%)*	3 (3.1%)	2 (2.7%)	7 (7.6%)	0 (0%)
	5 <sup>th</sup> incompletely ossified	69 (72.6%)	69 (65.1%)	65 (66.3%)	52 (70.3%)	67 (72.8%)	39 (54.2%)*

\* significantly different from control  $p \leq 0.05$

\*\* significantly different from control  $p \leq 0.01$

**Table B.6.6.3-6 Litter incidence of skeletal findings – selected parameter**

Parameter		Dose (mg/kg bw/day)					
		0	5	25	125	0	200
Number examined		14/18	17	16	14	18	14
Skull	Fontanelle enlarged	9 (64.3%)	8 (47.1)	9 (56.3)	7 (50.0)	13 (72.2%)	11 (78.6%)
	Bones incomplete ossified	9 (64.3%)	8 (47.1)	9 (56.3)	7 (50.0)	13 (72.2%)	11 (78.6%)
Ribs	Extra	14 (100.0%)	17 (100.0%)	16 (100.0%)	13 (92.6%)	14 (77.8%)	14 (100.0%)
Vertebrae	Lumbar-arches extra	4 (28.6%)	7 (41.2%)	6 (37.5%)	12 (85.7%)*	9 (50.0%)	12 (85.7%)
	Lumbar-centra extra	4 (28.6%)	7 (41.2%)	6 (37.5%)	12 (85.7%)*	9 (50.0%)	12 (85.7%)
Sternebrae	5 <sup>th</sup> unossified	2 (14.3%)/	6 (35.3%)	3 (18.8%)	2 (14.3%)	5 (27.8%)	0 (0%)
	5 <sup>th</sup> incompletely ossified	14/ (100.0%)	17 (100.0%)	14 (87.5%)	14 (100.0%)	17 (94.4%)	13 (92.9%)

\* significantly different from control  $p \leq 0.05$

\*\* significantly different from control  $p \leq 0.01$

### E. Affected Implants

The fetal and litter incidences of affected implants (nonviable implants plus malformed fetuses) were lower than the corresponding control groups for all treatment groups. On an individual basis there was a statistically significant ( $p \leq 0.01$ ) reduction in affected implants for the 25 mg/kg group when compared with control. This was not unfavorable, was not dose-dependent, and considered incidental in nature.

## III. Conclusion

FOE 5043 Technical promoted clear evidence of maternal toxicity at 125 and 200 mg/kg and borderline maternal toxicity at 25 mg/kg. The test article was free of adverse maternal reproductive effects at all dose levels tested. Developmental toxicity in the form of reduced fetal weights and ossification was observed at 200 mg/kg and increased skeletal variation was noted at 125 and 200 mg/kg.

NOAEL<sub>maternal toxicity</sub>: 5 mg/kg bw/day  
NOAEL<sub>development</sub>: 25 mg/kg bw/day

<b>Conclusion</b>	<p><b>The study is in line with current guidelines.</b></p> <p>No observable adverse effect level: 25 mg/kg/bw/day (maternal and reproduction parameters) Reduced fetal body weight at the high dose. An increase in skeletal variations was observed in the 125 and 200 mg/kg dose groups.</p> <p>Maternal toxicity was exhibited by clinical signs, reduced body weight gain during treatment, and an increase incidence of histopathological changes in the liver. The NOAELs established in the rabbit for maternal and developmental toxicity by oral administration were 25 mg/kg bw/day, respectively.</p>
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**Summary of Reproductive and Developmental Toxicity Studies**

The reproductive toxicity of FOE 5043 was studied in a 2-generation study in rats and developmental toxicity studies in rats and rabbits.

500 ppm, the highest dose tested, had no effect on reproduction when fed to rats over a period of 2 generations. In parental animals, there was a compound-related reduction in body weights for P generation females during the pre-mating phase. Other effects occurring in the P and F1 generation adults included increased absolute and relative liver weights and histopathological changes in the liver. The NOAEL obtained for reproductive toxicity were 500 ppm, respectively.

In an oral developmental toxicity study in rats, developmental effects were observed at 125 mg/kg bw/day (highest dose tested) as demonstrated by decreased fetal body weights. These effects were correlated with a reduction in body weight and food consumption in dams at 125 mg/kg bw/day. The NOAEL for both maternal and developmental toxicity in the rat via oral administration was 25 mg/kg bw/day.

In an oral rabbit developmental toxicity study, developmental effects occurred at doses of 125 and 200 mg/kg bw/day. Effects included reduced fetal weights, and increased incidences of delayed ossification and skeletal variation. Maternal toxicity was exhibited by clinical signs, reduced body weight gain during treatment, and an increased incidence of histopathological changes in the liver. The NOAELs established in the rabbit for maternal and developmental toxicity by oral administration were 5 mg/kg bw/day and 25 mg/kg bw/day, respectively.

It can be concluded that FOE 5043 is not a reproductive or developmental toxicant. The developmental effects observed were restricted to the higher dose levels which produced maternal toxicity.

## B.6.7 Neurotoxicity studies

### Summary of neurotoxicity studies

Flufenacet has been investigated in acute and subchronic oral neurotoxicity screening studies using rats. In an acute neurotoxicity screening study, all clinical and neurobehavioral effects observed following administration of a single dose of flufenacet were described to acute systemic toxicity. Complete recovery occurred in surviving animals with the exception of urine stains which persisted till termination in females. There were no correlative micro pathologic findings to indicate any evidence of an adverse effect on the nervous system.

In a subchronic neurotoxicity screening study, a dose-related increase in evidence of neurotoxicity was demonstrated following dietary exposure of flufenacet. Compound-related effects in the functional observation battery and motor activity assessments were evident in animals treated at higher concentrations. These findings were correlated with microscopic lesions (swollen axons) observed in the brain and spinal cord. These effects, however, occurred only at exposure levels that produced substantial evidence of systemic toxicity as demonstrated in a separate subchronic feeding study (see Monograph/baseline dossier, KCA. 5.3.2, M-004999-01-1) in which tissue damage involving liver-, kidney-, hematologic/spleen- and thyroid-related endpoints was observed at similar high dietary levels. Thus, the results of these studies taken collectively suggest that an increased incidence of axonal swelling occurred in animals exposed to high levels of flufenacet which saturate metabolic pathways.

**Table 5.7-1: Summary of neurotoxicity studies**

Study	Sex	NOAEL (mg/kg bw/d)	LOAEL	Main effects seen at LOAEL	Reference
Rat acute neurotoxicity, oral	M F	75 50	200 75	Unspecific clinical signs (uncoordinated gait, decreased activity) NOEL neurotoxicity 450/150 mg/kg bw (males/females highest doses tested with survivors).	██████, 1995 (amended 1998) M-004986-02-1
Rat 90-day neurotoxicity feeding	M F	7.3 8.4	38 43	Microscopic lesions in brain and spinal cord (increased incidence of swollen axons in the cerebellum-medulla oblongata)	██████., 1995 M-005014-01-2
Rat developmental neurotoxicity feeding	Dam Pup	1.7/3.0 (DG 6-21/DL 1-12)	8.3/15	Dam: BW ↓, food intake ↓ (gestation) Pup: BW/BWgain ↓, rel. food intake ↑, delayed development (eye opening, preputial separation)	██████, 2000 M-026105-01-1

**B.6.7 Acute Oral Neurotoxicity**

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  An Acute Oral Neurotoxicity Screening Study with Technical Grade FOE 5043 in Fischer 344 Rats <b>studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report 01:</b>	<b>[REDACTED]; 1995 (1998)</b>
<b>Title:</b>	An Acute Oral Neurotoxicity Screening Study with Technical Grade FOE 5043 in Fischer 344 Rats Supp.: A Special Acute Oral Neurotoxicity Study to Establish a No-Observed-Effect Level with Technical Grade Thiafluamide (FOE 5043) in Fischer 344 Rats
<b>Document No:</b>	Original: 94-412-XP (1995) Supplemental: 97-912-MC (1998)
<b>Report No:</b>	<b>106897-1</b>
<b>Guidelines:</b>	US-EPA-FIFRA, Guideline 81-8(SS)
<b>GLP</b>	Yes

**INTRODUCTION AND PURPOSE:**

Thiafluamide is a compound that Bayer has developed for use as a herbicide. The purpose of this study was to establish an overall No-Observed-Effect Level (NOEL) in female rats following acute exposure to thiafluamide by establishing a NOEL for clinical observations, which was the only test affected in females at the lowest dose of 78 mg/kg. Other endpoints, including motor activity, gross pathology and neuropathology were not included in the present study, since an acute NOEL was established in the main study for these tests at 78 mg/kg or a higher dose level. Males were not tested since an overall NOEL was established in males in the main study at a dose of 78 mg/kg

**GUIDELINE:**

This study was not conducted in accordance with any existing guideline. Instead, it was designed to supplement the information that was generated to satisfy the requirements of US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS, 1991, EPA 540/09-91-123, PB 91-154617.

**I. Materials and methods****A. Materials**

<b>1. Test material:</b>	FOE 5043
Synonym(s):	Thiafluamide, flufenacet
Specification no.:	n.a.
Description:	tan granules/cream powder
Lot/Batch no:	Fl. 036 from 7/4/91



Purity:	97.4-98.5%
Stability of test compound:	unknown at freezer conditions
<b>2. Vehicle and/or positive control</b>	Vehicle: 2% (v/v) Cremophor EL in deionized water Positive control: reference was made to historical laboratory validation studies using positive control materials
<b>3. Test animals</b>	
Species:	rat
Strain:	Fisher 344 CDF(F-344)/BR
Sex:	<u>Main study</u> : male and female <u>Supplementary study</u> <sup>a</sup> : female <sup>b</sup>
Age:	9 weeks
Weight (day 0):	Males: 148 – 215 g Females: 122 - 141 g
Source:	████████████████████
Acclimatisation period:	at least 6 days
Diet:	Purina Rodent Laboratory Chow #5001-4 in etts form, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually housed in stainless steel wire mesh cages providing a 66.5 square inch area with deotized Animal Cage Board.
Environmental conditions:	Temperature: 17.8 – 25.6°C Humditiy: 40-70% Air changes: not reported Photoperiod: 12 hr light/dark cycle

## B. Study design and methods

### 1. Animal assignment and treatment:

Route of administration:	Oral (gavage)
Exposure:	single dose after overnight fasting at a dosing volume of 10 mL/kg
Group size:	12/dose level
Dose levels (nominal):	<u>Main study</u> : Males: 0, 75, 200 and 450 mg/kg bw Females: 0, 75, 150 and 300 mg/kg bw <u>Supplementary study</u> : 0, 25 and 50 mg/kg bw
Dose levels (actual):	<u>Main study</u> : Males: 0, 78, 221 and 497 mg/kg bw Females: 0, 78, 163 and 326 mg/kg bw <u>Supplementary study</u> : 0, 25 and 48 mg/kg bw

Dose selection rationale:

Doses for the main study were established on the basis of the results from an acute oral toxicity study.

## 2. Dose preparation and analysis:

Dose preparation:

The test substance was administered by gavage as a single dose in 2% Cremophor EL in deionized water at a dosing volume of 10 mL/kg.

Analysis:

The concentration of thiafluamide in the vehicle was measured using liquid chromatographic (LC) analysis. The stability of thiafluamide in the vehicle, following storage at room temperature, was established using samples at nominal 2.5 and 5.0 mg/mL test concentrations (nominal 25 mg/kg and 50 mg/kg dose levels) used in the present study. Homogeneity was verified by analysis of three samples each of the nominal 2.5 and 5.0 mg/LI suspensions. Homogeneity was accepted if the coefficient of variation (C.V.) was  $\leq 6\%$ . Each dosing suspension was analyzed to measure the concentration of thiafluamide.

## 3. Observations:

Main study: clinical observations, mortality, body weight, automated measurements of activity (figure-eight maze), a functional observational battery, brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system were examined microscopically for lesions.

Supplementary study: Clinical observations, mortality checks and a functional observational battery<sup>c</sup>

## 4. Statistical Analysis:

In general, continuous data were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. For the FOB, continuous data were first analyzed using a one-way ANOVA. If there was a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different from the control group. Categorical data collected in the FOB were analyzed in a similar manner, using General Linear Modeling (GLM) and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

Statistical evaluations were performed using software from either INSTEM Computer Systems or SAS. With the exception of Bartlett's test, which was tested at  $p \leq 0.001$ , the level used to establish statistical significance was  $p \leq 0.05$ .

<sup>a</sup> the purpose of the supplementary study was to establish an overall No-Observed-Effect Level (NOEL) in female rats following acute exposure to thiafluamide by establishing a NOEL for clinical observations, which was the only test affected in females at the lowest dose of 78 mg/kg in the main study

<sup>b</sup> males were not tested since an overall NOEL was established in males in the main study at a dose of 78 mg/kg bw

<sup>c</sup> additional endpoints that are included in the standard screening battery were not included in the present study since a NOEL was established for these tests at higher dose levels in the main study

## II. Results and discussion

### A. Mortality

Compound-related deaths occurred in the main study at the high dose of 450 mg/kg for males and 300 mg/kg for females. Four high-dose males and all twelve high-dose females died on the day of exposure or within three days following treatment. There were no other deaths prior to terminal sacrifice.

### B. Clinical signs of toxicity

Clinical signs associated with treatment were evident in the main study in the mid-(200 mg/kg) and high-(450 mg/kg) dose males and in females at all three dose levels. Compound-related signs in males that received 200 mg/kg consisted of incoordinated gait and decreased activity. Additional signs in high-dose males (450 mg/kg bw) consisted of a hot-to-touch body, lacrimation and stains (oral, lacrimal and urine). Compound-related signs for the low-(75 mg/kg) and mid-(150 mg/kg) dose females consisted of incoordinated gait, decreased activity and urine stain.

Additional signs in the high-(300 mg/kg) dose females consisted of moribundity and a cool-to-touch body (both on the day before death), lacrimation, a hot-to-touch body and an increased incidence of oral stain.

Compound-related signs in males and females were apparent on the day of treatment (day 0) and generally resolved within days 0-5. Urine stain persisted in surviving females to the end of the observation period, 14 days following treatment.

**Table B.6.7-1 Summary of clinical observations for male rats – main study**

Observations	Nominal Dose (mg/kg bw/day)			
	0	75	200	450 <sup>b</sup>
Incoordinated Gait (Ataxia)	0	0	3 (0) <sup>a</sup>	7 (0)
Decreased Activity	0	0	2 (0)	8 (0)
Body, Hot to touch	0	0	0	4 (0)
Lacrimation, clear	0	0	0	4 (0)
Oral stain	0	0	0	10 (0-4)
Lacrimal stain, red	0	0	0	1 (1-2)
Urine stain	0	1 (1)	0	4 (0-4)
Feces, soft	0	1 (0)	0	1 (0)
Perianal stain	6 (0-12)	5 (0-12)	9 (0-10, 14)	7 (0-6)
Nasal stain, red	1 (1)	3 (0)	1 (1)	2 (1-2)

<sup>a</sup> Incidence for n=12 (days observed)

<sup>b</sup> Four high-dose males died on the day of treatment (day 0)

**Table B.6.7-2 Summary of clinical observations for female rats – main study**

Observations	Nominal Dose (mg/kg bw/day)			
	0	75	150	300 <sup>b</sup>
Incoordinated Gait (Ataxia)	0	1 (0)	9 (0)	11 (0-2)
Decreased Activity	0	1 (0)	7 (0)	11 (0-29)
Body, Hot to touch	0	0	0	9 (0)
Body, cool to touch	0	0	0	1 (2)
Lacrimation, clear	0	0	0	9 (0)
Oral stain	1 (1)	1 (1)	2 (0-1)	10 (0-2)
Lacrimal stain, red	1 (1)	3 (1, 11-12)	2 (1)	1 (1-2)
Urine stain	0	3 (0-11)	8 (0-14)	9 (0-2)
Moribund	0	0	0	1 (0)
Perianal stain	1 (1)	0	1 (1)	1 (0)
Nasal stain, red	4 (0-5)	6 (0-1)	1 (0-3)	1 (0-2)

<sup>a</sup> Incidence for n=12 (days observed)

<sup>b</sup> All 12 high-dose males died within 3 days following treatment

### C. Body weight

Body weight was not affected by treatment in surviving males or females in the main study.

### D. Functional observational battery (FOB)

For the functional observational battery (FOB), compound-related effects were evident in the main study on day 0 at the middle- and high-dose levels for males (200 and 450 mg/kg) and females (150 and 300 mg/kg). These effects are consistent with clinical observations.

The only effects that were statistically significant in males consisted of decreased activity at the high dose and elevated temperature at 200 and 450 mg/kg dose levels. An additional effect at the high dose for males consisted of an increased incidence of animals that were sitting or lying normally during open-field observation.

Increased body temperature was the only difference that was statistically significant for females. Additional effects at the high dose consisted of decreased activity in the home cage, sitting or lying normally during open field observation and gait incoordination. The incidence and/or severity of these effects generally increased with dose. Instances of urine stain in three mid-dose females on days 7 and 14 are also associated with treatment.

Except for the persistence of urine stain in surviving females (to day 14), all signs of toxicity resolved in both sexes by the next observation period on day 7. Forelimb and hindlimb grip strength and landing foot splay were not affected by treatment in males or females at any dose level. The remaining observations on day 0 are considered incidental and unrelated to treatment with FOE 5043.

**Table B.6.7-3 Summary of FOB results – males main study**

Observations		Nominal Dose (mg/kg bw/day) <sup>a</sup>			
		0	75	200	450
Home cage:	Decreased activity	0	0	2	4*
Open field:	Sitting or lying normally	0	1	0	3
Reflex/	Body temperature	37.1	37.1	37.8*	38.0*

Observations		Nominal Dose (mg/kg bw/day) <sup>a</sup>			
		0	75	200	450
Physiologic	(mean, °C)				

**Table B.6.7-4 Summary of FOB results – females main study**

Observations		Nominal Dose (mg/kg bw/day) <sup>a</sup>			
		0	75	150	300
Homecage:	Decreased activity	0	0	0	3*
Open field:	Sitting or lying normally	0	0	0	2
	Incoordinated gait	0	0	0	1
Reflex/ Physiologic	Body temperature (mean, °C)	36.6	37.0	37.6*	38.0*

<sup>a</sup> Incidence, based on 12 animals/group

\* p ≤ 0.05 (ANOVA)

**E. Motor and locomotor activity**

There were no statistically-significant differences in activity for males or females at any dose level during the main study. Compound-related decreases in motor and locomotor activity in the figure-eight maze occurred on day 0 in males that received doses of 200 or 450 mg/kg and females that received 150 or 300 mg/kg during the main study. Complete recovery occurred in surviving males and females by the end of the main study, 14 days following exposure. Habituation was not affected by treatment with FOE 5043.

**F. Organ weight**

Brain weight was not affected by treatment in surviving males or females at any dose level in the main study.

**G. Gross necropsy**

There were no gross lesions at terminal sacrifice of the main study for surviving males or females. Animals that were found dead prior to study termination did not undergo a necropsy examination.

**H. Histopathology**

There were no treatment-related microscopic lesions in the main study within skeletal muscle or neural tissues of males that survived the high dose of 450 mg/kg or females treated with the 150 mg/kg dose (the highest dose with survivors). Therefore, tissues from animals that received lower doses were not examined.

**Supplemental study in females****A. Mortality**

In the supplementary study there were no deaths prior to terminal sacrifice, one day following treatment.

**B. Clinical signs of toxicity**

Clinical signs were not evident in control or treated animals in the supplementary study.

**C. Body weight**

Body weight was not monitored since a NOEL for weight gain was established in the main study at higher dose levels.

**D. Functional observational battery (FOB)**

In the supplementary study compound-related effects were not evident in any dose group.

**III. Conclusion**

Based on treatment-related clinical signs, FOB observations and decreased motor- and locomotoractivity in the mid- and high-dose groups the NO(A)EL for males is 78 mg/kg bw. In the main study in females clinical signs of toxicity were also observed at the low-dose. In the supplemental study in females no clinical signs or compound-related effects were observed in the FOB in any dose group. Therefore, the NO(A)EL for females is 48 mg/kg bw.

All clinical signs and neurobehavioral effects are ascribed to acute systemic toxicity. Urine stain in females was the only effect that persisted to termination, 14 days following treatment in the main study. There were no correlative micropathologic findings. Based on these results, the NOEL for neurotoxicity is 450 mg/kg for males and 150 mg/kg for females (the highest doses with survivors).

NO(A)EL <sub>neurotoxicity</sub> :	497 mg/kg bw (nominal 450 mg/kg bw) for males and 163 mg/kg bw (150 mg/kg bw) for females (highest dose with survivors)
NO(A)EL <sub>overall</sub> :	78 mg/kg bw (nominal 75 mg/kg bw) for males and 48 mg/kg bw (nominal 50 mg/kg bw) for females

**METHODS:****Animals:****- Source, Number and Age**

Female (nulliparous and nonpregnant) Fischer 344 CDF(F-344)/BR rats from [REDACTED], W1 were used in this study. The rat was selected due to its general acceptance and suitability as a rodent species for toxicological testing of this type as well as the availability of a historical database on the Fischer 344 strain. The study design required a total of 36 female rats which were nine weeks of age when the treatment was administered.

**- Examination and Acclimation**

Upon receipt, animals were examined and sacrificed if general appearance and/or behavior were considered abnormal. Those animals considered acceptable were then placed into individual cages and acclimated to their ambient laboratory conditions (temperature 18-26°C, relative humidity 40- 70%, 12-hr light/dark cycle) for at least six days prior to placement on the study. Purina Mills Rodent Lab Chow 5001-4 in "etts" form and tap water were provided for *ad libitum* consumption during the acclimation period and throughout the study, except during the overnight fast prior to dosing and during neurobehavioral assessment when only water was available. For the holding period, animal care personnel observed the animals at least once daily for moribundity and mortality. Upon

completion of the acclimation period, a veterinarian reviewed the status of the animals prior to their release for study.

#### **- Care and Housing**

While on study, rats were individually housed in suspended stainless steel wire-mesh cages. The room, cages and cage racks were thoroughly cleaned and disinfected before arrival of the animals. Deotized Animal Cage Board was used in the bedding trays and changed at least three times weekly. The cages and racks were replaced at least once every two weeks with clean, disinfected cages and racks. The room was disinfected at least once every two weeks.

Randomization procedures utilized software from INSTEM Computer Systems. Following acclimation, the animals were weighed and those with body weights that were more or less than 20% of the mean weight were rejected. The remaining animals were randomly assigned to the control or treated group in order that groups had comparable body weights when treatment was initiated. This was done to facilitate comparisons following treatment.

#### **Analysis of Feed and Water**

A sample from each lot of feed was analyzed by Purina Mills Laboratories Inc., St. Louis, MO. Contaminant concentrations outlined in the *Certification Profile* for Purina Mills Certified Lab Chows were used as a general standard by which to gauge acceptable levels of contaminants in the feed. Tap water (Kansas City Missouri Municipal Water) was analyzed for contaminants by Pace Inc., Lenexa, KS.

#### **Test Substance Analysis**

The identity of thiaflumide was confirmed by analytical methods. The test substance was analyzed within one month of administration of the dose to verify the concentration of the active ingredient.

#### **Analysis of Test Substance in the Vehicle**

The concentration of thiaflumide in the vehicle was measured using liquid chromatographic (LC) analysis. The stability of thiaflumide in the vehicle, following storage at room temperature, was established using samples at nominal 2.5 and 5.0 mg/ml test concentrations (nominal 25 mg/kg and 50 mg/kg dose levels) used in the present study. Homogeneity was verified by analysis of three samples each of the nominal 2.5 and 5.0 mg/ml suspensions. Homogeneity was accepted if the coefficient of variation (C.V.) was <6%. Each dosing suspension was analyzed to measure the concentration of thiaflumide.

#### **Dose Selection**

The rationale for dose selection is based on the results of the main acute oral neurotoxicity screening study with the test substance at analytically-confirmed dose levels of 0, 78, 221 and 497 mg/kg for males and 0, 78, 163, and 326 mg/kg for females (12/sex/dose level). In that study, there was no evidence of exposure in males that received the low dose of 78 mg/kg. By comparison, evidence of exposure in females that received the low dose of 78 mg/kg consisted of in coordinated gait, decreased activity and urine stain observed by clinical observation. Based on these results, the doses selected to establish a NOEL in females are 0, 25 and 50 mg/kg. The FOB will be performed at the time when effects at higher dose levels are most evident (i.e., the time of peak effects), which is approximately three hours following treatment.

#### **Experimental Design**

**Route, Dose and Number of Animals**

The oral route of exposure was employed in accordance with the test guideline. Three dose groups (12 females/dose level) were administered the test substance at nominal doses of 0 (vehicle), 25 or 50 mg/kg. The test substance was administered by gavage as a single dose in 2% Cremophor EL in deionized water at a dosing volume of 10 ml/kg. These procedures are in accordance with those used in the main study. All 12 animals/dose level were used for neurobehavioral testing and survivors were sacrificed upon complete recovery from possible compound-related effects.

**Clinical Signs and Body Weights**

Cage-side observations were conducted at least once daily for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded once each day. This examination consisted of a series- of observations that parallel the principal observations performed in the functional observational battery (FOB). The detailed clinical examination was performed in a systematic fashion, proceeding from the least to most manipulative with respect to the animal. First, the animal was observed in its home cage for overt signs, such as unusual posture, coarse tremors, gross muscle fasciculations, activity level, and stereotypic or highly-repetitive behaviors. The bedding material was also inspected to assess the consistency of feces. Second, the cage was opened for an unobstructed view and the animal was observed for signs including clonic or tonic movements, response to stimuli, piloerection, gait abnormalities, respiratory abnormalities and level of activity. Next, the animal was retrieved and inspected for general appearance, evidence of injury, areas of coloration or alopecia, and for the presence of various stains and secretions. The eyes were examined for palpebral closure and if needed an inspection was conducted for broken teeth or malocclusion. The location, color and approximate size of gross lesions were also noted if present. Lastly, the animal was encouraged to move about for further observance of gait abnormalities, responses to stimuli, or for evidence of clonic or tonic movements. Animals were weighed just prior to treatment, to establish the appropriate dosing volume for each animal

**Functional Observational Battery (FOB) Testing**

This FOB closely follows the battery of tests with each animal tested individually. This FOB did not include some of the tests that are routinely performed in this laboratory by a second person; primarily involving animal manipulation (i.e., aerial righting) and various measurements (i.e., landing foot splay, grip strength and body temperature). These tests were not included in the present study because a NOEL was established for these tests at higher dose levels. Scoring criteria and explicitly-defined scales were used to rank the severity of observations that do not readily lend themselves to quantitation. The technicians who conducted the FOB were "blind" with respect to the animal's group assignment. Observations for all animals were performed by the same observer. Studies have been conducted with acrylamide, carbaryl and untreated rats to establish the sensitivity, reliability, and validity of these test procedures, the adequacy of training of technical personnel and to serve as a historical control.

All animals that were assigned to the study were tested using the FOB on one occasion - approximately 3 hours (minimum) after administration of the dose. On the day of FOB testing, the appropriate animals were placed in the sequence that was established for testing on that day. The dose group identification was concealed at that time to ensure that testing would be conducted without knowledge of the group assignment. Animals were then transferred to the room where testing took place and were allowed to acclimate with minimal disturbance for at least 30 minutes prior to testing. The test room was a standard animal room that was maintained on the same light: dark cycle and settings for temperature and relative humidity as the animal room, with tests conducted during the



light phase. Sets of nine animals were evaluated individually using the FOB. The order of testing was done in a semi-random order such that groups were balanced across test times. All animals were tested within the same day.

### Statistical Analysis

Statistical evaluations were performed using software from either INSTEM Computer Systems or SAS . With the exception of Bartlett's test, which was tested at  $p < 0.001$ , the level used to establish statistical significance was  $p < 0.05$ . In general, continuous data were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. For the FOB, continuous data were first analyzed using a one-way ANOVA. If there was a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different from the control group. Categorical data collected in the FOB were analyzed in a similar manner, using General Linear Modeling (GLM) and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

### Archival Procedures

The protocol, raw data, a sample of the test substance, and the final report are archived at locations specified by Bayer Co

## RESULTS AND DISCUSSION

### Animal Care

There were no excursions from the defined ranges in temperature or relative humidity.

### Analysis of Feed and Water

The results for feed were compared to the allowable limits in "*Lab Chows Animal Diet Reference Guide*" (Publication SP2437M-87010 dated 1987) from Ralston Purina Co., St. Louis, MO. No contaminant levels measured in the batches of Rodent Laboratory Chow used in this study were considered to have affected the outcome of this study. No contaminant levels were detected in water samples that were considered to affect the outcome of this study.

### Analysis of Test Substance in the Vehicle

The stability of thiafluamide in the dosing suspension was established using nominal concentrations of 2.5 and 5.0 mg/ml used in this study. The results from these analyses established that there was no appreciable decrease in concentration with either six or seven days of room temperature storage. Homogeneity of the test substance in the vehicle was also verified, with a coefficient of variation (C.V.) of 1% for the nominal 2.5 mg/ml and 3% for the nominal 5.0 mg/ml (equivalent to dose levels of 25 and 50 mg/kg, respectively) concentrations. Doses of 25 and 50 mg/kg were determined by analytical methods to be 98% and 95% of the nominal dose levels, respectively. Based on these results, the analytically-confirmed doses were 0, 25 and 48 mg/kg.

### Clinical Observations and Mortality

There were no deaths prior to terminal sacrifice, one day following treatment. Clinical signs were not evident in control or treated animals.

**Based on these results, the NOAEL for clinical signs in females is 48 mg/kg.**

### Conclusions

Technical grade thiafluamide was administered by gavage to fasted young-adult female Fischer 344 rats (12/dose level) at nominal doses of 0, 25, or 50 mg/kg. This study was conducted to provide information needed to establish an overall acute NOAEL in females. An overall NOAEL was

established at 78 mg/kg in males and at higher dose levels for selected endpoints (e.g., body weight, gross pathology and micropathology. Therefore, males and the aforementioned tests in females were not included in the present study. In summary, the following observations were noted:

1. Based on analytical results, the actual doses of thiafluamide were 0, 25 and 48 mg/kg.
2. Clinical signs were not evident in controls or treated animals. Based on these results, the NOEL is 48 mg/kg for females.
3. Body weight gain was not monitored in the present study since a NOAEL for weight gain was established at higher dose levels.
4. Results from the FOB revealed no compound-related effects at either dose. Thus, the present study established no evidence of toxicity in female rats following acute oral exposure to thiafluamide at dose levels of 25 or 48 mg/kg. Based on the combined results of the present study and the main study, the overall NOAEL for thiafluamide is 78 mg/kg for males and 48 mg/kg for females.

**Summary of Clinical Observations for Rats treated with Technical Grade Thiafluamide (FOE 5043) in an Acute Oral Neurotoxicity Study**

Signs within normal limits	Nominal Dose (mg/kg)		
	0	25	50
	12*(0-1)	12(0-1)	12(0-1)

*\*Incidence for n=12 (days observed)*

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  <b>A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Thiafluamide (FOE 5043) in Fischer 344 Rats studies were presented and evaluated during the EU process for Annex I listing.</b>
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<b>Report 02:</b>	<b>[REDACTED] (1998)</b>
Title:	A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Thiafluamide (FOE 5043) in Fischer 344 Rats
Document No:	M-005014-01-2
Report No:	94-472-YQ
Guidelines:	US-EPA-FIFRA, Guideline 82-5(b)
GLP	Yes

## I. Materials and methods

### A. Materials

#### 1. Test material:

	FOE 5043
Synonym(s):	Thiafluamide, flufenacet
Specification no.:	n.a.
Description:	cream powder
Lot/Batch no:	Fl. 036 from 7/4/91
Purity:	98.0-98.2%
Stability of test compound:	unknown at freezer conditions

#### 2. Vehicle:

Vehicle: Corn oil 1% (w/w) in diet  
Positive control: reference was made to historical laboratory validation studies using positive control materials

#### 3. Test animals:

Species:	rat
Strain:	Fisher 344 CDF(F-344)/BR
Sex:	Male and female (nulliparous and nonpregnant)
Age:	8 weeks
Weight:	Males: 178.1-248.5 g Females: 131.1-160.8 g
Source:	[REDACTED]
Acclimatisation period:	One week
Diet:	Purina Rodent Laboratory Chow #5001-4 in etts form, <i>ad libitum</i>

Water:	Tap water, <i>ad libitum</i>
Housing:	Individually housed in stainless steel wire mesh cages providing a 66.5 square inch area. Deotized Animal Cage Board was used in the bedding trays.
Environmental conditions:	Temperature: 18.3 – 25.6°C Humidity: 40-70% Air changes: not reported Photoperiod: 12 hr light/dark cycle

## B. Study design and methods

### 1. Animal assignment and treatment:

Route of administration:	Oral (diet)
Exposure:	13 weeks
Group size:	12/sex/dose level
Dose levels:	<u>Nominal</u> : 0, 120, 600 and 3000 ppm <u>Effective</u> : 0, 104, 527 and 2910 ppm equal to 0, 7.30, 38.1 and 219 mg/kg bw/day in males 0, 8.40, 42.6 and 247 mg/kg bw/day in females
Dose selection rationale:	The rationale for dose selection was based on the results of a subchronic (13-week) dietary exposure study with the test substance.

### 2. Dose preparation and analysis:

Dose preparation:	Corn oil was used as the vehicle for the test article at 1% by weight of the diet; a small amount of acetone served as a solvent in the diet preparation process and was allowed to evaporate. The control diet was prepared the same way, excluding the test substance.
Analysis:	The concentration of thiafluamide in the vehicle was measured using liquid chromatographic (LC) analysis. The stability (following both room temperature and freezer exposure) and homogeneity of the test substance in the feed were established by analysis of samples at nominal concentrations (100 and 4000 ppm) that bracketed the range of test concentrations that were used in the study. Homogeneity was verified by analysis of nine samples each of the nominal 100 and 4000 ppm dietary levels. The concentration of the test article in the ration was measured for the ration that was used during weeks 1, 6, 10 and 14 of the study.

### 3. Observations:

clinical observations, mortality, body weight, food consumption, automated measurements of activity (figure-eight maze), functional observational battery, quantitative electroencephalography (qEEG), ophthalmology, brain weight, and a gross necropsy. Skeletal muscle, peripheral

nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined microscopically for lesions

All twelve rats/sex/dietary level were used for neurobehavioral evaluation, with half used for neuropathology.

#### **4. Statistical Analysis:**

Statistical evaluations were performed using software from either INSTEM Computer Systems or SAS. With the exception of Bartlett's test, which was tested at  $p \leq 0.001$ , the level used to establish statistical significance was  $p \leq 0.05$ .

In general, continuous data were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA.

In the event of unequal variances, data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between group comparisons).

## **II. Results and discussion**

### **A. Mortality**

There were no compound-related deaths prior to terminal sacrifice. One (high-dose) male and two (one control and one mid-dose) females were found dead before the scheduled terminal sacrifice. The cause of these deaths is not known. However, since there was no dose-related increase in incidence and no compound related clinical signs, these deaths are considered incidental and not related to treatment with thiafluamide.

### **B. Clinical signs of toxicity**

There were no compound-related clinical signs. Based on these results.

### **C. Body weight and food consumption**

Body weight and food consumption were reduced by treatment at the high dose for males and females but not at lower dietary levels.

The high dose generally reduced body weight and food consumption throughout the exposure period, with an average reduction in body weight for the duration of exposure of 22% and 14% for males and females, respectively.

**Table B.6.7-5 Body Weight summary data in g (n = 11-12)**

Day	Dose group (ppm) males				Dose group (ppm) females			
	0	120	600	3000	0	120	600	3000
0	212.1	211.6	210.2	209.1	142.6	143.4	143.3	143.1
7	235.6	238.2	231.7	<b>202.1*</b>	151.2	152.1	150.5	<b>138.6*</b>
14	249.3	258.6	253.5	<b>208.6*</b>	159.7	160.0	158.3	<b>142.4*</b>
21	265.8	271.1	265.2	<b>215.9*</b>	165.4	166.7	165.6	<b>145.1*</b>
28	278.8	283.1	278.1	<b>227.3*</b>	167.2	168.1	166.7	<b>147.0*</b>
35	295.8	299.7	292.4	<b>237.7*</b>	174.3	171.8	172.2	<b>152.7*</b>
42	309.1	310.7	304.9	<b>243.2*</b>	178.3	176.1	175.9	<b>154.5*</b>
49	315.7	316.4	310.0	<b>245.5*</b>	183.4	180.7	180.0	<b>156.2*</b>
56	322.8	322.4	317.8	<b>245.3*</b>	182.3	181.4	179.4	<b>154.2*</b>
63	333.8	333.5	326.3	<b>251.4*</b>	185.1	184.2	183.6	<b>156.5*</b>
70	343.9	342.7	332.6	<b>255.4*</b>	187.9	188.0	186.0	<b>158.4*</b>
77	348.5	342.7	332.6	<b>255.4*</b>	189.7	190.5	188.0	<b>159.2*</b>
84	346.0	351.4	339.9	<b>257.7*</b>	192.3	193.0	191.6	<b>158.4*</b>
91	357.3	359.8	348.7	<b>271.2*</b>	196.0	195.3	193.0	<b>160.9*</b>

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

The high-dose males consumed an average 14% less food than controls and the high-dose females consumed an average 11% less food than controls for the duration of exposure. By comparison, daily food consumption, averaged over the duration of the study on a per kg body weight basis, was increased by 7% and 3% in high-dose males and females, respectively. This relative increase in both sexes is attributed to the reduction in body weight for the high-dose males and females. However, food spillage was evident throughout the study for the high-dose males and females. Therefore, the average quantity of food consumed at the high dose was actually less than these measurements indicate.

**Table B.6.7-6 Summary of food consumption in g/animal/day (n = 11-12)**

Day	Dose group (ppm) males				Dose group (ppm) females			
	0	120	600	3000	0	120	600	3000
7	21.53	21.74	20.75	<b>15.46*</b>	14.31	13.84	13.76	<b>11.47*</b>
14	21.01	21.95	21.64	<b>18.16*</b>	15.22	14.53	14.75	15.54
21	21.58	21.94	21.74	<b>17.92*</b>	15.27	14.60	14.58	14.23
28	21.41	21.33	22.36	<b>18.75*</b>	14.93	14.91	14.30	14.27
35	20.78	21.08	21.34	<b>18.70*</b>	14.41	13.95	13.60	<b>12.90*</b>
42	20.56	20.44	21.19	<b>18.07*</b>	14.47	13.92	14.02	<b>12.61*</b>
49	21.46	20.74	21.42	<b>17.58*</b>	14.55	14.17	14.20	<b>12.72*</b>
56	21.07	20.51	20.91	<b>16.74*</b>	14.23	13.77	13.95	<b>11.85*</b>
63	20.16	20.20	20.45	<b>17.98*</b>	13.44	13.36	13.32	12.44
70	19.93	20.33	20.18	<b>17.63*</b>	14.03	14.16	13.71	<b>12.36*</b>
77	19.91	20.23	20.53	<b>18.17*</b>	13.89	13.97	13.68	<b>12.18*</b>
84	20.13	20.65	20.47	<b>17.86*</b>	14.58	14.14	14.09	<b>12.67*</b>
91	20.94	20.27	20.26	<b>18.17*</b>	14.10	13.54	13.55	<b>12.22*</b>

\* p ≤ 0.05 significant by Anova + Dunnetts tests (two-sided)

#### D. Functional observational battery (FOB)

For the functional observational battery (FOB), compound-related effects were evident in high-dose males and females but not at lower dietary levels. Effects in males that are ascribed to treatment consisted of reduced forelimb grip strength and a slightly uncoordinated righting response. By comparison, compound-related effects in females that received the high dose consisted of reduced forelimb grip strength, decreased body temperature and increased hindlimb footsplay.

**Table B.6.7-7 Summary of selected FOB results –week 13 (n = 11-12)**

Observations		Dose group (ppm) males				Dose group (ppm) females			
		0	120	600	3000	0	120	600	3000
Reflex/ Physiologic	Forelimb grip strength (kg)	0.85	0.90	0.85	<b>0.74*</b>	0.68	0.62	0.69	<b>0.52*</b>
Sensorimotor coordination	Hindlimb footsplay (mm)	63	63	60	68	47	54	48	<b>65*</b>
	Righting reflex slightly uncoordinated (incidence)	0 (0%)	0 (0%)	0 (0%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)
	Body temperature (°C)	37.3	37.3	37.3	37.0	38.0	38.4	38.2	37.5

\* p ≤ 0.05 (ANOVA)

### E. Motor and locomotor activity

Automated measures of motor and locomotor activity were affected by treatment in high-dose males and females during week 13 but not at lower doses or on earlier test occasions. Males and females that received 3000 ppm of thiafluamide in the diet had increased activity on the final test occasion (week 13), demonstrating cumulative toxicity or neurotoxicity from 8 to 13 weeks of exposure. There was no effect on habituation for either sex.

**Table B.6.7-8 Summary session motor activity – week 13 (mean ± SD; n = 12)**

Timepoint	Dose group (ppm) males				Dose group (ppm) females			
	0	120	600	3000	0	120	600	3000
Pretreatment	547 ± 199	590 ± 160	589 ± 233	483 ± 149	978 ± 343	880 ± 289	961 ± 164	897 ± 259
Week 4	701 ± 305	698 ± 198	719 ± 293	584 ± 245	1077 ± 291	1006 ± 315	1093 ± 206	1007 ± 332
Week 8	652 ± 271	626 ± 156	720 ± 211	682 ± 285	982 ± 350	992 ± 453	1066 ± 301	1126 ± 332
Week 13	688 ± 419	581 ± 184	589 ± 205	1027 ± 493	1040 ± 332	1076 ± 365	1025 ± 334	<b>1621±428*</b>

\* p≤ 0.05 (ANOVA)

**Table B.6.7-9 Summary session locomotor activity – week 13 (mean ± SD; n = 12)**

Timepoint	Dose group (ppm) males				Dose group (ppm) females			
	0	120	600	3000	0	120	600	3000
Pretreatment	196 ± 51	206 ± 44	222 ± 69	179 ± 52	363 ± 343	309 ± 118	324 ± 54	304 ± 102
Week 4	257 ± 147	248 ± 68	273 ± 120	185 ± 73	351 ± 85	329 ± 111	354 ± 78	321 ± 119
Week 8	268 ± 128	249 ± 62	305 ± 125	237 ± 129	325 ± 108	342 ± 171	375 ± 108	359 ± 137
Week 13	303 ± 230	261 ± 87	255 ± 94	392 ± 196	351 ± 120	358 ± 133	344 ± 100	<b>584±173*</b>

\* p≤ 0.05 (ANOVA)

### F. Quantitative electroencephalography (qEEG)

There were no compound-related qEEG findings.

### G. Ophthalmology

There were no compound-related ophthalmic findings.

### H. Organ weight

Brain weight was reduced at the high dose in perfused and nonperfused males (average, 5-10%) and females (average, 6-8%) but not at lower dose levels. The reduction in brain weight was much less than the effect on terminal body weight. This difference resulted in an increase in relative brain weight at the high dose in both sexes.

**Table B.6.7-10 Summary of brain weights – perfused animals**

Parameter	Dose group (ppm)			
	0	120	600	3000
	Males			



Parameter		Dose group (ppm)			
		0	120	600	3000
Brain	a	1.849	1.855	1.722	1.663
	r	0.524	0.520	0.490	<b>0.615*</b>
		Females			
Brain	a	1.666	1.646	1.706	<b>1.541*</b>
	r	0.830	0.831	0.839	<b>0.917*</b>

\* significantly different from respective control  $p \leq 0.05$

a: absolute organ weight (g)

r: relative organ weights as % of body weight

**Table B.6.7-11 Summary of brain weights – non-perfused animals**

Parameter		Dose group (ppm)			
		0	120	600	3000
		Males			
Brain	a	1.971	1.989	1.959	<b>1.867*</b>
	r	0.558	0.552	0.578	<b>0.666*</b>
		Females			
Brain	a	1.832	1.856	1.803	<b>1.715*</b>
	r	0.957	0.974	0.986	<b>1.110*</b>

\* significantly different from respective control  $p \leq 0.05$

a: absolute organ weight (g)

r: relative organ weights as % of body weight

## I. Gross necropsy

Compound-related gross lesions were not evident in males or females at any dietary level.

## J. Histopathology

Compound-related microscopic lesions were evident in cerebellum – medulla oblongata and spinal cord tissues at the mid- and high-dose levels for both sexes. This consisted of an increased incidence, relative to controls, of swollen axona. Based on these results, the NOEL for microscopic lesions is 120 ppm for males and females.

**Table B.6.7-12 Summary axonal swellings frequency**

Parameter		Dose group (ppm)			
		0	120	600	3000
		Males			
Brain	Levels 7&8	0.2	0.5	1.0	<b>2.8*</b>
Spinal cord	Cervical	0.5	0.5	1.2	1.3
	Thoracic	1.7	1.2	1.7	3.2

Parameter		Dose group (ppm)			
		0	120	600	3000
	Lumbar	2.2	2.3	3.2	5.2
	Cauda equina	3.5	2.5	5.2	<b>9.7*</b>
		Females			
Brain	Levels 7&8	1.5	0.5	2.5	<b>5.5*</b>
Spinal cord	Cervical	0.8	0.8	0.5	<b>3.3<sup>s</sup></b>
	Thoracic	1.3	1.0	0.8	<b>8.2*</b>
	Lumbar	2.8	2.8	2.7	<b>9.8*</b>
	Cauda equina	3.7	4.0	7.5	<b>13.2*</b>

\*  $p \leq 0.05$  Anova + Dunnetts tests

<sup>s</sup>  $p \leq 0.05$  Kruskal-Wallis Anova + Mann-Whitney rank sum test

### III. Conclusion

In summary, the present study established a dose-related increase in evidence of neurotoxicity following subchronic dietary exposure to thiafluamide, with compound-related neurobehavioral effects evident at a dose of 3000 ppm for males and females and microscopic CNS lesions evident at 600 and 3000 ppm dietary levels.

However, compound-related effects only occurred at exposure levels that produced substantial evidence of systemic toxicity. The highest dietary concentration of 3000 ppm produced a marked reduction in body weight gain in both sexes. Furthermore, a separate subchronic study established evidence of tissue damage involving liver-, kidney-, hematologic/spleen- and thyroid-related endpoints at dietary levels of 400-3000 ppm. Compound-related effects persisted with continued exposure and there was evidence of cumulative toxicity after 4-8 weeks of continued exposure. The possible reversibility of these effects following cessation of exposure was not investigated in this study.

NOEL: 120 ppm equal to 7.30 mg/kg bw/day for males and 8.40 mg/kg bw/day for females

NOAEL: 600 ppm equal to 38.1 mg/kg bw/day for males, and 42.6 mg/kg bw/day for females.

#### Clinical observations:

There were no compound-related clinical signs.

#### Body weight:

The high dose of 3000 ppm for males and females produced a sustained, statistically-significant decrease in body weight, relative to controls. Body weight was not affected by treatment at lower doses in either sex. The difference in body weight, relative to controls, for the duration of exposure averaged 22 % and 14 % for the high-dose males and females, respectively.

**Mortality:**

One (high-dose) male and two (one control and one mid- dose) females were found dead before the scheduled terminal sacrifice. The cause of these deaths was not compound-related.

**Food consumption:**

Food consumption was significantly reduced by treatment in males and females that received 3000 ppm of thiafluamide in the diet but not at lower dose levels.

**Behavioral testing:**

Functional Observational Battery (FOB): There were compound-related effects in the high-dose males and females but not at lower doses. These effects consisted of reduced forelimb grip strength and a slightly uncoordinated righting response in males and reduced forelimb grip strength, decreased body temperature and increased hindlimb foot splay in females. These effects generally developed with continued exposure, with evidence of a cumulative effect beyond weeks 4-8.

**Motor and locomotor activity:**

Automated measures of activity were affected by treatment in the high-dose males and females but not at lower dose levels. Motor and locomotor activity were increased, relative to controls, for the high-dose males and females on the final (i.e., week 13) test occasion. Habituation was not affected by treatment at any dose level.

**Quantitative electroencephalography (qEEG):**

There were no qEEG findings ascribed to treatment with thiafluamide at any dietary level.

**Ophthalmology:**

All ophthalmologic findings were considered incidental and not related to exposure to FOE 5043.

**Necropsy, gross pathology, histopathology:**

Compound-related gross lesions were not evident at necropsy in either sex. Brain weight was reduced, relative to controls, in males and females that received the high dose but not at lower dose levels. Since brain weight was affected to a lesser extent than terminal body weight, relative brain weight was increased at the high dose in both sexes. There were compound-related microscopic lesions in the mid and high-dose males and females but not at the low dose. These lesions consisted of an increased incidence, relative to controls, of swollen axons in the cerebellum - medulla oblongata and spinal cord.

<b>Conclusion</b>	<b>The study is in line with current guidelines. RMS does not object to the NOAEL/NOEL, supports the proposal of the previous evaluation.</b>  This study established a dose-related increase in evidence of neurotoxicity, with compound-related neurobehavioral effects evident at a dose of 3000 ppm for males and females and microscopic CNS lesions evident at 600 and 3000 ppm dietary levels. All compound-related effects occurred at dose levels that produced substantial evidence of systemic toxicity. Compound-related effects persisted with
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	<p>continued exposure and there was evidence of cumulative toxicity after 4-8 weeks of continued exposure. The possible reversibility of these effects was not investigated.</p> <p>No-observed-effect level:</p> <p>For clinical signs: 3000 ppm for males and females.</p> <p>For body weight, food consumption, FOB, motor and locomotor activity: 600 ppm for males and females.</p> <p>For qEEG: 3000 ppm for males and females, the highest dose tested.</p> <p>For microscopic lesions: 120 ppm for males and females.</p> <p>For neurotoxicity: 120 ppm for males and females.</p>
<b>Summary for original approval (1997);</b>	<p>FOE 5043 has been investigated in acute and subchronic oral neurotoxicity screening studies using rats. In an acute neurotoxicity screening study, all clinical and neurobehavioral effects observed following administration of a single dose of FOE 5043 were ascribed to acute systemic toxicity. Complete recovery occurred in surviving animals with the exception of urine stains which persisted till termination in females. There were no correlative micropathologic findings to indicate any evidence of an adverse effect on the nervous system. In a subchronic neurotoxicity screening study, a dose-related increase in evidence of neurotoxicity was demonstrated following dietary exposure of FOE 5043. Compound-related effects in the functional observation battery and motor activity assessments were evident in animals treated at higher concentrations. These findings were correlated with microscopic lesions (swollen axons) observed in the brain and spinal cord. These effects, however, occurred only at exposure levels that produced substantial evidence of systemic toxicity as demonstrated in a separate subchronic feeding study (Christenson and Wahle, 1995b) in which tissue damage involving liver-, kidney-, hematologic/spleen- and thyroid-related endpoints was observed at similar high dietary levels. Thus, the results of these studies taken collectively suggest that an increased incidence of axonal swelling occurred in animals exposed to high levels of FOE 5043 which saturate metabolic pathways.</p>

**B.6.7.1 Neurotoxicity studies in rodents**

<b>New studies; not evaluated</b>	A new studies. Developmental neurotoxicity study of technical grade Flufenacet administered orally via diet to Crl:CD BR VAF/Plus presumed pregnant rats; <b>Non EU authorities</b>
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For registration of flufenacet in the United States (US), a developmental neurotoxicity study was conducted based on thyroid-related findings and therefore, the potential for affecting development of the nervous system. In this study dietary exposure to flufenacet did not cause any neurotoxic effect in parental and offspring animals. Treatment-related findings consisted of reduced food consumption and a reduction in maternal body weights during gestation and in males at mid- and high-dose. Body weights were also reduced in mid- and high-dose F1-males and high-dose F1-females. F1 offspring of these dose groups exhibited also a delay in development (eye opening, preputial separation).

<b>Report:</b>	<b>██████████; 2000</b>
<b>Title:</b>	Developmental neurotoxicity study of technical grade Flufenacet administered orally via diet to Crl:CD BR VAF/Plus presumed pregnant rats
<b>Document No:</b>	<b><u>M-026105-01</u></b>
<b>Report No:</b>	<u>BC9333</u>
<b>Guidelines:</b>	US-EPA guideline 83-3; US-EPA OPPTS 870.6300; PMRA DACO:4.5.12; Deviations: none
<b>GLP</b>	<u>Yes</u>

**Materials and methods****A. Materials****1. Test materials:**

Name:	Flufenacet
Description:	White powder
Lot/Batch no:	603-0013
Purity:	96.9 % - 96.0 %
Stability of test compound:	guaranteed for study duration

**2. Vehicle:**

1% corn oil

**3. Test animals:**

Species:	Rat
Strain:	Sprague-Dawley; Crl:CD®BR VAF/Plus
Age:	At least 60 days
Weight at dosing:	200 g – 225 g
Source:	██

Acclimatization period:	6 days
Diet:	Purina Mills Rodent Lab Chow® #5001-4 in "etts" Form (PMI Nutrition International Inc., St. Louis, Missouri, USA), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	individually in stainless-steel wire-bottomed cages; bedding: Bed-o'cobs® (The Andersons Industrial Products Group Maumee, Ohio, USA)

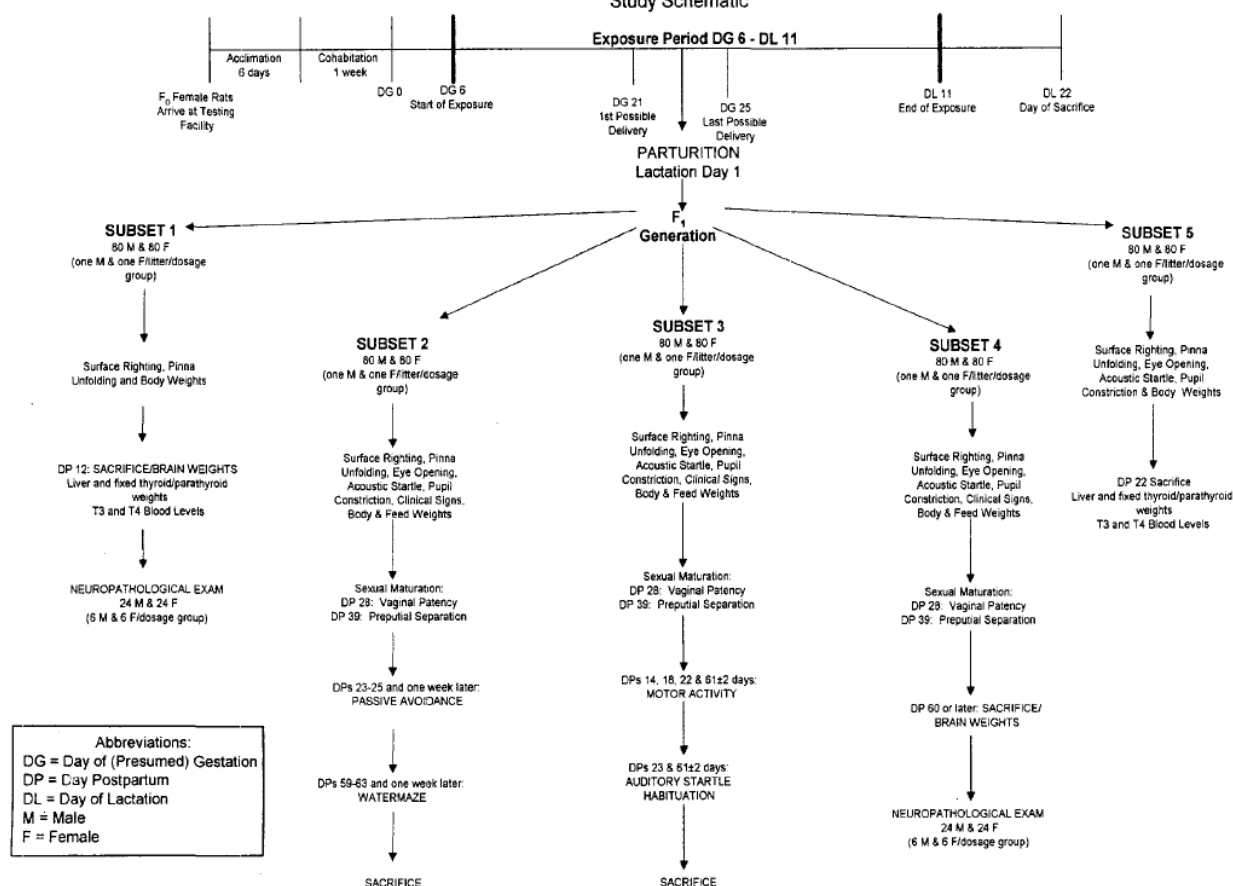
## B. Study design and methods

### 3. Animal assignment and treatment

Dose:	0 - 20 - 100 - 500 ppm equivalent to DG 6-21: 0 - 1.7 - 8.3 - 40.8 mg/kg bw/day DL 1-12: 0 - 3.0 - 15.4 - 76.7 mg/kg bw/day DG = gestation day; DL = lactation day
Exposure period:	DG 6-24 (dams that did not deliver a litter) or DL 11 (dams that did deliver a litter)
Application route:	oral, diet
Group size:	25 females/dose
Observations:	Mortality, clinical signs, body weight, food consumption, signs of autonomic dysfunction, abnormal postures, abnormal movements, abnormal behaviour patterns, unusual appearance, maternal behaviour, litter size, live litter size, pups: viability at birth, brain weights, neurohistology, liver weight, thyroid/parathyroid weight, gross pathology, histology, clinical chemistry, passive avoidance, water maze testing, motor activity, auditory startle habituation.

The study schematic can be found in the following.

Text Figure 1: Developmental Neurotoxicity Study of Technical Grade Flufenacet Administered Orally Via Diet to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats  
Study Schematic



## Results and discussion

### A. F<sub>0</sub> Generation (dams)

#### Mortality

All dams survived until scheduled sacrifice.

#### Clinical observation

All maternal clinical observations which occurred during gestation and lactation were not dose related, occurred only in one to three animals per group and/or the observation commonly occurs in this rat strain. Therefore, these findings are not considered to be test substance-related.

#### Body weight

There were no treatment-related changes in maternal body weights at 20 ppm. From gestation day 18-21 body weight gain was significantly reduced (81.2% of the control group value). This decrease was transient and not dose-related and, therefore, not considered related to the test substance.

During gestation treatment-related decreases in maternal body weights/body weight gains were observed at 100 and 500 ppm. No treatment-related effects on maternal body weights were observed during lactation.

**Table 6.7.1/03-1: Summary of maternal body weights changes during gestation**

Dose (ppm)	Maternal body weight changes during gestation – means (g)							
	Gestation days							
	0-6	6-9	9-12	12-15	15-18	18-21	6-21	0-21
0	+30.5	+19.0	+28.0	+25.4	+36.8	+46.2	+135.3	+166.7
20	+32.3	+13.8	+12.2	+21.2	+37.6	+37.5*	+122.4	+154.3
100	+29.1	+9.0**	+15.4*	+20.9	+39.6	+39.9	+124.4	+153.3
500	+30.8	+7.0**	+8.3	+28.8	+36.3	+36.8**	+116.2**	+147.0

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

### Food consumption

Absolute (g/day) and relative (g/kg/day) food consumption were reduced at the start of exposure at the 100 ppm and 500 ppm dietary levels. During gestation food consumption was reduced at 100 and 500 ppm on gestation day (DG) 6 to 9, and at 500 ppm also on DGs 9 to 12. However, from DG 12 to 15 food consumption at 500 ppm was significantly increased. This transient reduction when treated feed was introduced was probably due to palatability, rather than toxicity of the test substance.

The absolute and relative food consumption values were significantly reduced in all dose groups from lactation day (DL) 7 to 12. These transient fluctuations in absolute and relative food consumption values were considered unrelated to the test substance because they were not dose-dependent and no statistically significant differences occurred for the entire dosage period (DGs 6 to 21 and DLs 1 to 11).

**Table 6.7.1/03-2: Summary of maternal food consumption during gestation**

Dose (ppm)	Absolute food consumption during gestation – means (g/day)							
	Gestation days							
	0-6	6-9	9-12	12-15	15-18	18-21	6-21	0-21
0	22.3	25.7	26.1	26.0	28.9	27.3	26.8	25.5
20	22.7	24.2	24.5	26.1	29.0	27.0	26.0	25.0
100	22.2	21.5**	25.4	26.9	29.3	26.6	25.9	24.9
500	22.8	18.6**	21.9**	29.2**	29.8	26.4	25.1	24.4
	Relative food consumption during gestation – means (g/kg/day)							
0	89.0	92.9	89.9	85.4	86.1	73.6	84.8	81.9
20	90.3	87.6	85.2	86.0	86.9	74.2	83.5	81.4
100	88.9	79.4**	89.9	89.4	88.7	71.6	82.7	80.8
500	90.3	69.6**	78.7**	98.0**	90.3	72.6	81.6	80.6

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$



**Table 6.7.1/03-3: Summary of maternal food consumption during lactation**

Dose (ppm)	Absolute food consumption during lactation – means (g/day)							
	Lactation days							
	1-4	4-7	7-12	1-12	12-14	12-22	14-22	1-22
0	33.2	45.2	58.8	48.1	57.0	65.3	67.3	56.4
20	32.3	42.7	53.9*	45.0	54.3	64.5	67.0	55.0
100	33.3	43.8	52.8**	45.1	53.6	64.0	66.6	54.6
500	29.9	42.0	54.1*	44.2	56.5	63.6	65.4	53.4
	Relative food consumption during lactation – means (g/kg/day)							
0	116.5	155.4	189.4	161.3	179.9	201.7	207.0	182.5
20	113.2	146.1	173.4*	150.6	169.8	196.6	203.2	176.3
100	118.6	152.6	173.4*	153.8	170.4	198.4	205.4	177.8
500	107.9	149.7	181.2	153.4	178.4	197.0	201.6	175.5

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

### **Natural delivery observations, litter observations, pup clinical observations, reflex and physical development**

No treatment-related findings on natural delivery, litter observation, clinical observations, pup weights per litter, live born and stillborn pups, viability index and lactation index, litter size, sex ratio were observed in any dose group.

### **Natural delivery observations**

Pregnancy (implantation sites at necropsy) occurred in all mated female rats in controls as well as in the 20 and 100 ppm dose groups. At 500 ppm pregnancy occurred in 22 of 25 mated female rats. One control dam (11755) did not deliver a litter. This dam was pregnant at sacrifice on DG 25, with five live and five dead fetuses in utero and one partially delivered live fetus. A twelfth conceptus was presumed cannibalized. All other pregnant dams delivered litters. The number of pregnant dams was significantly reduced at 500 ppm. This significant difference was considered unrelated to the test substance because the number of pregnancies was determined prior to the initiation of treatment and the incidence was within the historical control range for this Testing Facility. The number of dams with stillborn pups was significantly reduced in all dose groups due to an increase in the number of dams with stillborn pups in the control group. This reduction was not considered test substance-related since an increase, not a reduction of stillborns is considered an expected toxicological effect.

**Table 6.7.1/03-4: Natural delivery observations**

Natural delivery observations - F0-females				
Dose (ppm)	0	20	100	500
Mated rats per group	25	25	25	25
Pregnant	25	25	25	22**
Delivered	24	25	25	22
Duration of gestation	22.7	22.6	22.8	22.9
Implantation sites / litter	16.3	15.0	15.0	16.4
Dams with stillborn pups	7	3**	2**	0**
Gestation index (%)	96.0	100.0	100.0	100.0

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

### **Litter observations**

No litter observations from birth to day 22 postpartum were affected by administration of the test substance in the maternal diet up to 500 ppm.

**Table 6.7.1/03-5: Litter observations**

<b>Litter observations</b>		<b>0</b>	<b>20</b>	<b>100</b>	<b>500</b>
<b>Dose (ppm)</b>					
Pups delivered		375	338 <sup>1)</sup>	350	332
Liveborn	mean	15.2	13.9 <sup>1)</sup>	13.9	15.0
	N (%)	97.3	98.8 <sup>1)</sup> *	99.4 **	99.7 **
Stillborn	mean	0.4	0.1	0.1	0.0
	N (%)	2.7	0.9 <sup>1)</sup> **	0.6 **	0.0 **
Unknown vital status		0	1 <sup>1)</sup>	0	1
Viability index (%)		97.5	96.7 <sup>1,2,3)</sup>	95.5 <sup>3)</sup>	96.7
Lactation index (%)		62.5	65.8	67.2	70.0
Mean pup weights/litter (g)					
Preculling	DP 1	6.6	6.2 <sup>1)</sup>	6.4	6.1
	DP 5	8.8	8.8 <sup>2)</sup>	9.0 <sup>3)</sup>	8.4
	DP 5	9.0	8.9	9.1 <sup>3)</sup>	8.4
Surviving pups/litter	DP 1	15.2	13.9	13.9	15.0
	DP 5	14.8	13.5	13.3	14.5
	DP 5	10.0	9.6	9.8	10.0
Postculling	DP 8	10.0	9.6	9.4*	10.0
	DP 12	6.6	6.3	6.6	7.2
	DP 14	7.8	7.9	7.9	7.8
Postculling	DP 18	7.5	7.9	7.7	7.7
	DP 22	7.5	7.9	7.7	7.7

<sup>1)</sup> excluded values for 1 litter as dam delivered one additional pup on DP 2

<sup>2)</sup> excluded values for 2 litters as 1 pup each was culled on DP 5

<sup>3)</sup> excluded values for 2 litters (dam 11790, the litter and 2 additional pups from litter 11796) sacrificed on DP 3

\* Statistically significant at p<0.05; \*\* statistically significant at p<0.01

Pup body weights per litter did not differ significantly for DPs 1 or 5 (pre- or post-culling).

The percentage of liveborn pups was significantly increased and the percentage of stillborn pups was significantly reduced in all dose groups. These values were considered unrelated to the test substance because an increase, not a reduction, in the number of stillborn pups is the expected toxicological effect.

The number of pups found dead or presumed cannibalized on DPs 6 to 8 was significantly increased in the 100 ppm dose group. However, this increase in pup mortality was considered unrelated to the test substance because the increase in pups found dead during this period was primarily from a single litter (11783) that had seven dead or missing pups on DPs 6 and 7 and the value was not dose-dependent.

The Viability Index (number of live pups on DP 5 divided by the number of liveborn pups on DP 1) and the Lactation Index (number of live pups on DP 22 divided by the number of live pups on DP 5) were comparable among the four groups and did not significantly differ.

The number of surviving pups per litter and the live litter size at weighing on DP 8 were significantly reduced (9.4 versus 10.0 surviving pups) in the 100 ppm dose group. These reductions were related to the pup deaths in one litter, as previously discussed, and considered unrelated to the test substance because the values were not dose-dependent. The percentage of male pups per number of pups sexed was comparable among the four dose groups and did not significantly differ.

### **Reflex and physical development**

The average day postpartum that at least 50% of the pups had open eyes was significantly increased (15.4 and 15.6 days, respectively, versus 14.8 days in the control group) in the 100 and 500 ppm dose groups. Reflecting this developmental delay, significant reductions in the mean percentage of pups

with eyes opened on DPs 14 and 15 occurred in the 100 and 500 ppm dose groups (11.6% and 7.5% versus 34.4% and 61.1% and 38.8% versus 85.4% in the respective control group).

There were no other biologically important differences among the four dose groups in the measures of reflex and physical development (surface righting, pinna folding, acoustic startle or pupil constriction). A significant reduction in the mean percentage of pups with eyes opened on DP 15 occurred in the 20 ppm dose group (51.8% versus 85.4% in the control group). A significant reduction in the mean percentage of pups responding to an acoustic startle on DP 13 occurred in the 500 ppm dose group (45.0% versus 66.7% in the control group). These transient observations were considered unrelated to the test substance because no treatment-related effect was noted in the average day postpartum that at least 50% of all the pups had eyes open in the 20 ppm dose group or had the acoustic startle reflex in the 500 ppm dose group; and/or the values were within the historical ranges of the Testing Facility.

**Table 6.7.1/03-6 Summary on eye opening F1-generation litters**

	HCD				Control		FFA 20 ppm		FFA 100 ppm		FFA 500 ppm	
N	10				20		20		20		20	
	Mean	SD	Min	Max	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 12	1.3	1.4	0.0	4.9	0.6	2.8	0.0	0.0	0.0	0.0	0.0	0.0
Day 13	8.3	6.3	0.0	21.5	9	12.9	2.5	5.1	2	6.8	3.9	8.7
Day 14	40.2	19.8	3.3	67.5	34.4	27.3	20.1	29	11.6*	18	7.5**	11.1
Day 15	82.2	15.4	50.0	98.6	85.4	24.5	51.8**	39.4	61.1*	41.1	38.8**	32.8
Day 16	98.8	1.3	96.0	100.0	98.1	6.1	90.6	20.6	89.5	24.8	81.3	29.2
Day 17	99.6	1.1	97.0	100.0	100	0.0	99.4	2.8	99.4	2.8	97.2	9.8
Day 18	100.0	0.0	100.0	100.0	100	0.0	100	0.0	100	0.0	99.3	3.2
Day 19	100.0	0.0	100.0	100.0	100	0.0	100	0.0	100	0.0	100	0.0
<b>Ave- rage#</b>	<b>14.7</b>	<b>0.4</b>	<b>14.1</b>	<b>15.4</b>	<b>14.8</b>	<b>0.7</b>	<b>15.3</b>	<b>0.9</b>	<b>15.4</b>	<b>0.8</b>	<b>15.6</b>	<b>0.7</b>

# Average day postpartum that at least 50% of the pups in a litter had the eyes opened

N = number of litters; for HCD number of studies

Eye opening (mean % of pups meeting the criterion for the developmental landmark tested on the specified postpartum day; excluded were values for litters that were sacrificed on day 12 postpartum)

\* Significantly different from the carrier group value ( $p \leq 0.05$ ).

\*\* Significantly different from the carrier group value ( $p \leq 0.01$ ).

### **Maternal and pup necropsy observations (through DP 22)**

All maternal clinical and necropsy observations were considered unrelated to the test substance. No necropsy observations in the pups were attributable to maternal consumption of the test substance at any concentration tested because the incidences were not statistically significant or the observation occurred in only one or two pups.

## **B. F1 Generation**

### **Mortality (subsets 1, 2, 3, 4, 5)**

Four, one, one and three F1 generation males and three, two, three and four F1 generation females in the 0, 20, 100 and 500 ppm maternal dose groups, respectively, were found dead during the study.

One male and two female offspring in the maternal control group were missing during the preweaning period and presumed cannibalized. One rat in the maternal control group with hypospadias was sacrificed on day 34 postpartum (DP 34). These deaths were considered unrelated to the maternal consumption of the test substance because the incidences did not differ significantly among groups, including the control group.

**Clinical observations (subsets 2, 3, 4)**

All clinical observations in the F1 generation male and female rats were considered unrelated to the test substance because they were not dose-dependent; they occurred in all dose groups, including the control group; and/or they occurred in only one or two rats in a dose group.

**Body weight (subsets 1, 2, 3, 4)**

Pup body weights per litter did not differ significantly for DPs 1 or 5 (pre- or post-culling), please refer also to Table 6.7.1/03-5.

Differences from the control group mean body weight and body weight changes for F1-males and F1-females were considered related to the test substance for the 500 ppm maternal dose group, because the differences were statistically significant and persisted throughout the postpartum period.

In the 100 ppm dose group, significant differences from the control group values occurred and did persist until sacrifice for the male rats. These changes may have been related to the test substance.

In the 20 ppm dose group, some significant differences from the control group values occurred, but the statistically significant differences represented only minimal differences (less than a few percent) and reflected statistical significance that occurs when larger than normal (75 to 100 animals, versus a more typical 25 to 30 animals per group) numbers of animals are compared. These small differences from the control group at the 20 ppm dietary level were considered incidental and unrelated to treatment because they were minimal and did not persist.

**Table 6.7.1/03-6: Summary of body weights in F1-generation**

Dose (ppm)	Body weights– means (g)							
	Males				Females			
	0	20	100	500	0	20	100	500
DP 5	9.3	9.2	9.4	8.6**	8.8	8.7	8.8	8.0**
DP 8	14.1	14.0	13.7	12.4**	13.3	13.3	13.0	11.7**
DP 12	20.4	19.0	18.5	17.4**	19.5	18.4*	17.8**	16.8**
DP 14	25.2	21.8**	21.0**	21.2**	24.2	21.1**	20.1**	20.6**
DP 18	34.5	30.5**	30.2**	30.7**	33.3	29.7**	29.4**	29.5**
DP 22	47.2	42.0**	41.4**	41.3**	44.8	40.9**	40.1**	39.7**
DP 23	49.4	43.2**	42.0**	41.0**	46.9	42.1**	40.7**	40.0**
DP 30	94.1	87.2**	83.0**	81.8**	85.6	80.4*	78.6**	75.1**
DP 37	152.9	145.4	138.2**	135.4**	129.3	124.7	121.2**	116.1**
DP 44	217.0	208.4	202.3**	199.0**	164.1	161.4	156.6*	151.2**
DP 51	273.5	263.8	256.2**	254.0**	190.0	187.0	182.0*	176.9**
DP 58	332.9	323.2	315.5**	310.8**	212.9	211.8	206.0	200.2**
DP 65	380.7	371.9	365.8**	358.9**	231.8	232.2	226.0	220.4**
DP 72	415.0	408.4	399.9**	394.1**	246.9	247.9	239.8	235.0**

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

Values were excluded for rats found dead, were missing or were assigned to subsets sacrificed on DP 12 / DP 22

**Table 6.7.1/03-7: Summary of body weights changes in F1-generation**

Dose (ppm)	Body weight changes– means (g) of F1-males			
	Study days			
	5-65	65-72	23-72	5-72
0	+371.4	+34.4	+366.0	+405.7
20	+362.7	+36.4	+365.0	+399.2
100	+356.4	+34.1	+357.7	+390.5*
500	+350.3**	+35.2	+352.4	+385.5**
Dose (ppm)	Body weight changes– means (g) of F1-females			
	Study days			
	5-65	65-72	23-72	5-72
0	+222.9	+15.1	+199.5	+238.0
20	+223.5	+15.7	+205.5	+239.2
100	+217.2	+13.8	+198.5	+231.0
500	+212.3**	+14.5	+193.9	+226.8**

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

Values were excluded for rats found dead, were missing or were assigned to subsets sacrificed on DP 12 / DP 22

Additional body weight tables according to new evaluation of pup bodyweight data based on the litter as the unit of measure (i.e. sample size from 20 litters was 20).

**Table 6.7.1/03-8 Body weights (g) for F1 offspring in the developmental neurotoxicity study with flufenacet**

	PND 4	PND 11	PND 13	PND 17	PND 21
Males					
Dose (ppm)					
0	9.20 ± 0.95	19.77 ± 2.93	24.4 ± 4.7	33.5 ± 6.1	45.4 ± 9.3
20	9.10 ± 0.97	18.89 ± 3.03	21.7 ± 2.8*	30.5 ± 2.6*	42.1 ± 3.8
100	9.38 ± 1.61	18.44 ± 3.68	20.9 ± 3.0*	30.1 ± 3.7*	41.2 ± 5.1
500	8.71 ± 0.97	17.60 ± 3.83*	20.9 ± 5.8*	30.3 ± 7.2	40.7 ± 8.9
Females					
0	8.73 ± 0.74	19.01 ± 2.38	23.6 ± 3.8	33.4 ± 3.4	44.9 ± 4.9
20	8.63 ± 1.15	18.16 ± 3.11	21.0 ± 2.6*	29.7 ± 2.9*	40.9 ± 4.2*
100	8.83 ± 1.58	18.11 ± 3.44	20.0 ± 3.2*	29.3 ± 3.5*	40.1 ± 5.1*
500	8.22 ± 0.95	16.95 ± 3.64*	20.4 ± 5.5*	29.2 ± 6.9*	39.4 ± 8.2*

Mean ± SD; \* $p \leq 0.05$  (20 litters per dose level)

**Table 6.7.1/03-8 Mean Body weight gain (g) from PND 4-11 of treatment in the developmental neurotoxicity study with Flufenacet**

Dose (ppm)	0	20	100	500
Males	10.58 ± 2.35	9.80 ± 2.76	9.06 ± 2.50*	8.89 ± 3.23*
Females	10.28 ± 2.01	9.74 ± 2.42	9.14 ± 2.45*	8.73 ± 3.04*

Mean ± SD; \* $p \leq 0.05$  (20 litters per dose level)

**Food consumption (subsets 2, 3, 4)**

Absolute and relative food consumption values for the F1 generation male and female rats were unaffected at the 20 ppm dose group. Differences from the control group (see below) that occurred on occasion were not considered related to the test substance because they were transient and/or not dose-related.

At maternal dose levels of 100 and 500 ppm absolute food consumptions were significantly decreased for the entire post-waning period (DP 23-72) in F1 –males as well as in F1-females (DP 23-30).

Due to the lower body weights in the 500 ppm maternal dose group, the relative food consumption values (see table below) were significantly increased for F1-males, as well as for F1-females of the 100 and 500 ppm maternal dose groups.

**Table 6.7.1/03-9: Summary of relative feed consumption in F1-generation**

Dose (ppm)	Feed consumption– means (g/kg/day)							
	Males				Females			
	0	20	100	500	0	20	100	500
DP 23-30	195.3	199.9	202.3*	198.7	198.0	203.4	206.3**	202.4
DP 30-37	190.7	192.8	202.2	213.1	204.2	208.9	214.3	215.7
DP 37-44	145.4	149.5	151.6**	152.5*	147.0	148.3*	160.2**	152.7*
DP 44-51	132.1	133.0	136.6	138.4	141.1	140.2	146.9	150.7*
DP 51-58	107.9	111.0	113.3**	113.4**	111.7	113.4	115.0	116.0
DP 58-65	94.2	95.7	96.5	96.4*	102.2	102.6	106.4*	104.5
DP 65-72	84.6	86.0	85.5	87.7	95.5	98.1	98.9	98.4
DP 23-72	117.4	119.0	120.3	122.4*	131.1	132.3	136.2	135.1

\* Statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

Values were excluded for rats found dead

Values were excluded that were associated with spillage, soiled feed or interrupted feed access or appeared associated with spillage

**Sexual maturation of F1-generation (subsets 2, 3, 4)**

The average day postpartum for preputial separation was significantly increased in F1-males of the 100 and 500 ppm maternal dose groups (48.4 days postpartum in both groups versus 47.2 days postpartum in the control group). Preputial separation was not affected in the 20 ppm dose group.

Maternal dose levels of up to and including 500 ppm did not affect the day of vaginal patency in F1-females.

**Table 6.7.1/03-10: Summary of sexual maturation**

Dose (ppm)	Sexual maturation (in days)	
	Preputial separation	Vaginal patency
0	47.2	32.0
20	47.7	31.5
100	48.4*	32.4
500	48.4*	32.5

\* Significantly different from the carrier group value ( $p \leq 0.05$ ).

**Passive avoidance testing and water maze performance F1-generation (subset 2)**

There were no biologically important differences in the values for learning, shortterm retention, long-term retention or response inhibition in the F1-generation male or female rats, as evaluated by

performance in a passive avoidance paradigm. The trials to criterion in Session 2 were significantly greater (3.4 seconds versus 3.2 seconds in the control group) for the male rats in the 20 ppm dose group. This significant increase was considered incidental and unrelated to the maternal consumption of the test substance because the value was not dose-related and occurred in only one sex. No other statistically significant differences occurred in the F1 generation males or females in the number of trials to criterion, trial latencies or numbers of rats that failed to learn.

No biologically important, dose-dependent differences occurred in the watermaze performance of the F1 generation male or female rats regarding learning, short-term retention, long-term retention or response inhibition. No statistically significant differences occurred in the F1 generation male or female rats in the number of trials to criterion, the number of errors per trial, trial latencies or numbers of rats that failed to learn.

#### **Motor activity F1-generation (subset 3)**

No treatment-related effects were observed in F1-males and females on DPs 14 (males only), 18, 22 and 60.

As expected, the motor activity results differed by age. The differences over time (five-minute blocks) that occurred (within-session habituation) reflected the normal accommodation of these rats to a novel environment. All other statistically significant increases or reductions in the number of movements or the time spent in movement were considered incidental and unrelated to treatment, because they were not dose-related and/or the changes did not persist across the four testing sessions.

#### **Auditory startled response F1-generation (subset 3)**

No treatment-related effects were observed in F1-males and females on DPs 23 and 61. Some statistically-significant increases in response magnitude were not considered treatment-related because each was an isolated event, occurred in only one sex, was not dose-dependent, and the values were within the historical ranges at the Testing Facility.

#### **Serum concentrations for T3 and T4 in F1-generation**

The serum concentrations of T3 and T4 in DP 12 and 22 pups were not affected by maternal administration of the test substance in the diet at doses up to 500 ppm. The values for T3 and T4 were increased in all groups on DP 22 from the DP 12 values (a normal change with age).

#### **Necropsy observations (subset 1, 2, 3, 4, 5)**

Necropsy observations in F1 generation male and female rats were considered unrelated to the treatment with the test substance.

#### **Terminal body weights and organ weights (subset 1, 4, 5)**

On DP 12 terminal body weights of F1-males of the 500 ppm maternal dose group were significantly reduced. Absolute liver weights were significantly reduced at 100 and 500 ppm. Relative brain weights were significantly increased in F1-males and F1-females. However, this relative increase was considered to reflect the reduced terminal body weight in this dose group.

There were no effects observed on relative thyroid-to-brain and relative liver-to-brain weight in any of the dose groups.

The results for F1-generation males and females sacrificed on day 12 postpartum are summarised in the following table.

**Table 5.7.1/03-11: Summary of terminal body and organ weights in F1-generation**

Dose (ppm)	Terminal body weight (g)	F1-males on DP 12					
		Absolute organ weight (g)			Relative organ weights (%) <sup>1)</sup>		
		Brain	Liver	Thyroid	Brain	Liver	Thyroid
0	20.4	1.133	0.74	0.005	5.618	3.635	24.637
20	18.9	1.124	0.68	0.005	6.017	3.658	24.593
100	18.8	1.093	0.63*	0.004	5.867	3.403	24.232
500	17.0**	1.054	0.58**	0.004	6.439*	3.516	23.386
Dose (ppm)	Terminal body weight (g)	F1-females on DP 12					
		Absolute organ weight (g)			Relative organ weights (%) <sup>1)</sup>		
		Brain	Liver	Thyroid	Brain	Liver	Thyroid
0	19.3	1.10	0.71	0.003	5.798	3.718	17.984
20	18.2	1.07	0.63	0.004	5.996	3.552	19.460
100	18.0	1.06	0.66	0.003	5.919	3.684	18.888
500	16.9#	1.05	0.61	0.003	6.389**	3.650	17.319

DP = day postpartum

<sup>1)</sup> Ratio (%) = (organ weight/terminal body weight) x 100

\* statistically significant at p&lt;0.05; \*\* statistically significant at p&lt;0.01

# Not statistical significant different, but considered to be treatment-related reduced

The terminal body weights, brain, liver and thyroid weights and ratios (%) of the liver and thyroid weight to the terminal body weight for the F1 generation male and female rats (subset 5) sacrificed on DP 22 were comparable among groups and did not significantly differ.

On DP 83 terminal body weights of F1-males (subset 4) in the 500 ppm dose group were reduced when compared to controls (90.6% of control, not statistically significant). Absolute and relative brain weights were not affected in F1-males and F1-female in any dose group.

#### **Histopathology - postpartum day 12 and 22 (subsets 1, 5)**

No test substance-related microscopic changes were observed in the liver or thyroid/parathyroid of F1-males or F1-females in any dose group. Also, microscopic examination of gross lesions from the F0 dams and F1 pups of the various groups revealed no changes considered to be related to the administration of or possible exposure to the test substance.

#### **Neurohistological evaluations - postpartum day 12 (subset 1)**

No treatment-related microscopic lesions were present within any of the brain sections. While some of the brain morphometric parameters are slightly lower for the postpartum day 12 male rats in the maternal high dose group than for the comparable male controls, these differences were not statistically significant. Furthermore, these differences are considered to be the result of slightly lower brain weights for the maternal high dose male group. These lower brain weights are, in turn, related to lower body weights in this group. These lower body weights are most likely the result of nutritional factors related to toxicity of the test substance to the dams. No such differences were noted for the postpartum day 12 female rats.

There is no evidence, therefore, that the test substance was neurotoxic under the conditions of this study.



**Neurohistological evaluations - postpartum day 83 (subset 5)**

Slightly decreased mean values (in comparison to control group values) for transverse and diagonal measures of the caudate-putamen (striatum) were present for the female rats in the maternal intermediate and high dose groups. However, there was no evidence of a dose enhancement, and no such differences were noted for the male rats. The intergroup differences in striatal measures for the female rats were, therefore, considered to be spurious.

No treatment-related histopathologic alterations were present, indicating lack of any evidence that the test substance produced neurotoxic effects in rats under the conditions of this study.

**Table 6.7.1/03-8: Summary of brain weights and morphometry data in F1-generation**

Postpartum day	Brain weight and morphometry data (mean values)								
	Males				Females				
	DP 12		DP 83		DP 12		DP 83		
Dose (ppm)	0	500	0	500	0	500	0	100	500
Brain weight (g)	1.148	1.087	2.127	2.050	1.1583	1.118	1.933	1.898	1.923
Anterior/Posterior Cerebrum (mm)	10.5	9.83	14.08	14.25	10.83	10.67	13.83	14.33	13.83
Anterior/Posterior Cerebellum (mm)	6.00	5.67	7.00	7.08	6.00	6.33	6.83	6.83	6.83
Frontal Cortex (μ)	1604	1508	1776	1764	1540	1604	1640	--	1548
Parietal Cortex (μ)	1620	1508	1868	1852	1592	1608	1704	--	1652
Caudate Putamen (μ)	2792	2592	3352	3288	2752	2664	3204 d	2868 d**	2900 d*
Caudate Putamen (μ)							2832 t	2688 t*	2590 t**
Corpus Callosum (μ)	291.2	248.0	284.8	249.7	248	281.7	252.5		248.0
Dentate Gyrus (μ)	1124	1068	1616	1556	1080	1068	1468		1432
Cerebellum (μ)	3328	3232	4600	4648	3609.6	3544	4512		4360
External Germinal Layer (μ)	37.8	37.8			41.5	42.3			

\* statistically significant at  $p < 0.05$ ; \*\* statistically significant at  $p < 0.01$

d = diagonal, t = transverse

**Table 6.7.1/03-13 Historical control data for caudate putamen (μm)**

Historical control data for caudate putamen (μm)					
		N	Min	Max	Mean
F1 generation males	DP 12	40	1938 - 2688	2544 - 2736	2311.0 - 2548.8
F1-generation males	Adults	40	2544 - 3360	3312 - 4032	2908.0 - 3624.0
F1 generation females	DP 12	40	2160	2688	2384.0 - 2529
F1-generation females	Adults	40	3072	3552	2770.0 - 3379.2

N = number examined, SD = standard deviation

d = diagonal, t = transverse

The following table summarizes the substance-related effects observed in F0-dams and F1-pups (F1-offspring).

**Table 6.7.1/03-14: Summary of test-substance-related effects**

<b>Dose (ppm)</b>	<b>F0 (dams)</b>	<b>F1 (offspring)</b>
500	decreased body weight (gestation) decreased body weight gains (gestation) decreased feed consumption (gestation)	decreased body weight decreased body weight/gains developmental delay (eye opening, preputial separation) decreased terminal body weight (DP 12 and adult males) decreased absolute feed consumption increased relative feed consumption
100	decreased body weight (gestation) decreased feed consumption (gestation)	decreased body weight/gains developmental delay (eye opening, preputial separation) decreased absolute feed consumption increased relative feed consumption
20	NOEL (no-observed-effect-level)	NOEL (no-observed-effect-level)

<b>Conclusion</b>	<p><b>The study is in line with current guidelines.</b></p> <p>The 500 ppm dose level was considered to be excessively toxic for F1 offspring. Compound-related effects were also evident in the offspring at the 100 ppm dietary level.</p> <p>Flufenacet did not cause any specific neurobehavioral effects in the offspring (developmental neurotoxicity) when administered to the dams during gestation and lactation at dietary concentrations up to 500 ppm.</p> <p>Thus, the <b>NOAEL for dams and offspring is 20 ppm corresponding to 1.7 mg/kg bw/day</b> based on effects of the body weight, feed consumption and a slight developmental delay at 100 ppm.</p>
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### **B.6.7.2 Delayed polyneuropathy studies**

<b>RMS Conclusion</b>	Flufenacet does not belong to a chemical class which is suspected to cause delayed neurotoxic effects (organophosphates, carbamates). Therefore, specific studies on delayed neurotoxicity are not necessary.
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## **B.6.8 Other toxicological studies**

### **B.6.8.1 Toxicity studies of metabolites**

#### **Summary of studies with metabolites**

During the previous EU review, the toxicological properties of several plant and/or soil metabolites (FOE-oxalate (M01), FOE-sulfonic acid (M02), FOE-thioglycolate sulfoxide (M04), and thiadone (M09)) were investigated in acute oral toxicity to rats and/or mutagenicity and/or their bioavailability in rats.

The data base on metabolites has been supplemented as the parent compound flufenacet shows an extensive metabolic behavior in rats, livestock and in the majority of crops and also in order to fulfill SANCO/221/2000 - rev. 10, 25th February 2003 requirements. Some plant metabolites were not detected as systemic metabolites in the rat ADME studies. Depending on the occurrence and the quantity of the metabolites to be addressed, a suitable approach has been chosen in order to meet the regulatory requirements and suffice the most recent scientific developments as addressed in the EFSA Scientific Opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment (EFSA Journal 2012;10(7):2799).

The toxicological profile and exposure assessment includes flufenacet metabolites

- (1) exceeding the trigger of 0.01 mg/kg in raw agricultural commodities relevant for human consumption
- (2) exceeding the trigger of 0.05 mg/kg of raw animal fodder (e.g. straw).

It has to be noted that individual metabolites occur in food items/feeding items and are predicted to reach groundwater in some scenarios.

For the detailed toxicological assessment the metabolites are grouped as follows:

- Metabolites containing the fluorophenylacetamide moiety  
FOE-oxalate (M01), FOE-sulfonic acid (M02), FOE-thioglycolate sulfoxide (M04),  
FOE-cysteine (M23), FOE- sulfinyl lactic acid (M33), FOE-sulfinyl lactic acid glucoside (M37), FOE sulfanyl lactic acid glucoside (M41), FOE malonylcysteine conjugate (M42)
- Rat metabolites containing/originating from the thiadiazole moiety  
FOE-thiadone (M09), ThN-glycoside (M25), Th-malonylalanyl-conjugate (M34), FOE-trifluoroethanesulfonic acid Na-salt (M44), Trifluoroacetate (TFA) (M45)

The detailed toxicological assessment of these metabolites can be found in the document M-476535-01-1 (“Flufenacet - Toxicological profile and exposure assessment of the plant metabolites”).

Based on commonality assessments, structure similarity considerations, evaluation of genotoxicity and further toxicological studies as well as exposure calculations, it is concluded that all plant metabolites are considered to be toxicologically adequately investigated and uncritical for human health.

A summary of the toxicological studies on several metabolites is provided below:

### **FOE-oxalate (M01)**

For a better understanding of the nature of some flufenacet metabolites, an investigation of the bioavailability of selected plant metabolites in rats was undertaken. The metabolite chosen to represent metabolites arising from the fluorophenylacetamide moiety was FOE-oxalate. Thiadiazole-*N*-glucoside was chosen to represent the thiadiazole metabolites. In this study unchanged FOE-oxalate was excreted with faeces (70%) and urine (28%), i.e. FOE-oxalate was not further metabolised. The study was already submitted for the first evaluation of flufenacet, please refer to the Monograph/baseline dossier KCA 6.2.1, additionally summarized in Monograph 5.1.2, M-002278-01-1).

The genotoxicity potential of FOE-oxalate (M01) was investigated in a battery of *in vitro* tests which were all negative with and without metabolic activation (+/- S9 mix). Therefore, FOE-oxalate (M01) is considered to be non-mutagenic and non-genotoxic.

**Table 6.8.1- 1: Summary of genotoxicity studies with FOE-oxalate (M01)\***

Study	Dose	Result	Reference
<b>Bacterial reverse mutation assay (<i>S. typhimurium</i>, TA1535, TA1537, TA98, TA100, TA102)</b>	<b>16 - 5000 µg/plate (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Herbold, 2009 M-358953-01-1</b>
<b>Mammalian cell gene mutation test (Chinese hamster V79 cells)</b>	<b>150 - 2400 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Wollny, 2010 M-361724-01-1</b>
<b>Mammalian chromosome aberration test (Chinese Hamster Ovary (CHO) cells)</b>	<b>600 - 2400 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Nern, 2009 M-358043-01-1</b>
Bioavailability study in rats [Fluorophenyl-UL- <sup>14</sup> C] FOE5043-oxalate	1 mg/kg bw	Excretion of unchanged FOE-oxalate 70% faeces, 28% urine	██████████, 1995 M-002278-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

### **FOE-sulfonic acid (M02)**

During the first EU review of flufenacet the bacterial reverse mutation assay, an acute oral toxicity study and a study investigating the bioavailability of the metabolite were submitted and evaluated. Based on the study results (non-mutagenic, acutely non-toxic, low oral absorption <10% and a high body clearance, high polarity) FOE-sulfonic acid (M02) was considered not relevant, please refer to the Evaluation table of flufenacet (7468/VI/98-rev. 10(27.12.2001)).

The genotoxicity potential of FOE-sulfonic acid (M02) has been further investigated in a battery of *in vitro* and *in vivo* tests. In these tests the metabolite has been tested as Na-salt as under physiological conditions FOE-sulfonic acid occurs mainly as an anionic molecule. Under environmental aqueous conditions the acid is promptly dissociated to the sulfonate and testing of the toxicological potential of the salt moiety which is representative for the real condition in water was considered to be more appropriate. Therefore, most of the toxicity studies with the metabolite have been conducted using the salt of the FOE-sulfonic acid, e.g. FOE-sulfonic acid Na-salt.

FOE-sulfonic acid (M02) resulted negative in the genotoxicity tests in bacteria and mammalian cells *in vitro* (bacterial reverse mutation, mammalian cell gene mutation). The *in vitro* chromosome aberration test resulted negative in the presence of metabolic activation, but showed a positive response in the absence of metabolic activation at cytotoxic concentrations. Due to the positive response in the *in vitro* chromosome aberration test, two *in vivo* genotoxicity tests were conducted. The micronucleus test and the unscheduled DNA synthesis (UDS) assay both showed clear negative results. These results confirm that the aberrations observed under extreme *in vitro* conditions are not reflecting chemical-specific genotoxicity. Overall, it can be concluded that FOE-sulfonic acid (M02) is considered to be non-genotoxic.

**Table 6.8.1- 2: Summary of studies with FOE-sulfonic acid (M02)\***

Study	Dose	Result	Reference
Bacterial reverse mutation assay ( <i>S. typhimurium</i> TA1535, TA100, TA1537, TA98, TA102)	16-5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Herbold, 2000 M-019064-01-1
<b>Mammalian cell gene mutation test (Chinese hamster V79 cells)</b>	<b>202-3230 µg/mL (+ S9 mix)</b> <b>101-808 µg/mL (- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Wollny, 2009 M-361158-01-1</b>
<b>Mammalian chromosome aberration test (Chinese hamster V79 cells)</b>	<b>250-3000 µg/mL (+ S9 mix)</b> <b>200-1000 µg/mL (- S9 mix)</b>	<b>Negative (+ S9 mix)</b> <b>Positive (- S9 mix)</b>	<b>Nern, 2010 M-366380-01-1</b>
<b><i>In vivo</i> Micronucleus test (Mouse bone marrow)</b>	<b>500-2000 mg/kg bw (2x intraperitoneal)</b>	<b>Negative</b>	<b>██████, 2010 M-368627-01-1</b>
<b><i>In vivo</i> Unscheduled DNA synthesis (UDS) assay (rat primary hepatocytes)</b>	<b>1000-2000 mg/kg bw (oral)</b>	<b>Negative</b>	<b>██████, 2010 M-397810-01-1</b>
Rat Acute oral (fasted)	500-2000 mg/kg bw/day	LD <sub>50</sub> > 2000 mg/kg bw	██████, 1998 M-004749-01-1
Rat Plasma kinetics and excretion	1 x 100 mg/kg bw (intravenous) 1 x 1000 mg/kg bw (oral)	Low oral absorption (<10%) rapid renal clearance (i.v: t <sub>1/2</sub> ≈ 30 min)	██████ 2000 M-042251-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

### FOE-thioglycolate sulfoxide (M04)

The metabolite FOE-thioglycolate sulfoxide (M04) was tested for its mutagenic potential in the bacterial reverse mutation test. There was no indication of a mutagenic effect with and without metabolic activation.

**Table 6.8.1- 3: Summary of studies with FOE-thioglycolate sulfoxide (M04)**

Study	Dose	Result	Reference
Bacterial reverse mutation assay ( <i>S. typhimurium</i> TA1535, TA100, TA1537, TA98, TA102)	16 - 5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Herbold, 2000 M-032500-01-1

### FOE-methylsulfone (M07)

The genotoxicity potential of FOE-methylsulfone (M07) has been investigated in a battery of *in vitro* tests. FOE-methylsulfone (M07) did not induce mutations in bacteria and mammalian cell, both with and without metabolic activation. There was also no evidence of a clastogenic potential in mammalian

cells *in vitro* without and with metabolic activation. Thus, FOE-methylsulfone (M07) is considered to be non-mutagenic and non-genotoxic.

**Table 6.8.1- 4: Summary of studies with FOE-methylsulfone (M07)\***

Study	Dose	Result	Reference
<b>Bacterial reverse mutation assay (<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100, TA102)</b>	<b>3 - 5000 µg/plate (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Sokolowski, 2012 M-422370-01-1</b>
<b>Mammalian cell gene mutation test (Chinese hamster V79 cells)</b>	<b>43.3 - 2800 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Wollny, 2012 M-430571-01-1</b>
<b>Mammalian chromosome aberration test (Chinese Hamster V79 cells)</b>	<b>170.6 - 2730.0 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Bohnenberger, 2012 M-437250-01-1</b>

\* New studies, i.e. studies that were not previously submitted, are written in bold

### **FOE-thiadone (M09)**

The acute oral toxicity test revealed that FOE-thiadone (M09) is more toxic than the parent compound flufenacet, with LD<sub>50</sub> values of < 1650 and <600 mg/kg bw for males and females, respectively. In 2011 for registration of flufenacet in Japan, a bacterial reverse mutation assay was conducted on the metabolite FOE-thiadone (M09) itself. In this study no evidence for point mutations in the bacterial reverse mutation test occurred. Thus, FOE-thiadone (M09) is considered to be non-mutagenic.

**Table 6.8.1- 5: Summary of studies with FOE-thiadone (M09)\***

Study	Dose	Result	Reference
<b>Bacterial reverse mutation assay (<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100, TA102)</b>	<b>3 - 5000 µg/plate (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Sokolowski, 2011 M-413989-01-1</b>
Rat acute oral	1650 mg/kg bw (males) 600 mg/kg bw (females)	LD <sub>50</sub> <1650 /<600 mg/kg bw (males/females):	██████████, 1993 M-004951-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

### **FOE 5043-trifluoroethanesulfonic acid Na-salt (M44)**

The genotoxicity potential of FOE 5043-trifluoroethanesulfonic acid Na-salt (M44) has been investigated in a battery of *in vitro* tests. The metabolite did not induce mutations in bacteria and mammalian cell, both with and without metabolic activation. There was also no evidence of a clastogenic potential in mammalian cells *in vitro* without and with metabolic activation. Thus, FOE 5043-trifluoroethanesulfonic acid Na-salt (M44) is considered to be non-genotoxic.

**Table 6.8.1- 6: Summary of studies with FOE 5043-trifluoroethanesulfonic acid Na-salt (M44)\***

Study	Dose	Result	Reference
<b>Bacterial reverse mutation assay (<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100, TA102)</b>	<b>3-5000 µg/plate (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Sokolowski, 2012 M-434728-01-1</b>
<b>Mammalian cell gene mutation test (Chinese hamster V79 cells)</b>	<b>125-2000 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Wollny, 2013 M-446033-01-1</b>
<b>Mammalian chromosome aberration test (Chinese Hamster V79 cells)</b>	<b>465-1860 µg/mL (+/- S9 mix)</b>	<b>Negative (+/- S9 mix)</b>	<b>Bohnenberger, 2013 M-447404-01-1</b>

\* New studies, i.e. studies that were not previously submitted, are written in bold

### **Trifluoroacetate (TFA) (M45)**

TFA is a plant and a soil metabolite of several plant protection products and a metabolite of other chemicals. TFA is a ubiquitous molecule with multiple sources. It has been found in surface water, groundwater and rain. TFA is also a metabolite of the inhalation anaesthetic halothane (used in animals and humans); a broad toxicology data base exists for halothane which did not reveal any adverse effect related to its metabolite TFA.

The toxicological properties of TFA were further assessed in an *in vitro* genotoxicity battery, acute and repeated dose oral toxicity studies, and in a developmental toxicity study.

Most of the toxicity studies with TFA have been conducted using the salt of trifluoroacetic acid, e.g. sodium trifluoroacetate. The reason for this is that under environmental aqueous conditions the acid is promptly dissociated to trifluoroacetate and therefore, it is considered to be appropriate to assess the toxicological potential of the salt moiety which is representative for the real condition in water.

Three *in vitro* genotoxicity studies have been conducted with trifluoroacetate sodium. These studies showed no evidence for mutagenicity in the reverse mutation assay in bacteria as well as in the mammalian cell gene mutation test. The mammalian chromosome aberration assay in human lymphocytes revealed no evidence for a clastogenic potential. Thus, TFA is considered to be non-mutagenic and non-genotoxic.

Furthermore, TFA is of low acute toxicity with a LD<sub>50</sub> above 2000 mg/kg bw without any evidence of acute effects based on clinical signs and necropsy findings. After repeated administration the liver was the target organ, with effects that were adaptive and reversible. Moreover, the 14-day mechanistic study showed that liver effects are related to peroxisome proliferation, a mode of action not relevant for humans. Furthermore, the developmental toxicity study in rats showed neither maternal nor developmental effects which are considered to be adverse up to the highest dose tested.

**Table 6.8.1- 7: Summary of acute and genotoxicity studies with trifluoroacetate (TFA) (M45)\***

Study	Dose	Result	Reference
Bacterial reverse mutation assay ( <i>S. typhimurium</i> TA1535, TA1537, TA98, TA100, TA102)	16-5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Johnson, 2005 M-256628-01-1
Mammalian cell gene mutation test (mouse lymphoma L5178Y cells)	360-1360 µg/mL (+/- S9 mix)	Negative (+/- S9 mix)	Ballantyne, 2005 M-260699-01-1
Mammalian chromosome aberration test (human lymphocytes)	85-1360 µg/mL (+/- S9 mix)	Negative (+/- S9 mix)	Clare, 2005 M-260807-01-1
Rat acute oral (fasted)	2000 mg/kg bw	LD <sub>50</sub> : >2000 mg/kg bw	<b>██████████</b> , 2013 M-444479-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

**Table 6.8.1- 8: Summary of repeated toxicity studies with trifluoroacetate (TFA) (M45)\***

Study	Sex	NO(A)EL mg/kg bw/day	LO(A)EL	Main findings seen at LO(A)EL	Reference
Rat 14-day feeding	M F	43 45	85 >190	Liver findings (increased organ weight in correlation with hepatocellular hypertrophy, increased cytochrome P-450, lauric acid hydroxylation activity, specific and total palmitoyl-CoA oxidation activities).	<b>██████████</b> , 2001 M-202165-01-1
Rat 28-day feeding	M F	1315 1344	-- --	No adverse effects observed. (liver weight changes without histopathological correlates)	<b>██████████</b> , 2005 M-259106-01-1
Rat 90-day feeding	M F	10 12	98 123	Changes in haematological and clinical chemistry parameters, organ weights and histopathological liver findings	<b>██████████</b> , 2007 M-283994-01-1
Rat developmental toxicity gavage	D Fet	150 150	-- --	No adverse effects at 150 mg/kg bw/d	<b>██████████</b> 2010 M-411209-01-1

\* New studies, i.e. studies that were not previously submitted, are written in bold

The current toxicity database for assessing TFA effects after acute and subacute exposure comprise the critical appropriate and GLP studies and information (including reproductive, developmental and neurotoxic effects) supporting that an Acute Reference Dose (ARfD) is not triggered for this compound. The rationale for the waiver of an ARfD of TFA can be found in the document M-480037-01-1 (“Trifluoroacetate (TFA) – Waiver of an Acute Reference Dose (ARfD)”).

#### **Acceptable Daily Intake (ADI) derivation for TFA**

Due to the aforementioned uncritical toxicological profile and the fact that humans are exposed to TFA without known negative consequences (TFA is a ubiquitous product and a metabolite of the inhalation anaesthetic halothane) the ADI can be established based on the repeated-exposure toxicological data base. The lowest NOAEL of 10 mg/kg bw/day observed in the 90-day rat study is considered appropriate to derive the ADI. This NOAEL is corrected by a safety factor of 100 for intra- and inter-species variation and an additional safety factor of 2 (EFSA default value in EFSA Scientific Opinion “Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data“, EFSA Journal 2012;10(3):2579) to



extrapolate from subchronic to chronic study duration. This results in a proposed ADI of 0.05 mg/kg bw/day.

In their reasoned opinion on setting the MRL for saflufenacil (EFSA Journal 2014; 12(2):3585) EFSA experts agreed to the proposal made by Bayer CropScience to derive an ADI of 0.05 mg/kg bw/day for TFA on the basis of the NOAEL of the subchronic rat study and the application of an extra uncertainty factor (UF) of 2.

## FOE-oxalate (M01)

New studies; not evaluated	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /07; Herbold, B.; 2009</b>
<b>Title:</b>	FOE 5043-Oxalate (Project: FOE 5043 (Flufenacet/AE F133402)) - Salmonella/microsome test - Plate incorporation and preincubation method
<b>Document No:</b>	M-358953-01-1
<b>Report No:</b>	AT05640
<b>Guidelines:</b>	OECD 471; Council Regulation 440/2008/EEC, Method B.13/14.; US-EPA712-C-98-247, OPPTS 870.5100; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- Test material:** FOE 5043-oxalate  
 Description: white crystalline powder  
 Lot/Batch no: SES10564-3-1  
 Purity: 95.3%  
 Stability of test compound: guaranteed for study duration; expiry date: 2010-02-03
- Vehicle and/or positive control:** DMSO, deionised water (MMC) / Sodium azide (Na-azide), Nitrofurantoin (NF), 4-nitro-1,2-phenylene diamine (4-NPDA), mitomycin C (MMC), Cumene hydroperoxide (Cumene), 2-aminoanthracene (2-AA)
- Test system:** *Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98, TA102  
**metabolic activation:** S9 mix

### B. Study design and methods

- Dose:** 0-16-50-158-500-1581-5000 µg/plate  
 positive controls:  
 Na-azide: 10-20 µg/plate  
 NF: 0.2-0.4 µg/plate  
 4-NPDA: 0.5-1-10-20 µg/plate  
 MMC: 0.2-0.4 µg/plate  
 Cumene: 50-75 µg/plate  
 2-AA: 3-6 µg/plate
- Application volume:** 0.1 mL/plate  
**Incubation time:** 48 hours, 37 °C

## Results and discussion

Doses up to and including 5000 µg per plate FOE 5043-oxalate produced weak bacteriotoxic effects, starting at 158 µg per plate in the plate incorporation trial only.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls.

In spite of the low doses used, positive controls increased the mutant counts significantly compared with negative controls, and thus demonstrated the system's high sensitivity.

Despite this sensitivity, no indications of mutagenic effects of FOE 5043-oxalate could be found at assessable doses of up to 5000 µg per plate in any of the *Salmonella typhimurium* strains used.

**Table 5.8.1/07- 1: Summary of results**

Mean revertants per plate						
Substance Dose (µg/plate)	S9 mix	TA1535	TA100	Strain TA1537	TA98	TA102
Plate incorporation						
FOE 5043-oxalate 0	–	9	106	6	17	191
16	–	7	101	5	19	204
50	–	9	115	7	21	207
158	–	8	93	6	19	200
500	–	9	109	7	19	193
1581	–	7	108	6	21	202
5000	–	8	118	2	22	229
Na-azide 10	–	877				
20	–	1085				
NF 0.2	–		302			
0.4	–		541			
4-NPDA 10	–			32		
20	–			54		
0.5	–				63	
1	–				92	
MMC 0.2	–					705
0.4	–					896
FOE 5043-oxalate 0	+	9	177	10	30	254
16	+	10	170	8	29	248
50	+	10	150	8	35	279
158	+	9	176	9	31	289
500	+	9	167	9	30	247
1581	+	9	187	6	30	245
5000	+	8	150	8	30	236
2-AA 3	+	135	2270	283	1527	601
6	+	103	1598	80	1748	1112
Pre-incubation						
FOE 5043-oxalate 0	–	9	99	6	21	149
16	–	10	112	6	17	164
50	–	8	123	7	21	159
158	–	9	118	7	17	182
500	–	9	101	6	18	158
1581	–	8	112	5	19	176
5000	–	10	103	5	21	180
Na-azide 10	–	761				
20	–	884				
NF 0.2	–		428			
0.4	–		775			

4-NPDA	10	–			34		
	20	–			77		
	0.5	–				71	
	1	–				100	
Cumene	50	–					335
	75	–					335
FOE 5043-oxalate	0	+	10	141	11	30	244
	16	+	10	125	9	27	264
	50	+	8	135	10	31	247
	158	+	12	145	9	26	254
	500	+	9	120	9	31	238
	1581	+	10	144	8	28	221
	5000	+	10	123	8	31	224
2-AA	3	+	100	2254	275	1534	488
	6	+	62	2023	196	2180	801

<b>Conclusion</b>	FOE 5043-oxalate has to be regarded as non-mutagenic.
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New studies; not evaluated	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /08; Wollny, H. E.; 2002</b>
Title:	FOE 5043-Oxalate - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Document No:	<b>M-361724-01</b>
Report No:	1277301
Guidelines:	OECD 476; Commission Regulation (EC) No. 440/2008, B17; US-EPA 712-C-98-221, OPPTS870.5300; Deviations: none
GLP	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-oxalate
  - Description: white powder
  - Lot/Batch no: SES 10564-3-1
  - Purity: 95.3%
  - Stability of test compound: guaranteed for study duration; expiry date: 2009-09-24
- 2. Vehicle and/or positive control:**
  - Vehicle: DMSO
  - Positive controls: ethylmethane sulfonate (EMS), 7,12-dimethylbenz(a)anthracene (DMBA)
- 3. Test system:**
  - metabolic activation: S9 Mix

### B. Study design and methods

#### 1. Treatment

- Dose: 0-300-600-1200-1800-2400 µg/mL
- Positive controls:
  - EMS: 0.15 mg/mL
  - DMBA: 1.1 µg/mL
- Treatment duration: 5 hours

## Results and discussion

No precipitation of the test item was observed up to the maximal concentration in all experimental parts.

No relevant cytotoxic effects occurred up to the maximal concentration of 2400 µg/mL.

No relevant and reproducible increase in mutant colony numbers/10<sup>6</sup> cells was observed in the main experiments up to the maximal concentration. The mutation frequency generally remained within the historical range of solvent controls; the induction factor did not reach or exceed the threshold of 3.0.

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 groups. A significant trend

detected in the first culture of the first experiment with metabolic activation was judged as irrelevant since it actually was reciprocal, going down versus increasing concentrations.

In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 13.2 up to 34.6 mutant colonies per  $10^6$  cells; the range of the groups treated with the test item was from 5.7 up to 26.5 mutant colonies per  $10^6$  cells.

The highest solvent controls (32.2 and 34.6 colonies per  $10^6$  cells) of the first experiment with metabolic activation slightly exceeded the historical range of solvent controls (0.8 – 31.3 colonies per  $10^6$  cells). However, this effect was judged as irrelevant since it is very minor and the solvent controls of the second experiment with metabolic activation remained well within the range of historical controls. The solvent control of the second culture of the first experiment slightly exceeded the historical range but the solvent control of the parallel culture was completely acceptable.

EMS (0.15 mg/mL) and DMBA (1.1  $\mu$ g/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table 6.8.1/08- 1: Summary of results

	concentration µg/mL	S9	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
<b>Experiment I / 5 h treatment</b>			<b>culture I</b>				<b>culture II</b>			
Solvent control DMSO		–	100.0	100.0	14.7	1.0	100.0	100.0	34.5	1.0
Positive control EMS	150.0	–	109.8	60.3	96.7	6.6	85.9	74.9	187.7	5.4
FOE 5043-oxalate	150.0	–	87.5	culture was not continued#			96.6	culture was not continued#		
	300.0	–	106.1	98.4	6.9	0.5	94.8	83.1	15.0	0.4
	600.0	–	123.5	99.6	11.3	0.8	97.2	77.8	12.4	0.4
	1200.0	–	108.8	79.0	7.8	0.5	106.0	77.4	16.8	0.5
	1800.0	–	63.3	78.0	16.1	1.1	91.5	71.3	23.8	0.7
	2400.0	–	10.6	59.2	24.2	1.6	82.5	75.6	18.4	0.5
Solvent control DMSO		+	100.0	100.0	32.2	1.0	100.0	100.0	34.6	1.0
Positive control DMBA	1.1	+	63.2	71.1	1103.2	34.2	47.5	74.0	1265.3	36.6
FOE 5043-oxalate	150.0	+	94.9	culture was not continued#			91.6	culture was not continued#		
	300.0	+	106.1	83.7	21.1	0.7	86.1	105.5	21.7	0.6
	600.0	+	102.0	86.2	20.6	0.6	87.0	107.1	20.5	0.6
	1200.0	+	96.5	126.1	19.4	0.6	84.1	103.0	13.3	0.4
	1800.0	+	97.8	107.2	16.4	0.5	82.9	102.5	17.5	0.5
	2400.0	+	99.7	110.3	14.8	0.5	74.8	74.1	14.8	0.4

	concentration µg/mL	S9	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
<b>Experiment II / 5 h treatment</b>			<b>culture I</b>				<b>culture II</b>			
Solvent control DMSO		–	100.0	100.0	17.6	1.0	100.0	100.0	18.5	1.0
Positive control EMS	150.0	–	70.9	82.4	108.5	6.2	74.2	96.3	96.9	5.2
FOE 5043-Oxalate	150.0	–	82.3	culture was not continued#			98.9	culture was not continued#		
	300.0	–	77.9	90.5	21.1	1.2	93.8	86.0	8.1	0.4
	600.0	–	88.1	78.2	18.7	1.1	99.0	87.9	8.3	0.4
	1200.0	–	76.9	71.6	5.7	0.3	99.0	85.2	12.3	0.7
	1800.0	–	35.7	77.4	26.5	1.5	39.0	97.4	16.4	0.9
	2400.0	–	3.1	81.1	22.5	1.3	12.0	86.8	11.4	0.6
Solvent control DMSO		+	100.0	100.0	13.2	1.0	100.0	100.0	1511	1.0
Positive control DMBA	1.1	+	28.1	54.0	1104.7	83.9	52.3	70.8	617.5	41.0
FOE 5043-Oxalate	150.0	+	68.0	culture was not continued#			89.9	culture was not continued#		
	300.0	+	68.8	78.4	15.3	1.2	87.8	89.6	12.1	0.8
	600.0	+	75.3	83.1	17.6	1.3	89.7	98.7	6.3	0.4
	1200.0	+	85.1	70.0	11.9	0.9	86.7	64.2	24.0	1.6
	1800.0	+	83.0	95.0	15.5	1.2	89.1	67.9	10.9	0.7
	2400.0	+	75.0	92.9	7.5	0.6	92.2	72.2	15.5	1.0

Conc. = concentration

# Culture was discontinued since a minimum of only four analysable concentrations is required

<b>Conclusion</b>	It can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-oxalate is considered to be non-mutagenic in this HPRT assay.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /09; Nern, M.; 2009</b>
<b>Title:</b>	FOE 5043-oxalate (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
<b>Document No:</b>	M-358043-01
<b>Report No:</b>	AT05598
<b>Guidelines:</b>	Directive 2000/32/EC, Method B.10; OECD 473; US-EPA 712-C-98-223, OPPTS 870.5375; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-oxalate  
 Description: fine white powder  
 Lot/Batch no: SES 10564-3-1  
 Purity: 95.3 %  
 Stability of test compound: guaranteed for study duration; expiry date: 2009-09-24
- 2. Vehicle and/or positive control:** DMSO, Hanks' balanced salt solution (positive control) / mitomycin C, cyclophosphamide
- 3. Test system:** Chinese hamster V79 cells  
 metabolic activation: S9 mix

### B. Study design and methods

- Dose: 0-150-300-600-1200-2400 mg/mL (+/- S9 mix)  
 positive controls:  
 mitomycin C: 0.1 µg/mL (4 h treatment), 0.03 µg/mL (18 h treatment),  
 cyclophosphamide, 2.0 µg/mL
- Treatment duration: With S9 mix: 4 hours  
 Without S9 mix: 4 and 18 hours
- Harvest: 18 and 30 hours

## Results and discussion

Chinese hamster V79 cells were treated with FOE 5043-oxalate concentrations of 600, 1200 and 2400 µg/mL for 4 hours without and with S9 mix for assessment of the clastogenic potential of FOE 5043-oxalate. In addition, after 18 hours treatment with FOE 5043-oxalate concentrations of 600, 1200 and 2400 µg/mL were read without S9 mix.

None of these cultures treated with FOE 5043-oxalate in the absence or presence of S9 mix showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations.

The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system and in the case of cyclophosphamide the activity of the used S9 mix.

**Table 6.8.1/09-1: Summary of cells with structural aberrations**

Substance Dose (µg/mL)	+/- S9	Cells scored	Metaphases with aberrations (%)		Mitotic Index (%)
			Including gaps	Excluding gaps	
Experiment 1 (4 hour treatment + 18 hour harvest, +/- S9)					
Solvent (DMSO)	–	200	3.5	3.5	100.0
FOE 5043-oxalate    600	–	200	3.0	2.5	97.9
1200	–	200	3.5	3.5	105.7
2400	–	200	3.0	3.0	94.3
Mitomycin C        0.1	–	168	38.5	37.0 <sup>a</sup>	134.2
Solvent (DMSO)	+	200	5.0	4.0	100.0
FOE 5043-oxalate    600	+	200	4.0	3.5	106.3
1200	+	200	3.0	2.5	127.7
2400	+	200	4.0	3.5	110.7
Cyclophosphamide    2	+	186	75.5	75.5 <sup>a</sup>	39.9 <sup>a</sup>
Experiment 2 (4 hour treatment + 30 hour recovery, +/- S9)					
Solvent (DMSO)	–	200	1.5	1.5	100.0
FOE 5043-oxalate    2400	–	200	2.5	2.5	99.4
Solvent (DMSO)	+	200	3.5	3.0	100.0
FOE 5043-oxalate    2400	+	200	4.5	4.0	106.4

<sup>a</sup> statistical significant at  $p \leq 0.01$

**Table 6.8.1/09-2: Additionally observed polyploid metaphases**

Substance Concentration [µg/mL]		Harvest time [h]	Polyploid Metaphases	
			without metabolic activation	with metabolic activation
4 hours Treatment				
Solvent (DMSO)	0	18	12 7	13 10
FOA 5043-oxalate	600	18	9 7	13 12
FOA 5043-oxalate	1200	18	11 13	7 14
FOA 5043-oxalate	2400	18	12 12	8 15
Mitomycin C	0.1	18	7 5	--
Cyclophosphamide	2.0	18	--	9 10
Solvent (DMSO)	0	30	2 7	7 7
FOA 5043-oxalate	2400	30	8 15	11 5
Substance Concentration [µg/mL]		Harvest time [h]	Polyploid Metaphases without metabolic activation	
18 hours Treatment				
Solvent (DMSO)	0	18	3 8	
FOA 5043-oxalate	600	18	8 9	
FOA 5043-oxalate	1200	18	6 7	
FOA 5043-oxalate	2400	18	5 5	
Mitomycin C	0.03	18	7 8	

<b>Conclusion</b>	<b>FOE 5043-oxalate is considered not to be clastogenic for mammalian cells <i>in vitro</i>.</b>
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**FOE-sulfonic acid (M02)**

The bacterial reverse mutation assay *in vitro*, the acute oral toxicity study and the plasma kinetics and excretion study in rats were already presented and evaluated during the EU process for Annex I listing. Please refer to the Evaluation table of flufenacet (7468/VI/98-rev. 10(27.12.2001) and the baseline dossier of flufenacet.

<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /01; ██████████ 1998</b>
<b>Title:</b>	FOE 5043 Sulfonsäure (plant metabolite of FOE 5043) - Study for acute oral toxicity in rats
<b>Document No:</b>	<b>M-004749-01-1</b>
<b>Report No:</b>	27317
<b>Guidelines:</b>	<b>OECD 401; Directive 67/548/EEC, Annex V, Part B.1.; US-EPA Series 81-1; Deviations: none</b>
<b>GLP</b>	Yes

**I. Materials and methods****A. Materials****1. Test material:**

	FOE 5043 Sulfonsäure
Synonym(s):	FOE 5043-sulfonic-acid, M02
Specification no.:	n.a.
Description:	white powder
Lot/Batch no:	WAK 6622-3
Purity:	99.4 %
Stability of test compound:	Unknown

**2. Vehicle:**

formulated in aqueous Cremophor EL, 2% v/v

**3. Test animals**

Species:	rat
Strain:	HsdCpb:WU
Sex:	male and female
Age:	6-8 weeks
Weight at dosing:	Males: 162-188 g; females: 166-171 g
Source:	██
Acclimatisation period:	At least 6 days
Diet:	Altromin® 1324 Diet for Rats and Mice, <i>ad libitum</i> (2 hours after administration)
Water:	Tap water, <i>ad libitum</i>

Housing: During the test period the animals were kept conventionally in polycarbonate cages type III (five animals per cage) on the first study day and in type IIA cages (one animal per cage) afterwards. The bedding consisted of low-dust wood granules type S 8/15 (supplier: [REDACTED]).

## B. Study design and methods

### 1. Animal assignment and treatment

Dose: Males/females: 500 and 2000 mg/kg bw  
 Application route: Oral (gavage)  
 Application volume: 10 mL/kg bw  
 Fasting time: Overnight fasting (approx. 17 hours)  
 Group size: 5/sex/dose  
 Post-treatment observation period: 15 days  
 Observations: Mortality, clinical signs, body weight, gross necropsy

## II. Results and discussion

### A. Mortality and Clinical Observations

Doses of 500 and 2,000 mg/kg body weight were tolerated by male and female rats without mortalities and 500 mg/kg also without clinical signs. At 2000 mg/kg in both sexes diarrhea occurred and anuses were moistened. The signs observed started 4 hours and lasted up to 5 hours after administration.

**Table 6.8.1/09-3 Doses, mortality, clinical signs / animals treated**

Dose (mg/kg bw)	Toxicological result*			Occurrence of clinical signs	Time of death	Mortality (%)
Male rats						
500	0	0	5	--	--	0
2000	0	5	5	4h – 5h	--	0
LD <sub>50</sub> > 2000 mg/kg bw						
Female rats						
500	0	0	4	--	--	0
2000	0	4	5	4h – 5h	--	0
LD <sub>50</sub> > 2000 mg/kg bw						

\* 1<sup>st</sup> number = number of dead animals,  
 2<sup>nd</sup> number = number of animals with toxic signs,  
 3<sup>rd</sup> number = number of animals used

### B. Body Weight

Body weight and body weight gain of male and female rats were not test substance related affected.

### C. Necropsy

The gross pathology investigations performed at the end of the post-treatment observation period did not afford any treatment-related findings.

## III. Conclusion

FOE 5043-sulfonic acid is non-toxic after acute oral administration. The acute oral LD<sub>50</sub> was > 2000 mg/kg bw both for male and female rats.

<b>Report:</b>	<b>KCA 5.8.1 /02; Herbold, B.A., 2000</b>
<b>Title:</b>	FOE 5043-sulfonic-acid - Salmonella/microsome test - Plate incorporation and preincubation method
<b>Document No:</b>	<b>M-019064-01-1</b>
<b>Report No:</b>	29473
<b>Guidelines:</b>	<b>Directive 92/69/EEC, B.14.; OECD 471 (1997); US-EPA 712-C-98-247, OPPTS 870.5100 (1998), Deviations: none</b>
<b>GLP</b>	Yes

## I. Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-sulfonic-acid
Synonym:	M02
Lot/Batch no:	KTS 9465-1-2
Purity:	94.1%
Stability of test compound:	A stability test in the solvent did not reveal significant degradation of the active ingredient.

#### 2. Control Materials:

Vehicle/negative control:	Test substance: deionized water Positive controls: DMSO
Positive controls:	<u>Non-activation:</u> Sodium azide (Na-azide): 10 µg/plate TA 1535 Nitrofurantoin (NF): 0.2 µg/plate TA 100 4-nitro-1,2-phenylene diamine (4-NPDA): 10 µg/plate TA 1537, 0.5 µg/plate TA 98 Cumene hydroperoxide: 50 µg/plate TA 102 <u>Activation:</u> 2-aminoanthracene (2-AA): 3 µg/plate all strains <i>Salmonella typhimurium</i> strains: TA1535, TA1537, TA100, TA98 and TA102
Metabolic activation:	S9 derived from male Sprague-Dawley rats (Aroclor 1254 induced rat liver)

### B. Study design and methods

Test concentrations:	Experiment I, plate incorporation:
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16-50-158-500-1581-5000 µg/plate  
 Experiment II, pre-incubation:  
 16-50-158-500-1581-5000 µg/plate

Application volume: 0.1 mL

Incubation time /temperature: Pre-incubation: 20 min at 37°C  
 Plate incorporation: 48 hours at 37°C

## II. Results and discussion

The potential of FOE 5043 to induce gene mutations was investigated according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) in two independent experiments both with and without liver microsomal activation (S9 mix).

Doses up to and including 5000 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed.

Evidence of mutagenic activity of FOE 5043-sulfonic-acid was not seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

**Table 6.8.1/09-4 Summary of results of the plate incorporation experiment (experiment I)**

Metabolic activation	Test Group	Mean revertant colony counts					
		(µg/plate)	TA1535	TA100	TA1537	TA98	TA102
Without activation	Vehicle control	6	91	7	19	168	
	FOE 5043-sulfonic-acid	16	4	97	5	24	166
		50	5	104	5	26	195
		158	5	95	5	27	188
		500	6	110	2	21	188
		1581	6	101	6	22	180
		5000	6	101	6	24	180
	Na-azide	10	677				
	NF	0.2	206				
	4-NPDA	10			96		
	4-NPDA	0.5				121	
	Cumene	50					315
With activation	Vehicle control	6	151	7	26	254	
	FOE 5043-sulfonic-acid	16	6	153	8	24	267
		50	11	151	9	25	257
		158	9	153	6	18	232
		500	7	159	9	26	254
		1581	6	143	6	18	245
		5000	6	144	8	28	229
	2-AA	2.5	164	1165	124	1128	350

Na-azide = sodium azide; 2-AA = 2-aminoanthracene, NF = nitrofurantoin, 4-NPDA = 4-nitro-1,2-phenylene diamine

**Table 6.8.1/09-5 Summary of results of the pre-incubation experiment (experiment II)**

Metabolic activation	Test Group	Mean revertant colony counts					
		(µg/plate)	TA1535	TA100	TA1537	TA98	TA102
Without activation	Vehicle control	9	105	7	15	228	
	FOE 5043-sulfonic-acid	16	7	117	8	11	220
		50	7	93	6	10	231
		158	7	116	6	16	229
		500	9	124	7	16	225
		1581	8	128	7	15	216
		5000	7	125	7	12	216
	Na-azide	10	603				
	NF	0.2		347			
	4-NPDA	10			124		
	4-NPDA	0.5				141	
	Cumene	50					554
With activation	Vehicle control	10	151	7	19	250	
	FOE 5043-sulfonic-acid	16	10	145	7	26	299
		50	7	152	9	25	299
		158	10	148	6	22	294
		500	8	172	8	19	226
		1581	9	159	9	24	252
		5000	8	144	9	21	265
	2-AA	2.5	120	1366	235	1204	332

Na-azide = sodium azide; 2-AA = 2-aminoanthracene, NF = nitrofurantoin, 4-NPDA = 4-nitro-l,2-phenylene diamine

### III. Conclusion

Based on the study results and under the experimental conditions described FOE 5043-sulfonic-acid did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used in this *Salmonella typhimurium* reverse mutation assay. Therefore, FOE 5043-sulfonic-acid was considered to be non-mutagenic without and with S9 mix.



<b>Report:</b>	<b>KCA 5.8.1 /10; Wollny, H. E.; 2009</b>
<b>Title:</b>	FOE 5043-Sulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
<b>Document No:</b>	<b>M-361158-01</b>
<b>Report No:</b>	1277302
<b>Guidelines:</b>	<b>OECD 476; Commission Regulation 440/2008/EC, Method B.17; US-EPA 712-C-98-221, OPPTS 870.5300; Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-sulfonic acid Na-salt
  - Description: white solid
  - Lot/Batch no: SES 10294-6-2
  - Purity: 92.4%
  - Stability of test compound: guaranteed for study duration; expiry date: 2010-02-11
- 2. Vehicle and/or positive control:** deionised water; DMSO (positive controls) / ethylmethane sulfonate (EMS), 7,12-dimethylbenz(a)anthracene (DMBA)
- 3. Test system:** Chinese hamster V79 cells (V79/HPRT)
  - metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose: Experiment I and II:  
0-201.9-403.8-807.5-1615.0-3230 µg/mL (+ S9 mix)  
0-101.0-201.9-403.8-604.8-807.5 µg/mL (- S9 mix)  
(highest applied conc. equal to approximately 10 mM)  
positive controls: EMS: 0.15 mg/mL, DMBA: 1.1 µg/mL
- Treatment time: 5 hours
- Incubation time: 8 days, 37 °C

## Results and discussion

No precipitation of the test item was observed up to the maximal concentration in all experimental parts.

Relevant cytotoxic effects defined as a reduction of the relative cloning efficiency I to values below 50% in both parallel cultures were noted in the first experiment without metabolic activation at 604.8 µg/mL and above. In the second experiment cytotoxic effects as described above occurred at 807.5 µg/mL. The recommended toxic range of the relative cloning efficiency of approximately 10-20% was covered without metabolic activation.

No relevant cytotoxic effects were observed in the presence of metabolic activation up to the maximum concentration.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in both main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test item and the activity of the metabolic activation system.

Under the experimental conditions the test item did not induce gene mutations at the HPRT locus in V79 cells.

Results are summarised in the following table.

**Table 6.8.1/10-1: Summary of results**

	concentration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
Experiment I / 5 hr treatment										
Solvent control		–	100.0	100.0	18.9	1.0	100.0	100.0	16.0	1.0
Positive control EMS	150.0	–	66.7	83.8	109.9	5.8	63.0	37.4	608.2	38.1
FOE 5043-sulfonic acid Na-salt	50.5	–	93.0	culture not continued#			90.8	culture not continued#		
	101.0	–	96.6	57.2	10.0	0.5	95.5	101.3	29.1	1.8
	201.9	–	96.1	55.9	27.2	1.4	97.1	112.4	9.5	0.6
	403.8	–	78.9	39.6	35.6	1.9	89.0	128.4	12.0	0.7
	604.8	–	33.7	53.1	13.2	0.7	36.0	120.0	13.7	0.9
	807.5	–	25.5	41.8	45.3	2.4	14.7	124.8	9.5	0.6
Solvent control		+	100.0	100.0	12.7	1.0	100.0	100.0	19.4	1.0
Positive control DMBA	1.1	+	45.3	65.9	1082.2	85.3	55.2	74.5	660.9	34.1
FOE 5043-sulfonic acid Na-salt	101.0	+	100.9	culture not continued#			94.3	culture not continued#		
	201.9	+	100.4	85.7	24.6	1.9	86.4	106.4	18.7	1.0
	403.8	+	101.2	95.1	15.8	1.2	85.7	105.5	17.5	0.9
	807.5	+	102.2	95.9	23.7	1.9	88.3	109.5	9.2	0.5
	1615.0	+	99.1	91.3	7.0	0.5	95.0	99.3	6.0	0.3
	3230.0	+	78.3	94.6	10.4	0.8	55.5	93.4	12.1	0.6

	concentration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
Experiment II/ 5 hr treatment										
Solvent control		--	100.0	100.0	16.7	1.0	100.0	100.0	14.2	1.0
Positive control EMS	150.0	--	70.8	89.0	171.1	10.3	61.5	81.9	139.6	9.8
FOE 5043-sulfonic acid	50.0	--	91.5	culture not continued#			91.7	culture not continued#		
Na-salt	101.0	--	84.5	78.6	17.8	1.1	93.1	105.2	14.2	1.0
	201.9	--	80.4	76.4	18.7	1.1	90.2	98.5	9.4	0.7
	403.8	--	70.3	102.1	14.4	0.9	93.7	100.1	17.0	1.2
	604.8	--	28.9	76.1	11.6	0.7	52.4	99.6	8.0	0.6
	807.5	--	18.8	87.9	7.5	0.5	21.5	103.6	20.6	1.5
Experiment II/ 5 hr treatment										
Solvent control		+	100.0	100.0	14.1	1.0	100.0	100.0	9.4	1.0
Positive control DMBA	1.1	+	60.8	48.7	1399.1	99.1	41.7	66.6	879.6	93.2
FOE 5043-sulfonic acid	101.0	+	103.5	culture not continued#			105.0	culture not continued#		
Na-salt	201.9	+	98.8	87.7	18.3	1.3	104.2	91.3	10.3	1.1
	403.8	+	102.8	80.7	11.5	0.8	96.8	80.7	12.9	1.4
	807.5	+	99.9	119.8	10.4	0.7	97.6	92.7	117	1.2
	1615.0	+	94.0	119.2	10.7	0.8	96.3	92.7	13.7	1.4
	3230.0	+	70.4	120.1	19.9	1.4	65.7	92.8	20.0	2.1

# Culture was not continued since a minimum of only four analysable concentrations is required

<b>Conclusion</b>	Based on the study results FOE 5043-sulfonic acid Na-salt is considered to be non-mutagenic in this HPRT assay.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /11; Nern, M.; 2010a</b>
<b>Title:</b>	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
<b>Document No:</b>	<b>M-366380-01</b>
<b>Report No:</b>	AT05870
<b>Guidelines:</b>	OECD 473; Directive 2000/32/EC, Method B.10; US-EPA 712-C-98-223, OPPTS 870.5375;none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-sulfonic acid Na-salt  
 Description: fine white powder  
 Lot/Batch no: SES 10294-6-2  
 Purity: 92.4 %  
 Stability of test compound: guaranteed for study duration; expiry date: 2010-02-11
- 2. Vehicle and/or positive control:** deionised water, Hanks' balanced salt solution (positive control) / mitomycin C, cyclophosphamide
- 3. Test system:** Chinese hamster V79 cells  
 Metabolic activation: S9 mix

### B. Study design and methods

- Dose: 0-200-400-600-700-800-900-1000 µg/mL (- S9 mix)  
 0-250-500-1000-2000-3000 µg/mL (+ S9 mix)  
 mitomycin C: 0.1 µg/mL, cyclophosphamide: 2.0 µg/mL
- Treatment duration: 4 hours
- Harvest: 18 and 30 hours
- Incubation temperature: 37 °C
- Replicates evaluated: At least 2 slides for each culture

## Results and discussion

Chinese hamster V79 cells were treated with FOE 5043-sulfonic acid Na-salt at concentrations of 200, 400 and 800 µg/mL without S9 mix for assessment of the clastogenic potential of FOE 5043-sulfonic acid Na-salt. In an independent repeat, concentrations of 600, 700 and 800 µg/mL of the test substance were used for assessment. With S9 mix concentrations of 500, 1000 and 3000 µg/mL were employed. Cultures treated with FOE 5043-sulfonic acid Na-salt in the absence of S9 mix showed statistically significant and biologically relevant increases of numbers of metaphases with aberrations, starting at a concentration of 700 µg/mL.

In contrast cultures treated in the presence of S9 mix showed no statistically significant or biologically relevant increases of numbers of metaphases with aberrations.

The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system and in the case of cyclophosphamide the activity of the used S9 mix.

**Table 6.8.1/11-1: Summary of cells with structural aberrations**

Substance		Cells	Metaphases with aberrations (%)		Mitotic Index
Dose (µg/mL)	+/- S9	scored	Including gaps	Excluding gaps	(%)
Experiment 1A (4 hour treatment + 18 hour harvest, +/- S9)					
Solvent (water)	—	200	1.5	1.5	100.0
FOE 5043-sulfonic acid Na-salt					
200	—	200	1.5	1.5	96.9
400	—	200	2.0	1.5	109.7
800	—	200	4.5	4.5	93.3
Mitomycin C 0.1	—	168	79.0	79.0**	91.3
Solvent (water)	+	200	3.5	3.0	100.0
FOE 5043-sulfonic acid Na-salt					
500	+	200	5.5	5.0	89.3
1000	+	200	5.0	4.5	102.5
3000	+	200	3.5	3.5	135.8
Cyclophosphamide 2	+	186	69.0	68.0**	44.0**
Experiment 1B (4 hour treatment + 30 hour harvest, +/- S9)					
Solvent (water)	—	200	2.0	2.0	100.0
FOE 5043-sulfonic acid Na-salt					
800	—	200	13.5	13.0**	79.8*
Solvent (water)	+	200	3.0	2.5	100.0
FOE 5043-sulfonic acid Na-salt					
3000	+	200	1.0	0.5	118.7
Experiment 2 (4 hour treatment + 30 hour harvest, — S9)					
Solvent (water)	—	200	1.5	1.5	100.0
FOE 5043-sulfonic acid Na-salt					
600	—	200	1.5	1.5	119.3
700	—	200	10.0	10.0**	108.4
800	—	200	12.5	12.5**	67.2*
Mitomycin C 0.1	—	200	50.0	50.0**	107.6

\* Statistical significant at p &lt; 0.05;

\*\* statistical significant at p &lt; 0.01

**Table 6.8.1/11-2: Additionally observed polyploid metaphases - 4 hours treatment – Experiment 1**

without metabolic Activation			with metabolic Activation		
Concentration [µg/mL]	Harvest time [h]	Polyploid Metaphases	Concentration [µg/mL]	Harvest time [h]	Polyploid Metaphases
Control (water)	18	3 8	Control (water)	18	10 11
FOE 5043-sulfonic acid Na-salt 200	18	3 5	FOE 5043-sulfonic acid Na-salt 500	18	9 2
400	18	6 9	1000	18	5 7
800	18	5 9	3000	18	8 7
Mitomycin C 0.1	18	5 3	cyclophosphamide 2	18	7 4
Control (water)	30	5 4	Control (water)	30	4 3
FOE 5043-sulfonic acid Na-salt 800	30	9 8	FOE 5043-sulfonic acid Na-salt 3000	30	3 10

**Table 6.8.1/11-3: Additionally observed polyploid metaphases - 4 hours treatment – Experiment 2**

without metabolic Activation		
Concentration [µg/mL]	Harvest time [h]	Polyploid Metaphases
Control (water)	30	9 8
FOE 5043-sulfonic acid Na-salt 600	30	9 9
700	30	17 14
800	30	12 14
Mitomycin C 0.1	30	12 11

<b>Conclusion</b>	FOE 5043-sulfonic acid Na-salt is considered to be clastogenic without S9 mix for mammalian cells <i>in vitro</i> .
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /12; [REDACTED] 2010b</b>
<b>Title:</b>	FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse
<b>Document No:</b>	<b>M-368627-01</b>
<b>Report No:</b>	AT05913
<b>Guidelines:</b>	<b>OECD 474; Council Regulation 440/2008, Method B.12.; US-EPA 712-C-98-226, OPPTS 870.5395; Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

<b>1. Test material:</b>	FOE 5043-sulfonic acid Na-salt
Description:	fine white powder
Lot/Batch no:	SES 10294-6-2
Content:	92.4 %
Stability of test compound:	guaranteed for study duration; expiry date: 2010-07-08
<b>2. Vehicle / positive control:</b>	deionised water, phys. saline solution (positive control)/ cyclophosphamide
<b>3. Test animals</b>	
Species:	mouse
Strain:	NMRI BR
Age:	approx. 6 - 12 weeks
Weight at dosing:	39 g - 47 g
Source:	[REDACTED]
Acclimatisation period:	at least five days
Diet:	fixed-formula feed 3883 (Provimi Kliba SA, Kaiseraugst, Switzerland), <i>ad libitum</i>
Water:	tap water , <i>ad libitum</i>
Housing:	singly in type II cages; bedding: soft wood granules, type BK8/15 (J. Rettenmaier & Soehne, Fuellstoff-Fabriken, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0-500-1000-2000 mg/kg bw; positive control: 20 mg/kg bw
Application route:	intraperitoneal
Application volume:	20 mL/kg bw (test item, negative control); 10 mL/kg bw (positive control)
Group size:	5 males/dose group
Observations:	mortality, clinical signs

## Results and discussion

### A. Clinical observations



After two intraperitoneal administrations of 500, 1000 and 2000 mg/kg bw FOE 5043-sulfonic acid Na-salt treated males showed compound-related symptoms such as apathy, spasm and difficulty in breathing. Symptoms were recorded for up to 4 hours after the second treatment. These symptoms demonstrate relevant systemic exposure of males to FOE 5043-sulfonic acid Na-salt. Thereafter, their external appearance and physical activity remained unaffected. There was no substance-induced mortality. For the control group animals no symptoms were recorded.

### B. Microscopic Evaluation

Normally, cells with micronuclei (Howell-Jolly bodies) occur in polychromatic erythrocytes with an incidence of up to approximately 6.0/2000. The increase in micronucleated polychromatic erythrocytes, due, for example, to chromosome breaks or spindle disorders, is the criterion for clastogenic effects in this test model.

The results with FOE 5043-sulfonic acid Na-salt gave no indications of clastogenic effects for male mice after two intraperitoneal treatments with doses of up to and including 2000 mg/kg bw. The number of micronucleated normochromatic erythrocytes did not increase relevantly in any of the groups.

The known mutagen and clastogen cyclophosphamide had a clear clastogenic effect at an intraperitoneal dose of 20 mg/kg. The number of micronucleated polychromatic erythrocytes increased to a biologically relevant degree.

Furthermore, the ratio of polychromatic to normochromatic erythrocytes was not altered by treatment in any of the groups.

**Table 6.8.1/12-1: Summary of results**

Experimental groups	Number of evaluated PCE	Number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
negative control	10000	2110 ± 557	4.9 ± 2.2	5.0 ± 2.6
FOE 5043-sulfonic acid Na-salt				
500 mg/kg bw	10000	1807 ± 418	4.5 ± 2.3	4.0 ± 2.3
1000 mg/kg bw	10000	1879 ± 493	4.2 ± 2.7	5.2 ± 2.8
2000 mg/kg bw	10000	1725 ± 448	4.6 ± 1.6	5.6 ± 2.1
positive control CPA 20 mg/kg	10000	1990 ± 397	5.2 ± 3.8	26.2* ± 5.1

PCE = polychromatic erythrocytes; NCE = normochromatic erythrocytes; MNNCE = micronucleated NCE;

MNPCE = micronucleated PCE

CPA = cyclophosphamide

\*Statistical significant at  $p < 0.01$  in non-parametric Wilcoxon ranking test

<b>Conclusion</b>	There was no indication of a clastogenic effect of intraperitoneally administered FOE 5043-sulfonic acid Na-salt in the micronucleus test on the male mouse, i.e. in a somatic test system <i>in vivo</i> .
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /13; █████, M.; 2010c</b>
<b>Title:</b>	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - Unscheduled DNA synthesis test with male rat liver cells in vivo
<b>Document No:</b>	<b>M-397810-01</b>
<b>Report No:</b>	AT06167
<b>Guidelines:</b>	<b>Council Regulation No. 440/2008, B.39.; OECD 486; Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

<b>1. Test material:</b>	FOE 5043-sulfonic acid Na-salt
Description:	white powder
Lot/Batch no:	SES 10294-6-2
Content:	92.4 %
Stability of test compound:	guaranteed for study duration; expiry date: 2010-07-08
<b>2. Vehicle / positive control:</b>	deionised water, corn oil, phys. saline solution / 2-Acetylaminofluorene (2-AAF), 1,2-Dimethylhydrazine (DMH)
<b>3. Test animals</b>	
Species:	Wistar rat
Strain:	CrI:(WI)BR
Age:	approx. 6 - 7 weeks
Weight at dosing:	146 g - 183 g
Source:	██
Acclimatisation period:	at least 5 days
Diet:	Fixed-formula diet 3883 (10 mm cubes) (Provimi Kliba SA. Switzerland), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	singly in type III H cages; bedding: soft wood granules, type BK 8/15 (J. Rettenmaier & Soehne, Fuellstoff-Fabriken, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0-1000-2000 mg/kg bw; positive control: 2-AAF: 100 mg/kg bw, DMH: 40 mg/kg bw
Application route:	oral
Application volume:	20 mL/kg bw (test item, negative control); 10 mL/kg bw (positive control)
Sacrifice time:	Negative control and test item: 4 and 16 hours after treatment 2-AAF: 16 hours after treatment DMH: 4 hours after treatment
Group size:	4 males/dose group
Fasting time:	before administration: approx. 6 hours – 16 hours after administration: approx. 30 minutes
Observations:	clinical signs

## Results and discussion

After single oral administrations of 1000 and 2000 mg/kg bw FOE 5043-sulfonic acid Na-salt, treated animals showed no symptoms and there was no substance-induced mortality. No symptoms and no mortality were recorded for the control groups.

No treatment related cytotoxic effects were observed. The availability of a high quality cell population for the *in vitro* part of the assay was demonstrated.

After treatment with FOE 5043-sulfonic acid Na-salt no biologically relevant increase in nuclear labelling was induced.

The positive controls (2-AAF, DMH) induced significant increases in NNG (net grain count) and in the percentage of cells ion repair and thus demonstrated the sensitivity of the test system for the detection of induced DNA-damage.

**Table 6.8.1/13-1: Mean grain values per dose group**

Dose group	Mean NNG + SD	Mean NG + SD	Mean CG + SD
<b>Sacrifice interval 16 hours</b>			
Negative control	-0.71 ± 0.40	1.54 ± 0.93	2.25 ± 0.88
1000 mg/kg bw	-0.55 ± 0.37	1.90 ± 1.25	2.45 ± 1.46
2000 mg/kg bw	-0.60 ± 0.25	1.49 ± 0.63	2.08 ± 0.59
Positive control 2-AFF 100 mg/kg bw	4.15#* ± 0.73	6.69 ± 1.50	2.54 ± 0.85
<b>Sacrifice interval 4 hours</b>			
Negative control	-0.60 ± 0.37	3.34 ± 0.42	3.94 ± 0.78
1000 mg/kg bw	-0.67 ± 0.18	2.60 ± 0.31	3.27 ± 0.41
2000 mg/kg bw	-0.81 ± 0.10	2.74 ± 1.08	3.55 ± 1.13
Positive control DMH 40 mg/kg bw	11.28#* ± 2.10	13.55 ± 2.52	2.27 ± 0.46

NNG =nuclear net grains ; NG = nuclear grains ; CG = cytoplasmic grains ; SD = standard deviation

\*p ≤ 0.05

# biologically relevant increase

<b>Conclusion</b>	FOE 5043-sulfonic acid Na-salt is considered negative in the <i>in vivo</i> UDS Assay with rat liver cells.
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**FOE-thioglycolate sulfoxide (M04)**

The bacterial reverse mutation assay *in vitro* was already presented and evaluated during the EU process for Annex I listing. Please refer to the Evaluation table of flufenacet (7468/VI/98-rev. 10(27.12.2001) and the baseline dossier of flufenacet.

<b>Report:</b>	<b>KCA 5.8.1 /03;Herbold, B., 2000</b>
<b>Title:</b>	FOE 5043-Thioglycolate Sulfoxide - Salmonella/microsome test - plate incorporation and preincubation method
<b>Document No:</b>	<b>M-032500-01-1</b>
<b>Report No:</b>	29871
<b>Guidelines:</b>	<b>Directive 92/69/EEC, Method B.14.; OECD 471; US-EPA 712-C-98-247, OPPTS 870.5100; Deviations: none</b>
<b>GLP</b>	Yes

**I. Materials and methods****A. Materials****1. Test material:**

Description:	FOE 5043-Thioglycolate Sulfoxide
Synonym:	M04
Lot/Batch no:	KTS 9468-3-3
Purity:	96.1- 96.5%
Stability of test compound:	A stability test in the solvent did not reveal significant degradation of the active ingredient.

**2. Control Materials:**

Vehicle/negative control:	Test substance and positive control substances: DMSO
Positive controls:	<u>Non-activation:</u> Sodium azide (Na-azide): 10 µg/plate TA 1535 Nitrofurantoin (NF): 0.2 µg/plate TA 100 4-nitro-1,2-phenylene diamine (4-NPDA): 10 µg/plate TA 1537, 0.5 µg/plate TA 98 Cumene hydroperoxide: 50 µg/plate TA 102 <u>Activation:</u> 2-aminoanthracene (2-AA): 3 µg/plate all strains <i>Salmonella typhimurium</i> strains: TA1535, TA1537, TA100, TA98 and TA102 Metabolic activation: S9 derived from male Sprague-Dawley rats (Aroclor 1254 induced rat liver)

**3. Test system:****B. Study design and methods**

Test concentrations:	Experiment I, plate incorporation: 16-50-158-500-1581-5000 µg/plate
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Experiment II, pre-incubation:  
16-50-158-500-1581-5000 µg/plate

Application volume: 0.1 mL

Incubation time /temperature: Pre-incubation: 20 min at 37°C  
Plate incorporation: 48 hours at 37°C

## II. Results and discussion

Doses up to and including 5000 ug per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. Substance precipitation occurred at the dose 1581 ug per plate and above.

Evidence of mutagenic activity of FOE 5043-Thioglycolate Sulfoxide was not seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

**Table 6.8.1/13-2 Summary of results of the plate incorporation experiment (experiment I)**

Metabolic activation	Test Group	Mean revertant colony counts					
		(µg/plate)	TA1535	TA100	TA1537	TA98	TA102
Without activation	Vehicle control	11	69	6	16	198	
	FOE 5043-Thioglycolate sulfoxide	16	11	60	5	15	227
		50	11	63	6	16	235
		158	8	54	5	16	230
		500	9	55	6	15	216
		1581	12	70	5	16	231
		5000	9	67	7	16	220
	Na-azide	10	700				
	NF	0.2	212				
	4-NPDA	10	164				
	4-NPDA	0.5	206				
	Cumene	50	342				
With activation	Vehicle control	9	84	7	22	276	
	FOE 5043-Thioglycolate sulfoxide	16	11	65	7	24	288
		50	9	79	6	26	287
		158	9	81	6	30	286
		500	11	74	8	26	263
		1581	10	89	7	19	282
		5000	11	68	7	25	278
	2-AA	2.5	329	1561	439	1706	550

Na-azide = sodium azide; 2-AA = 2-aminoanthracene, NF = nitrofurantoin, 4-NPDA = 4-nitro-1,2-phenylene diamine

**Table 6.8.1/13-3 Summary of results of the pre-incubation experiment (experiment II)**

Metabolic activation	Test Group	Mean revertant colony counts					
		(µg/plate)	TA1535	TA100	TA1537	TA98	TA102
Without activation	Vehicle control	7	65	8	15	263	
	FOE	16	6	66	9	13	276
	5043-	50	8	74	7	15	278
	Thioglyco	158	7	69	8	17	262
	late	500	7	74	8	14	258
	sulfoxide	1581	8	83	8	16	283
		5000	9	84	8	17	281
	Na-azide	10	636				
	NF	0.2		259			
	4-NPDA	10			150		
	4-NPDA	0.5				174	
	Cumene	50					494
With activation	Vehicle control	9	85	9	20	321	
	FOE	16	9	76	10	24	329
	5043-	50	7	75	7	16	345
	Thioglyco	158	8	83	9	22	343
	late	500	7	89	8	28	300
	sulfoxide	1581	9	87	8	24	341
		5000	7	79	5	22	281
	2-AA	2.5	168	1140	174	1101	413

Na-azide = sodium azide; 2-AA = 2-aminoanthracene, NF = nitrofurantoin, 4-NPDA = 4-nitro-1,2-phenylene diamine

### III. Conclusion

Based on the study results and under the experimental conditions described FOE 5043-thioglycolate sulfoxide did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used in this *Salmonella typhimurium* reverse mutation assay. Therefore, FOE 5043-Thioglycolate-sulfoxide was considered to be non-mutagenic without and with S9 mix.

#### FOE-methylsulfone (M07)

New studies; not evaluated	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /14; Sokolowski, A.; 2012</b>
Title:	Salmonella typhimurium reverse mutation assay with FOE 5043-methylsulfone
Document No:	<b>M-422370-01</b>
Report No:	1454201
Guidelines:	OECD 471; Commission Regulation (EC) No. 440/2008, Method B13/14; US-EPA 712-C-98-247, OPPTS 870.5100; Deviations: Test substance and reference compounds were not analyzed to verify concentration, homogeneity or stability.
GLP	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-methylsulfone
  - Description: white granules
  - Lot/Batch no: NLL 8856-1-3
  - Purity: 98.0%
  - Stability of test compound: guaranteed for study duration; expiry date:2012-04-28
- 2. Vehicle and/or positive control:** acetone
  - positive controls: Strains: sodium azide, (NaN<sub>3</sub>), 4-nitro-o-phenylene-diamine (4-NOPD), methyl methane sulfonate, (MMS), 2-aminoanthracene (2-AA)
- 3. Test system:** Salmonella typhimurium strains TA1535, TA100, TA102, TA1537, TA98
  - metabolic activation: S9 Mix

### B. Study design and methods

#### 1. Treatment

- Dose: Pre-Experiment/Experiment I:  
0-3-10-33-100-333-1000-2500-5000 µg/plate  
Experiment II: 0-33-100-333-1000-2500-5000 µg/plate
- Application volume: Plate incorporation assay: 0.1 mL  
Pre-incubation assay: 0.05 mL (test substance, solvent), 0.1 mL (positive control)
- Incubation time: 48 hours

### Results and discussion

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used in experiment I. In experiment II, reduced background growth was observed at the highest concentration with and without metabolic activation in all strains used.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation in experiment I. In experiment II, toxic effects evident as a reduction in the number of revertants (below the indication factor of 0.5) were observed at the highest concentration without metabolic activation in strain TA 1535, and with and without metabolic activation in strains TA 1537, TA 98, TA 100, and TA 102.

No substantial increase in revertant colony numbers of any of the five tester strains was observed

following treatment with FOE 5043-methylsulfone at any dose level, neither in the presence nor 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.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

**Table 6.8.1-14-1: Summary of results of the pre-experiment and experiment I**

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean $\pm$ SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	Acetone		13 $\pm$ 1	12 $\pm$ 5	26 $\pm$ 2	111 $\pm$ 16	492 $\pm$ 8
	Untreated		17 $\pm$ 6	14 $\pm$ 4	24 $\pm$ 4	113 $\pm$ 2	458 $\pm$ 38
	FOE 5043-methyl-sulfone	3 $\mu$ g	13 $\pm$ 4	11 $\pm$ 3	27 $\pm$ 7	112 $\pm$ 4	460 $\pm$ 47
		10 $\mu$ g	17 $\pm$ 2	13 $\pm$ 1	23 $\pm$ 4	111 $\pm$ 22	490 $\pm$ 24
		33 $\mu$ g	21 $\pm$ 9	10 $\pm$ 3	29 $\pm$ 2	119 $\pm$ 11	499 $\pm$ 13
		100 $\mu$ g	17 $\pm$ 3	11 $\pm$ 4	26 $\pm$ 3	117 $\pm$ 2	479 $\pm$ 10
		333 $\mu$ g	13 $\pm$ 7	14 $\pm$ 4	32 $\pm$ 8	110 $\pm$ 12	437 $\pm$ 8
		1000 $\mu$ g	12 $\pm$ 5	8 $\pm$ 2	23 $\pm$ 5	95 $\pm$ 9	424 $\pm$ 6
		2500 $\mu$ g	17 $\pm$ 1	17 $\pm$ 6	25 $\pm$ 4	100 $\pm$ 13	382 $\pm$ 32
		5000 $\mu$ g	17 $\pm$ 4	8 $\pm$ 4	26 $\pm$ 5	87 $\pm$ 1	296 $\pm$ 21
	NaN <sub>3</sub>	10 $\mu$ g	1880 $\pm$ 145			2233 $\pm$ 73	
	4-NOPD	10 $\mu$ g			286 $\pm$ 12		
	4-NOPD	50 $\mu$ g		72 $\pm$ 11			
	MMS	3.0 $\mu$ L					5294 $\pm$ 106
With Activation	Acetone		19 $\pm$ 3	20 $\pm$ 0	34 $\pm$ 4	131 $\pm$ 3	611 $\pm$ 12
	Untreated		16 $\pm$ 6	17 $\pm$ 3	31 $\pm$ 6	116 $\pm$ 17	624 $\pm$ 21
	FOE 5043-methyl-sulfone	3 $\mu$ g	19 $\pm$ 2	21 $\pm$ 4	30 $\pm$ 2	123 $\pm$ 8	596 $\pm$ 18
		10 $\mu$ g	23 $\pm$ 8	20 $\pm$ 3	42 $\pm$ 3	136 $\pm$ 14	633 $\pm$ 17
		33 $\mu$ g	19 $\pm$ 3	23 $\pm$ 4	40 $\pm$ 9	135 $\pm$ 9	656 $\pm$ 13
		100 $\mu$ g	21 $\pm$ 3	20 $\pm$ 6	43 $\pm$ 4	123 $\pm$ 5	637 $\pm$ 15
		333 $\mu$ g	18 $\pm$ 7	19 $\pm$ 4	35 $\pm$ 6	135 $\pm$ 9	573 $\pm$ 28
		1000 $\mu$ g	17 $\pm$ 6	21 $\pm$ 5	36 $\pm$ 3	122 $\pm$ 10	533 $\pm$ 33
		2500 $\mu$ g	14 $\pm$ 1	19 $\pm$ 4	29 $\pm$ 8	99 $\pm$ 9	481 $\pm$ 9
		5000 $\mu$ g	14 $\pm$ 2	12 $\pm$ 3	33 $\pm$ 5	93 $\pm$ 10	353 $\pm$ 76
	2-AA	2.5 $\mu$ g	484 $\pm$ 24	358 $\pm$ 21	2621 $\pm$ 162	2940 $\pm$ 32	
	2-AA	10.0 $\mu$ g					2946 $\pm$ 105

NaN<sub>3</sub> = sodium azide

MMS = methyl methane sulfonate

2-AA= 2-aminoanthracene

4-NOPD = 4-nitro-o-phenylene-diamine



**Table 6.8.1-14-2: Summary of results of experiment II**

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	Acetone		18 ± 5	14 ± 1	28 ± 7	114 ± 16	443 ± 8
	Untreated		16 ± 1	17 ± 6	42 ± 5	110 ± 10	436 ± 25
	FOE 5043-methyl-sulfone	33 µg	20 ± 3	11 ± 4	32 ± 3	104 ± 11	429 ± 30
		100 µg	19 ± 4	11 ± 1	26 ± 2	106 ± 15	416 ± 21
		333 µg	22 ± 2	13 ± 3	31 ± 8	111 ± 10	437 ± 25
		1000 µg	15 ± 6	19 ± 4	33 ± 6	130 ± 5	328 ± 11
		2500 µg	16 ± 4	14 ± 4	31 ± 5	119 ± 4	287 ± 51
		5000 µg	7 ± 2 <sup>MR</sup>	2 ± 1 <sup>MR</sup>	9 ± 2 <sup>MR</sup>	17 ± 2 <sup>MR</sup>	2 ± 2 <sup>MR</sup>
	NaN <sub>3</sub>	10 µg	2004 ± 48			2136 ± 56	
	4-NOPD	10 µg			318 ± 17		
	4-NOPD	50 µg		71 ± 6			
	MMS	3.0 µL					3700 ± 20
With Activation	Acetone		17 ± 2	17 ± 5	42 ± 11	127 ± 12	608 ± 14
	Untreated		21 ± 3	16 ± 4	44 ± 4	135 ± 3	600 ± 6
	FOE 5043-methyl-sulfone	33 µg	19 ± 3	17 ± 3	44 ± 7	136 ± 5	577 ± 39
		100 µg	15 ± 3	19 ± 6	36 ± 5	131 ± 15	532 ± 94
		333 µg	18 ± 3	14 ± 4	44 ± 4	123 ± 8	502 ± 57
		1000 µg	22 ± 6	18 ± 4	31 ± 5	125 ± 8	466 ± 59
		2500 µg	17 ± 3	15 ± 2	38 ± 8	130 ± 14	380 ± 25
		5000 µg	9 ± 2 <sup>RM</sup>	4 ± 1 <sup>R</sup>	13 ± 3 <sup>MR</sup>	54 ± 7 <sup>MR</sup>	121 ± 15 <sup>MR</sup>
	2-AA	2.5 µg	368 ± 14	243 ± 25	1751 ± 466	1756 ± 125	
	2-AA	10.0 µg					2957 ± 115

NaN<sub>3</sub> = sodium azide

MMS = methyl methane sulfonate

M = Manual count

2-AA = 2-aminoanthracene

4-NOPD = 4-nitro-o-phenylene-diamine

R = Reduced background growth

<b>Conclusion</b>	FOE 5043-methylsulfone is considered to be non-mutagenic in the <i>Salmonella typhimurium</i> reverse mutation assay.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /15; Wollny, H. E.; 2012;</b>
Title:	FOE 5043-methylsulfone - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Document No:	1454202
Report No:	<b>M-430571-01</b>
Guidelines:	<b>OECD 476; Commission Regulation (EC) No. 440/2008, B.17; US-EPA 712-C-98-221; Deviations: none</b>
GLP	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-methylsulfone
- Description: light yellow granules
- Lot/Batch no: NLL 8856-1-3
- Purity: 98%
- Stability of test compound: guaranteed for study duration; expiry date: 2012-08-07
- 2. Vehicle and/or positive control:** acetone /ethylmethane sulfonate (EMS), 7,12-dimethylbenz(a)anthracene (DMBA)
- 3. Test system:** Chinese hamster V79 cells
- metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

Dose:	exposure period	S9 mix	concentrations in µg/mL				
			Experiment I				
	4 hours	–	175.0	350.0	700.0	1050.0	1400.0
	4 hours	+	175.0	350.0	700.0	1400.0	2800.0
			Experiment II				
	24 hours	–	43.8	87.5	175.0	350.0	525.0
	4 hours	+	175.0	350.0	700.0	1400.0	2800.0

Incubation time: 8 days, 37°C

### Results and discussion

Precipitation was observed at the maximum concentration of 700 µg/mL in the second experiment without metabolic activation. As no precipitation was noted in any other experimental part even at higher concentrations, this observation may well be based on precipitation of denatured proteins rather than test item. The protein concentration during 24 hours treatment is considerably higher due to the 15% horse serum added.

Relevant cytotoxic effects indicated by a relative cloning efficiency I or a relative cell density below 50% occurred in the first experiment without metabolic activation at 700 µg/mL and above in the

absence of metabolic activation. In the second experiment cytotoxic effects as described above occurred at 350 µg/mL and above in the absence of metabolic activation. The recommended cytotoxic range of approximately 10-20% relative cloning efficiency I was covered in the absence of metabolic activation. In the presence of metabolic activation no relevant cytotoxicity was noted up to the maximum concentration of 2800 µg/mL or 10 mM.

No relevant and reproducible increase in mutant colony numbers/ $10^6$  cells was observed in the main experiments up to the maximum concentration. The mutation frequency generally remained well within the historical range of solvent controls. The induction factor did not reach or exceed the threshold of three times the mutation frequency of the corresponding solvent control at any of the concentrations with and without metabolic activation.

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 groups.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

**Table 6.8.1/15-1: Summary of results of experiment I and II**

	Concen- tration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
<b>Experiment I / 4 hr treatment</b>										
Solvent control		–	100.0	100.0	10.6	1.0	100.0	100.0	30.3	1.0
Positive control EMS	150.0	–	91.1	109.7	128.5	12.1	90.1	90.6	126.0	4.2
FOE 5043- methylsulfone	87.5	–	92.6	culture not continued#			96.3	culture not continued#		
	175.0	–	91.5	124.5	11.6	1.1	92.1	91.8	7.7	0.3
	350.0	–	88.9	111.0	11.6	1.1	71.6	124.0	11.6	0.4
	700.0	–	26.0	122.2	179	1.7	16.6	94.9	32.0	1.1
	1050.0	–	0.0	129.7	12.2	1.1	2.4	85.2	51.4	1.7
	1400.0	–	0.0	115.6	18.6	1.8	0.0	91.3	34.6	1.1
Solvent control		+	100.0	100.0	19.9	1.0	100.0	100.0	17.8	1.0
Positive control DMBA	1.1	+	39.6	84.8	1001.5	50.3	44.3	94.4	628.2	35.4
FOE 5043- methylsulfone	87.5	+	94.9	culture not continued#			98.1	culture not continued#		
	175.0	+	93.8	96.5	6.0	0.3	97.1	99.5	28.8	1.6
	350.0	+	95.3	94.7	26.5	1.3	94.8	120.0	10.0	0.6
	700.0	+	93.8	91.0	20.4	1.0	92.8	107.4	14.0	0.8
	1050.0	+	93.3	100.0	24.7	1.2	95.0	103.3	9.7	0.5
	1400.0	+	95.1	107.0	20.3	1.0	93.4	118.5	3.7	0.2

	Concen- tration  µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10 <sup>6</sup> cells	induction factor
Experiment II/ 24 hr treatment										
Solvent control		–	100.0	100.0	16.6	1.0	100.0	100.0	12.5	1.0
Positive control EMS	150.0	–	98.1	96.3	178.0	10.7	93.7	90.5	192.6	15.5
FOE 5043- methylsulfone	43.8	–	101.2	99.1	17.8	1.1	94.3	95.2	15.0	1.2
	87.5	–	99.6	102.6	3.3	0.2	94.0	95.3	4.9	0.4
	175.0	–	68.7	101.4	12.6	0.8	68.6	97.0	10.4	0.8
	350.0	–	12.6	96.6	14.9	0.9	15.6	92.1	17.7	1.4
	525.0	–	0.0	94.0	14.6	0.9	0.0	94.5	15.7	1.3
	700.0P	–	0.0	culture not continued##			0.0	culture not continued##		
Experiment II/ 4 hr treatment										
Solvent control		+	100.0	100.0	7.0	1.0	100.0	100.0	16.4	1.0
Positive control DMBA	1.1	+	88.7	98.6	244.4	34.9	75.5	83.5	605.9	36.9
FOE 5043- methylsulfone	87.5	+	103.3	culture not continued#			100.1	culture not continued#		
	175.0	+	104.8	97.8	14.9	2.1	97.3	103.7	8.2	0.5
	350.0	+	99.9	98.2	5.8	0.8	91.5	113.2	9.6	0.6
	700.0	+	98.4	95.4	11.0	1.6	88.6	101.0	10.9	0.7
	1050.0	+	96.9	96.1	8.1	1.2	91.1	96.3	8.7	0.5
	1400.0	+	79.3	91.1	8.8	1.3	82.7	87.1	9.4	0.6

# Culture was not continued since a minimum of only four analysable concentrations is required

## Culture was not continued due to strong toxic effects P Precipitation

<b>Conclusion</b>	The test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-methylsulfone is considered to be non-mutagenic in this HPRT assay
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /16; Bohnenberger, S.; 2012</b>
<b>Title:</b>	In vitro chromosome aberration test in Chinese hamster V79 cells with FOE 5043-methylsulfone
<b>Document No:</b>	<b>M-437250-01</b>
<b>Report No:</b>	1454203
<b>Guidelines:</b>	OECD 473; Commission Regulation (EC) No. 440/2008, B10; US-EPA 712-C-98-223, OPPTS 870.5375; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-methylsulfone
  - Description: light yellow granules
  - Lot/Batch no: NLL 8856-1-3
  - Purity: 98%
  - Stability of test compound: guaranteed for study duration; expiry date: 2012-08-07
- 2. Vehicle and/or positive control:** Acetone / ethylmethane sulfonate (EMS), cyclophosphamide (CPA)
- 3. Test system:** Chinese hamster V79 cells
  - metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose: 0, 170.6 – 2730 µg/mL (- S9 mix)  
0, 250 – 2730 µg/mL (+ S9 mix)  
positive controls:  
EMS: 500-600-1000 µg/mL  
CPA: 1.0 µg/mL
- Treatment duration: 4 hr, 18 hr (only without S9 mix)
- Chromosome preparation: 18 hr after start of treatment
- Incubation temperature: 37 °C

## Results and discussion

In Experiment IA and IB in the absence of S9 mix no cytotoxicity was observed up to the highest applied concentration. In Experiment IA in the presence of S9 mix, cytotoxicity of approx. 50% was observed at the highest evaluated concentration, indicated by reduced cell numbers. In Experiment IIA in the absence of S9 mix concentrations showing clear cytotoxicity were not evaluable for cytogenetic damage. In the presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. In Experiment IIB in the absence of S9 mix clear cytotoxicity was observed at the highest evaluated concentration, indicated by reduced cell numbers.

In Experiment IA in the absence of S9 mix one statistically significant and dose-dependent increase in

chromosomal aberrations (4.8% aberrant cells, excluding gaps) slightly above the historical solvent control data range (0.0 - 4.0% aberrant cells, excluding gaps) was observed after treatment with 2000.0 µg/mL. In Experiment IB in the absence of S9 mix two statistically significant increases in chromosomal aberrations (4.8 and 4.5% aberrant cells, excluding gaps) slightly above the historical solvent control data range were observed after treatment with 1750.0 and 2000.0 µg/mL. However, no dose-dependent increase was observed, no precipitation or cytotoxicity occurred and no relevant increase after continuous treatment with the test item was observed. Therefore, the findings are considered as being biologically irrelevant. In the presence of S9 mix no statistically significant increases in chromosomal aberrations were observed.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with structural chromosome aberrations.

**Table 6.8.1/16-1: Summary of cells with structural aberrations**

Substance Dose (µg/mL)	+/- S9 mix	Cells scored	Metaphases with aberrations (%)		Cell numbers % of control	Mitotic Index (%)
			Including gaps*	Excluding gaps*		
Experiment IA (4 hour treatment; preparation after 18 hours, + / – S9 mix)						
Solvent (acetone, 0.5 % (v/v))	–	200	2.5	2.0	100.0	100.0
Positive control (EMS) 1000.0	–	200	20.5	20.5 <sup>S</sup>	n.d	96.7
FOE 5043-methylsulfone 500.0	–	200	1.0	1.0	86.3	93.8
1000.0	–	200	2.5	2.5	77.1	100.0
2000.0#	–	400	5.0	4.8 <sup>S</sup>	68.6	100.4
Solvent (acetone, 0.5 % (v/v))	+	200	1.0	1.0	100.0	100.0
Positive control (EMS) 1000.0	+	200	18.5	18.0 <sup>S</sup>	n.d.	71.6
FOE 5043-methylsulfone 250.0	+	200	3.0	2.5	83.2	92.6
500.0	+	200	2.0	2.0	85.4	110.9
1000.0P	+	200	2.5	1.5	51.3	113.1
Experiment IB (4 hour treatment; preparation after 18 hours, – S9 mix)						
Solvent (acetone, 0.5 % (v/v))	–	200	1.0	1.0	100.0	100.0
Positive control (EMS) 1000.0	–	200	15.0	14.5 <sup>S</sup>	n.d	48.1
FOE 5043-methylsulfone 1500.0	–	200	1.5	1.5	101.6	70.3
1750.0#	–	400	4.8	4.8 <sup>S</sup>	90.4	107.0
2000.0#	–	400	5.8	4.5 <sup>S</sup>	88.1	90.5
2250.0	–	200	5.5	3.0	90.7	94.9
2500.0	–	200	2.5	2.0	102.5	81.0
2730.0	–	200	2.5	2.5	91.3	108.9

Substance Dose (µg/mL)	+/- S9 mix	Cells scored	Metaphases with aberrations (%)		Cell numbers % of control	Mitotic Index (%)
			Including gaps*	Excluding gaps*		
Experiment IIA (4 hour treatment; preparation after 18 hours, + S9 mix)						
Solvent (acetone, 0.5 % (v/v))	+	200	3.0	2.5	100.0	100.0
Positive control (CPA) 1.0	+	200	9.0	8.5 <sup>S</sup>	n.d.	81.8
FOE 5043-methylsulfone 1023.8	+	200	4.5	4.0	106.5	105.5
1365.0	+	200	3.5	2.5	91.3	97.3
2047.5P	+	200	4.0	3.0	97.0	120.5
2730.0P	+	200	2.0	2.0	91.3	113.2
Experiment IIA (18 hour treatment; preparation after 18 hours, – S9 mix)						
Solvent (acetone, 0.5 % (v/v))	–	200	2.5	1.0	100.0	100.0
Positive control (EMS) 500.0	–	200	14.5	14.0 <sup>S</sup>	n.d.	106.8
FOE 5043-methylsulfone 170.6	–	200	2.0	1.5	124.1	100.9
341.3	–	200	1.5	1.5	87.7	124.0
682.5	–	200	3.5	3.5	83.3	75.1
Experiment IIB (18 hour treatment; preparation after 18 hours, – S9 mix)						
Solvent (acetone, 0.5 % (v/v))	–	200	2.5	1.5	100.0	100.0
Positive control (EMS) 600.0	–	200	24.0	20.0 <sup>S</sup>	n.d.	80.3
FOE 5043-methylsulfone 170.6	–	200	3.5	3.5	89.7	105.7
341.3	–	200	2.5	1.5	69.0	102.5
682.5	–	200	3.5	3.0	38.0	62.4

# Evaluation of 200 metaphases per culture; 2 cultures per concentration

\* inclusive cells carrying exchanges

s statistical significant at  $p < 0.05$

P precipitation occurred at the end of the treatment

EMS = ethyl methane sulfonate; CPA = cyclophosphamide

<b>Conclusion</b>	<p>The test item did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) <i>in vitro</i>.</p> <p>Therefore, FOE 5043-methylsulfone is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation.</p>
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**FOE-thiadone (M09)**

<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /06; [REDACTED] 1993</b>
<b>Title:</b>	Acute oral toxicity study with FOE 6457 (Thiadone, an FOE 5043 metabolite) in rats
<b>Document No:</b>	<b>M-004951-01</b>
<b>Report No:</b>	BC6979
<b>Guidelines:</b>	<b>S-EPA-FIFRA, Guideline 81-1; US-EPA-TSCA Health Effects Testing Guidelines, 40 CFR Section 798.1175; OECD 401; JMAFF , 59 NohSan No. 4200; Deviations: none</b>
<b>GLP</b>	Yes

**I. Materials and methods****A. Materials****1. Test material:**

FOE 6457

Synonym(s):

thiadone, M09

Specification no.:

n.a.

Description:

white crystals

Lot/Batch no:

9213125-184

Purity:

98.5 %

Stability of test compound:

Unknown

**2. Vehicle:**

deionized water

**3. Test animals**

Species:

rat

Strain:

Sprague-Dawley (Sas:CD(SD)BR)

Sex:

male and female

Age:

8-10 weeks

Weight at dosing:

Males: 182-197 g; females: 170-187 g

Source:

[REDACTED]

Acclimatisation period:

At least 6 days

Diet:

Purina Rodent Lab Chow 5001-4, *ad libitum*

Water:

Tap water, *ad libitum*

Housing:

Individually in stainless steel cages suspended over Deotized Animal Cage Board bedding material

**B. Study design and methods****1. Animal assignment and treatment**

Dose:	Males: 1650 mg/kg bw Females: 600 mg/kg bw
Application route:	Oral (gavage)
Application volume:	20 mL/kg bw
Fasting time:	Overnight fasting
Group size:	5/sex/dose
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, body weight, gross necropsy

## **II. Results and discussion**

### **A. Mortality and Clinical Observations**

All animals died within 10 minutes after dosing. Only one animal was observed to have clinical signs after dosing: one female had tremors prior to death.

### **B. Body Weight**

No body weight changes were noted.

### **C. Necropsy**

The only gross observation noted during necropsy was white mucosa of the glandular stomach in all animals; in male rats, this condition extended to the duodenum, as well.

## **III. Conclusion**

LD50 values were not determined. However, the data suggest that the LD50 of FOE 6457 is < 1650 mg/kg for males and < 600 mg/kg for females. Therefore, FOE 6457 is more toxic than its parent compound, FOE 5043, after acute oral administration.

<b>Report:</b>	<b>KCA 5.8.1 /17; Sokolowski, A.; 2011</b>
<b>Title:</b>	Salmonella typhimurium reverse mutation assay with FOE 5043-Thiadone
<b>Document No:</b>	<b>M-413989-01</b>
<b>Report No:</b>	1423000
<b>Guidelines:</b>	<b>OECD 471; Commission Regulation (EC) No. 440/008, Method B13/14; US-EPA 712-C-98-227, OPPTS 870.5100; Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-thiadone  
 Description: white solid  
 Lot/Batch no: SES 10558-3-5  
 Purity: 98.6%  
 Stability of test compound: guaranteed for study duration; expiry date: 2012-05-24
- 2. Vehicle and/or positive control:** DMSO / sodium azide (NaN<sub>3</sub>); 4-nitro-o-phenylene-diamine (4-NOPD); methyl methane sulfonate (MMS); 2-aminoanthracene (2-AA)
- 3. Test system:** Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, TA102  
 metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose:
- |                                       |   |
|---------------------------------------|---|
| experiment I:                         | 3 – 5000 µg/plate                       |
| experiment II:                        | 3-10-33-100-333-1000-2500-5000 µg/plate |
| sodium azide (NaN <sub>3</sub> ):     | 10 µg/plate                             |
| 4-nitro-o-phenylene-diamine (4-NOPD): | 10 µg/plate                             |
| methyl methane sulfonate (MMS):       | 3.0 µg/plate                            |
| 2-aminoanthracene (2-AA):             | 2.5 µg/plate                            |
- Application volume: 100 µL
- Incubation time: pre-incubation: 60 minutes; at least 48 hours

### Results and discussion

The plates incubated with the test item showed reduced background growth in all strains used in experiment II at higher concentrations.

Toxic effects evident as a reduction in the number of revertants (below the indication factor of 0.5) occurred in all strains at higher concentrations.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with FOE 5043-Thiadone at any dose level, neither in the presence nor 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.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

**Table 6.8.1/17-1: Summary of results for pre-experiment and experiment I**

Metabolic Activation	Test Group	Dose	Revertant Colony Counts (Mean $\pm$ SD)				
			Strain				
		(per plate)	TA1535	TA1537	TA98	TA100	TA102
Without Activation	DMSO	--	12 $\pm$ 2 <sup>BM</sup>	12 $\pm$ 5	29 $\pm$ 8	126 $\pm$ 4	399 $\pm$ 6
	Untreated	--	14 $\pm$ 3 <sup>BM</sup>	12 $\pm$ 5	25 $\pm$ 1	117 $\pm$ 22	389 $\pm$ 23
	FOE 5043-Thiadone	3 $\mu$ g	11 $\pm$ 3 <sup>BM</sup>	12 $\pm$ 3	27 $\pm$ 3	117 $\pm$ 2	362 $\pm$ 40
		10 $\mu$ g	11 $\pm$ 1 <sup>BM</sup>	15 $\pm$ 5	29 $\pm$ 4	119 $\pm$ 3	400 $\pm$ 9
		33 $\mu$ g	12 $\pm$ 2 <sup>BM</sup>	14 $\pm$ 1	27 $\pm$ 7	116 $\pm$ 17	374 $\pm$ 23
		100 $\mu$ g	12 $\pm$ 2 <sup>BM</sup>	13 $\pm$ 1	26 $\pm$ 4	121 $\pm$ 2	365 $\pm$ 39
		333 $\mu$ g	11 $\pm$ 3 <sup>BM</sup>	15 $\pm$ 2	27 $\pm$ 8	136 $\pm$ 18	308 $\pm$ 33
		1000 $\mu$ g	9 $\pm$ 2 <sup>BM</sup>	14 $\pm$ 1	29 $\pm$ 6	120 $\pm$ 15	97 $\pm$ 17
		2500 $\mu$ g	8 $\pm$ 2 <sup>BM</sup>	11 $\pm$ 3	31 $\pm$ 3	92 $\pm$ 6	9 $\pm$ 3
		5000 $\mu$ g	6 $\pm$ 2 <sup>BM</sup>	5 $\pm$ 2	13 $\pm$ 3 <sup>UM</sup>	76 $\pm$ 13	2 $\pm$ 1
	NaN3	10 $\mu$ g	1822 $\pm$ 44			1743 $\pm$ 210	
	4-NOPD	10 $\mu$ g			286 $\pm$ 34		
	4-NOPD	50 $\mu$ g		76 $\pm$ 10			
	MMS	3.0 $\mu$ L					4674 $\pm$ 98
With Activation	DMSO	--	18 $\pm$ 4 <sup>BM</sup>	18 $\pm$ 3	37 $\pm$ 13	138 $\pm$ 4	522 $\pm$ 22
	Untreated	--	19 $\pm$ 4 <sup>BM</sup>	16 $\pm$ 1	42 $\pm$ 13	140 $\pm$ 4	502 $\pm$ 60
	FOE 5043-Thiadone	3 $\mu$ g	17 $\pm$ 3 <sup>BM</sup>	14 $\pm$ 2	41 $\pm$ 5	116 $\pm$ 3	492 $\pm$ 43
		10 $\mu$ g	16 $\pm$ 4 <sup>BM</sup>	18 $\pm$ 4	41 $\pm$ 8	141 $\pm$ 13	510 $\pm$ 23
		33 $\mu$ g	17 $\pm$ 2 <sup>BM</sup>	16 $\pm$ 5	34 $\pm$ 11	140 $\pm$ 13	492 $\pm$ 1
		100 $\mu$ g	18 $\pm$ 3 <sup>BM</sup>	19 $\pm$ 3	43 $\pm$ 1	138 $\pm$ 8	407 $\pm$ 18
		333 $\mu$ g	16 $\pm$ 3 <sup>BM</sup>	14 $\pm$ 5	35 $\pm$ 4	131 $\pm$ 17	353 $\pm$ 50
		1000 $\mu$ g	13 $\pm$ 4 <sup>BM</sup>	16 $\pm$ 1	32 $\pm$ 5	129 $\pm$ 10	144 $\pm$ 10
		2500 $\mu$ g	11 $\pm$ 4 <sup>BM</sup>	15 $\pm$ 2	36 $\pm$ 2	120 $\pm$ 19	15 $\pm$ 5
		5000 $\mu$ g	9 $\pm$ 1 <sup>BM</sup>	6 $\pm$ 3	23 $\pm$ 3	84 $\pm$ 13	5 $\pm$ 2
	2-AA	2.5 $\mu$ g	300 $\pm$ 60	224 $\pm$ 10	2112 $\pm$ 88	2400 $\pm$ 76	
	2-AA	10.0 $\mu$ g					2944 $\pm$ 55

**Table 6.8.1/17-2: Summary of results for pre-experiment and experiment II**

Metabolic Activation	Test Group	Dose	Revertant Colony Counts (Mean ±SD)				
			Strain				
		(per plate)	TA1535	TA1537	TA98	TA100	TA102
Without Activation	DMSO		17 ± 4	11 ± 3	33 ± 8	107 ± 13	337 ± 16
	Untreated		14 ± 2	12 ± 3	23 ± 1	137 ± 27	296 ± 6
	FOE 5043-Thiadone	3 µg	16 ± 7	11 ± 3	27 ± 4	98 ± 12	297 ± 22
		10 µg	15 ± 5	10 ± 2	27 ± 3	111 ± 17	326 ± 17
		33 µg	13 ± 4	13 ± 4	31 ± 3	116 ± 6	309 ± 29
		100 µg	17 ± 6	13 ± 3	30 ± 9	110 ± 21	294 ± 38
		333 µg	14 ± 1	11 ± 5	28 ± 8	84 ± 3	231 ± 17
		1000 µg	12 ± 4	14 ± 3	23 ± 6	91 ± 4	94 ± 3
		2500 µg	11 ± 2	10 ± 4	22 ± 4	86 ± 5	29 ± 1 R
		5000 µg	3 ± 0 R	2 ± 1 R	1 ± 0 M R	31 ± 10 R	2 ± 1 R
Without Activation	NaN3	10 µg	1425 ± 85			1591 ± 84	
	4-NOPD	10 µg			349 ± 28		
	4-NOPD	50 µg		89 ± 6			
	MMS	3.0 µL					2290 ± 41
With Activation	DMSO		19 ± 3	21 ± 2	39 ± 7	115 ± 17	346 ± 13
	Untreated		21 ± 9	15 ± 5	41 ± 16	151 ± 7	363 ± 29
	FOE 5043-Thiadone	3 µg	20 ± 2	21 ± 5	44 ± 1	111 ± 16	298 ± 18
		10 µg	20 ± 6	22 ± 2	35 ± 6	107 ± 15	347 ± 25
		33 µg	20 ± 4	23 ± 2	37 ± 4	116 ± 5	341 ± 52
		100 µg	17 ± 3	21 ± 2	36 ± 10	106 ± 13	331 ± 30
		333 µg	19 ± 4	22 ± 3	39 ± 9	125 ± 7	215 ± 29
		1000 µg	19 ± 3	16 ± 6	25 ± 1	120 ± 13	108 ± 13
		2500 µg	14 ± 1	14 ± 2	18 ± 5	70 ± 10	36 ± 12 R
		5000 µg	7 ± 1	5 ± 3	16 ± 3	49 ± 2	1 ± 1 M R
	2-AA	2.5 µg	208 ± 7	143 ± 13	924 ± 35	977 ± 121	
	2-AA	10.0 µg					1257 ± 6
Key to Positive Controls				Key to Plate Postfix Codes			
NaN3	sodium azide			R	Reduced background growth		
2-AA	2-aminoanthracene			M	Manual count		
MMS	methyl methane sulfonate			B	Extensive bacterial growth		
4-NOPD	4-nitro-o-phenylene-diamine						

<b>Conclusion</b>	FOE 5043-thiadone is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.
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**FOE-trifluoroethanesulfonic acid Na-salt (M44)**

<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /18; Sokolowski, A.; 2012</b>
<b>Title:</b>	<i>Salmonella typhimurium</i> reverse mutation assay with FOE 5043-trifluoroethanesulfonic acid Na-salt
<b>Document No:</b>	<b>M-434728-01</b>
<b>Report No:</b>	1486601
<b>Guidelines:</b>	<b>OECD 471; Commission Regulation (EC) No. 440/2008, Method B.13/14; US-EPA 712-C98-247. OPPTS 870.5100</b>
<b>GLP</b>	Yes

**Materials and methods****A. Materials****1. Test material:**

FOE 5043-trifluoroethanesulfonic acid Na-salt
Description: white solid
Lot/Batch no: NLL 8865-4-1
Purity: 99.4%
Stability of test compound: guaranteed for study duration; expiry date:

**2. Vehicle and/or positive control:**

deionised water  
sodium azide (NaN<sub>3</sub>), 4-nitro-o-phenylene-diamine (4-NOPD),  
methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)  
Salmonella typhimurium TA1535, TA1537, TA98, TA100,  
TA102

**3. Test system:**

metabolic activation: S9 mix

**B. Study design and methods****1. Treatment**

Dose:	0-3-10-33-100-333-1000-2500-5000 µg/plate
	positive controls:
	NaN <sub>3</sub> : 10 µg/plate
	4-NOPD: 10-50 µg/plate
	MMS: 3.0 µg/plate
	2-AA: 2.5-10 µg/plate
Application volume:	0.1 mL/plate
Incubation time:	48 hours, 37 °C

**Results and discussion**

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with FOE 5043-trifluoroethanesulfonic acid Na-salt at any dose level, neither in

the presence nor absence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

**Table 6.8.1/18-1: Revertant counts (mean  $\pm$ SD)**

Dose (µg/plate)		S9 mix	Strain				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Experiment I							
Vehicle control		–	12 ± 0	15 ± 5	28 ± 8	91 ± 4	371 ± 13
Untreated		–	10 ± 2	13 ± 3	30 ± 7	96 ± 5	349 ± 25
FOE 5043-trifluoroethanesulfonic acid Na-salt							
3	–	14 ± 2	15 ± 4	26 ± 8	92 ± 1	365 ± 14	
10	–	11 ± 5	14 ± 5	29 ± 6	110 ± 10	353 ± 29	
33	–	12 ± 5	19 ± 1	29 ± 2	94 ± 12	364 ± 3	
100	–	13 ± 1	15 ± 3	30 ± 1	100 ± 16	348 ± 42	
333	–	14 ± 7	16 ± 1	29 ± 6	97 ± 3	379 ± 29	
1000	–	12 ± 3	14 ± 5	30 ± 4	95 ± 4	363 ± 11	
2500	–	12 ± 3	19 ± 5	28 ± 5	96 ± 11	351 ± 14	
5000	–	12 ± 4	20 ± 2	30 ± 7	102 ± 2	358 ± 14	
NaN <sub>3</sub> 10	–	1916 ± 55			2171 ± 50		
4-NOPD 10	–			330 ± 15			
4-NOPD 50	–		68 ± 11				
MMS 3.0	–					4404 ± 61	
Vehicle control		+	29 ± 3	29 ± 1	45 ± 11	136 ± 9	482 ± 37
Untreated		+	23 ± 6	28 ± 5	53 ± 11	128 ± 6	491 ± 18
FOE 5043-trifluoroethanesulfonic acid Na-salt							
3	+	29 ± 8	30 ± 4	47 ± 6	130 ± 9	474 ± 51	
10	+	29 ± 2	29 ± 3	53 ± 12	120 ± 17	427 ± 33	
33	+	28 ± 4	25 ± 2	49 ± 1	118 ± 2	504 ± 38	
100	+	27 ± 4	31 ± 6	48 ± 7	117 ± 14	472 ± 23	
333	+	30 ± 3	30 ± 3	48 ± 9	120 ± 12	521 ± 41	
1000	+	27 ± 7	25 ± 6	53 ± 10	119 ± 17	520 ± 64	
2500	+	25 ± 7	25 ± 3	51 ± 8	128 ± 25	461 ± 57	
5000	+	30 ± 7	30 ± 7	58 ± 2	100 ± 11	483 ± 24	
2-AA 2.5	+	325 ± 16	202 ± 13	1771 ± 107	1444 ± 90		
2-AA 10	+					1511 ± 160	

Dose (µg/plate)		S9 mix	Strain				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Experiment II							
vehicle control		–	14 ± 6	24 ± 0	32 ± 3	177 ± 17	425 ± 14
untreated		–	15 ± 5	21 ± 2	34 ± 5	194 ± 20	399 ± 12
FOE 5043-trifluoroethanesulfonic acid Na-salt							
33		–	13 ± 2	28 ± 6	32 ± 5	197 ± 12	423 ± 11
100		–	11 ± 3	29 ± 2	27 ± 5	211 ± 21	446 ± 13
333		–	16 ± 1	22 ± 5	26 ± 2	203 ± 8	467 ± 18
1000		–	17 ± 4	27 ± 7	27 ± 6	189 ± 2	404 ± 10
2500		–	15 ± 1	28 ± 1	26 ± 4	203 ± 20	387 ± 26
5000		–	16 ± 1	31 ± 2	24 ± 6	202 ± 10	420 ± 17
NaN <sub>3</sub>	10	–	2088 ± 72			2006 ± 173	
4-NOPD	10	–			317 ± 27		
4-NOPD	50	–		93 ± 10			
MMS	3.0	–					1690 ± 459
vehicle control		+	25 ± 5	29 ± 1	40 ± 7	240 ± 11	646 ± 21
untreated		+	18 ± 4	30 ± 3	44 ± 3	256 ± 39	632 ± 6
FOE 5043-trifluoroethanesulfonic acid Na-salt							
33		+	20 ± 7	34 ± 1	41 ± 5	250 ± 12	659 ± 36
100		+	24 ± 7	32 ± 4	36 ± 3	247 ± 22	685 ± 18
333		+	22 ± 4	32 ± 2	42 ± 5	245 ± 7	682 ± 54
1000		+	19 ± 4	34 ± 4	40 ± 8	244 ± 9	642 ± 20
2500		+	20 ± 4	29 ± 7	44 ± 6	229 ± 10	628 ± 6
5000		+	27 ± 8	29 ± 1	45 ± 7	251 ± 17	704 ± 26
2-AA	2,5	+	311 ± 27	232 ± 13	1574 ± 146	2217 ± 96	
2-AA	10.5	+					3019 ± 251

NaN<sub>3</sub> sodium azide

2-AA 2-aminoanthracene

MMS methyl methane sulfonate

4-NOPD 4-nitro-o-phenylene-diamine

<b>Conclusion</b>	FOE 5043-trifluoroethanesulfonic acid Na-salt is non-mutagenic in this <i>Salmonella typhimurium</i> reverse mutation assay.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /19; Wollny, H. E.; 2013</b>
<b>Title:</b>	FOE 5043-trifluoroethanesulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
<b>Document No:</b>	M-446033-01-1
<b>Report No:</b>	1486603
<b>Guidelines:</b>	<b>OECD 476; Commission Regulation (EC) No. 440/2008, B17; US-EPA 712-C-98-221, OPPTS 870.5300 Deviations: none</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-trifluoroethanesulfonic acid Na-salt  
 Description: white solid  
 Lot/Batch no: NLL 8865-4-1  
 Purity: 94.7%  
 Stability of test compound: guaranteed for study duration; expiry date: 2013-03-06
- 2. Vehicle and/or positive control:** deionised water / ethylmethane sulfonate (EMS),  
 7,12-dimethylbenz(a)anthracene (DMBA)
- 3. Test system:** Chinese hamster V79 cells  
 metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

Dose:	exposure period	S9 mix	concentrations in µg/mL				
			Experiment I				
	4 hours	–	125	250	500	1000	2000
	4 hours	+	125	250	500	1000	2000
			Experiment II				
	24 hours	–	125	250	500	1000	2000
	4 hours	+	125	250	500	1000	2000
Positive control:			ethylmethane sulfonate (EMS):			0.15 mg/mL	
			7,12-dimethylbenz(a)anthracene (DMBA):			1.1 µg/mL	
Incubation time:			8 days after exposure, 37°C				

## Results and discussion

No relevant toxic effects indicated by a relative cloning efficiency I or a relative cell density below 50% was noted up to the maximum concentration of 2000 µg/mL with and without metabolic activation following 4 and 24 hours treatment.

No relevant and reproducible increase in mutant colony numbers/10<sup>6</sup> cells was observed in the main experiments up to the maximum concentration.

The historical solvent control range and the threshold of three times the mutation frequency of the corresponding solvent control was reached or exceeded in the second culture of the first experiment without metabolic activation at 500 µg/mL. The isolated increase was judged as biologically irrelevant, as it was neither reproduced in the parallel culture under identical experimental conditions nor dose dependent as indicated by the lacking statistical significance. The historical solvent control range (2.8 - 43.5 mutant colonies/10<sup>6</sup> cells) but not the threshold was also exceeded in culture II of the first experiment without metabolic activation at 250 and 1000 µg/mL (48.9 and 47.5 mutant colonies/10<sup>6</sup> cells). In culture II of the first experiment with metabolic activation the range of the historical solvent control data (3.4 - 36.6 mutant colonies/10<sup>6</sup> cells) was slightly exceeded at 1000 µg/mL (41.5 mutant colonies/10<sup>6</sup> cells). However, all of the increased mutation frequency values listed above were judged as biologically irrelevant, as they were neither reproduced in the parallel cultures performed under identical experimental conditions nor dose dependent.

No statistically 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.

**Table 6.8.1/19-1: Summary of results for experiment I and II**

	conc.	S9 mix	relative cloning efficiency I %	relative cell density %	relative cloning efficiency II %	mutant colonies/ 10 <sup>6</sup> cells	induction factor	relative cloning efficiency I %	relative cell density %	relative cloning efficiency II %	mutant colonies/ 10 <sup>6</sup> cells	induction factor
Column	1	2	3	4	5	6	7	8	9	10	11	12
<b>Experiment I / 4 h treatment</b>			culture I					culture II				
Solvent control with water		-	100.0	100.0	100.0	25.0	1.0	100.0	100.0	100.0	27.1	1.0
Positive control (EMS)	150.0	-	101.3	98.7	110.4	162.8	6.5	99.7	97.8	90.2	148.6	5.5
Test item	62.5	-	101.6	culture was not continued <sup>#</sup>				93.4	culture was not continued <sup>#</sup>			
Test item	125.0	-	101.2	87.2	122.6	23.5	0.9	95.3	90.8	94.3	19.6	0.7
Test item	250.0	-	100.4	89.7	106.0	30.8	1.2	92.6	107.5	81.5	48.9	1.8
Test item	500.0	-	96.2	94.0	113.5	18.7	0.7	93.4	109.0	86.7	81.1	3.0
Test item	1000.0	-	100.0	93.7	102.7	32.8	1.3	93.0	109.4	84.5	47.5	1.8
Test item	2000.0	-	100.7	95.6	113.8	34.0	1.4	91.3	95.7	87.5	24.6	0.9
Solvent control with water		+	100.0	100.0	100.0	14.0	1.0	100.0	100.0	100.0	36.4	1.0
Positive control (DMBA)	1.1	+	50.6	94.1	68.1	539.4	38.5	50.7	122.0	81.4	850.2	23.3
Test item	62.5	+	89.2	culture was not continued <sup>#</sup>				91.9	culture was not continued <sup>#</sup>			
Test item	125.0	+	75.9	79.4	81.3	24.3	1.7	97.6	111.3	103.1	22.8	0.6
Test item	250.0	+	80.2	80.6	77.4	11.9	0.8	99.2	92.9	100.1	16.0	0.4
Test item	500.0	+	77.4	69.0	67.1	11.2	0.8	97.9	79.9	99.6	22.8	0.6
Test item	1000.0	+	78.8	94.2	67.3	30.0	2.1	100.3	75.1	84.8	41.5	1.1
Test item	2000.0	+	80.4	57.5	63.8	21.5	1.5	94.4	80.3	83.6	15.2	0.4
<b>Experiment II / 24 h treatment</b>			culture I					culture II				
Solvent control with water		-	100.0	100.0	100.0	10.6	1.0	100.0	100.0	100.0	15.7	1.0
Positive control (EMS)	150.0	-	97.4	124.5	86.8	403.5	38.0	98.9	101.0	88.1	320.6	20.4
Test item	62.5	-	98.7	culture was not continued <sup>#</sup>				102.1	culture was not continued <sup>#</sup>			
Test item	125.0	-	100.5	124.1	81.7	14.3	1.3	102.0	131.9	101.0	18.2	1.2
Test item	250.0	-	96.9	132.5	87.5	17.5	1.6	100.1	133.0	97.7	14.4	0.9
Test item	500.0	-	94.7	95.5	91.1	22.1	2.1	99.8	114.8	102.4	19.0	1.2
Test item	1000.0	-	96.9	98.6	85.8	13.0	1.2	96.8	119.8	88.6	19.5	1.2
Test item	2000.0	-	98.1	110.8	90.1	21.0	2.0	99.1	127.4	101.8	16.8	1.1
<b>Experiment II / 4 h treatment</b>			culture I					culture II				
Solvent control with water		+	100.0	100.0	100.0	8.7	1.0	100.0	100.0	100.0	10.6	1.0
Positive control (DMBA)	1.1	+	92.7	72.2	69.1	676.0	78.1	69.9	83.1	92.7	594.3	56.3
Test item	62.5	+	97.0	culture was not continued <sup>#</sup>				81.5	culture was not continued <sup>#</sup>			
Test item	125.0	+	99.4	91.2	79.5	10.1	1.2	86.4	101.2	97.3	18.3	1.7
Test item	250.0	+	94.7	90.8	75.2	19.5	2.3	88.5	96.3	86.3	9.2	0.9
Test item	500.0	+	93.6	98.0	74.9	15.6	1.8	87.7	98.3	98.8	21.5	2.0
Test item	1000.0	+	99.1	104.3	81.8	12.4	1.4	86.9	95.3	93.1	12.6	1.2
Test item	2000.0	+	94.6	96.7	96.2	14.0	1.6	84.1	109.1	91.1	23.0	2.2

# culture was not continued since a minimum of only four analyzable concentrations is required

<b>Conclusion</b>	Under the experimental conditions the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-trifluoroethanesulfonic acid Na-salt is considered to be non-mutagenic in this HPRT assay.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /20; Bohnenberger, S.; 2013</b>
<b>Title:</b>	<i>In vitro</i> chromosome aberration test in Chinese hamster V79 cells with FOE 5043-trifluoroethanesulfonic acid Na-salt
<b>Document No:</b>	1486602
<b>Report No:</b>	M-447404-01-1
<b>Guidelines:</b>	OECD 473; Commission Regulation No. 440/2008, B10; US-EPA 712-C-98-223; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** FOE 5043-trifluoroethanesulfonic acid Na-salt
  - Description: white solid
  - Lot/Batch no: NLL 8865-4-1
  - Purity: 94.7%
  - Stability of test compound: guaranteed for study duration; expiry date: 2013-03-06
- 2. Vehicle and/or positive control:** deionised water  
ethylmethane sulfonate (EMS), cyclophosphamide (CPA)
- 3. Test system:** Chinese hamster V79 cells
  - metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose: 0-465-930-1860 µg/mL (- S9 mix)  
0-465-930-1395-1627.5-1743.8-1860 µg/mL (+ S9 mix)  
positive controls  
EMS: 600-1000 µg/mL  
CPA: 2.0 µg/mL
- Treatment time: 4 hours; 18 hours (only without S9 mix)
- Chromosome preparation: 18 hours after start of treatment
- Incubation: 37 °C

## Results and discussion

Four independent experiments were performed. In Experiment IA the exposure period was 4 hours without S9 mix. In Experiment IB and IIB the exposure period was 4 hours with S9 mix. In Experiment IIA the exposure period was 4 hours with S9 mix and 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, except for the positive controls in Experiment IIA and IIB in the presence of S9 mix, where only 50 metaphases were evaluated.

The highest treatment concentration in this study, 1860.0 µg/mL (approx. 10 mM) was chosen with regard to molecular weight of the test item and with respect to the OECD Guideline for in vitro mammalian cytogenetic tests.

No precipitation of the test item in the culture medium was observed. No relevant influence on osmolarity or pH value was observed.

In Experiment IA, IB and IIA in the absence and presence of S9 mix no cytotoxicity was observed up to the highest required concentration. In Experiment IIB in the presence of S9 mix cytotoxicity indicated as reduced cell numbers was observed at the highest evaluated concentration (54.2% of control).

In Experiment IA and IB in the absence and presence of S9 mix and in Experiment IIA in the absence of S9 mix, no clastogenicity was observed at the concentrations evaluated. The aberration rates of the cells after treatment with the test item (1.0 – 2.5% aberrant cells, excluding gaps) did not exceed the range of the solvent control values (2.0 – 2.5% aberrant cells, excluding gaps) and were within the range of the laboratory historical solvent control data. In Experiment IIA in the presence of S9 mix, one single statistically significant increase in chromosomal aberrations (5.3% aberrant cells, excluding gaps), slightly exceeding the historical solvent control data range (0.0 - 4.0% aberrant cells, excluding gaps) was observed at the highest required concentration. In the confirmatory Experiment IIB three statistically significant increases (3.5, 3.0, and 3.5% aberrant cells, excluding gaps) were observed after treatment with 930.0, 1395.0 and 1743.8 µg/mL, respectively. These values were in the range of the historical solvent control data and are therefore regarded as biologically irrelevant. The statistically significant increase in chromosomal aberrations of Experiment IIA could not be confirmed.

No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (1.1 – 4.4%) as compared to the rates of the solvent controls (1.7 - 4.1%).

No biologically relevant increase in the rate of endomitotic metaphases was found after treatment with the test item (0.0 – 1.2%) as compared to the rates of the solvent controls (0.0 – 1.3%).

Either EMS (600.0 or 1000.0 µg/mL) or CPA (2.0 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

**Table 6.8.1/20-1: Summary of results**

Test item concentration	Polyploid cells	Endomitotic cells	Cell numbers	Mitotic indices	Aberrant cells (%)		
					incl. gaps*	excl. gaps*	with exchanges
(µg/mL)	(%)	(%)	(% of control)	(% of control)			
Experiment IA: Exposure period 4 hrs without S9 mix; preparation interval 18 hrs							
Solvent control <sup>1</sup>	2.8	0.0	100.0	100.0	2.5	2.5	0.0
Positive control (EMS) 1000.0	n.d.	n.d.	n.d.	99.1	14.5	<b>14.5<sup>S</sup></b>	5.5
FOE 5043-trifluoroethanesulfonic acid Na-salt							
465.0	1.8	0.0	123.1	87.0	2.0	1.5	0.0
930.0	2.5	0.0	107.5	99.1	1.5	1.5	0.0
1860.0	1.6	0.0	119.5	95.0	1.5	1.5	1.0

Test item concentration  (µg/mL)	Polyploid cells  (%)	Endomitotic cells  (%)	Cell numbers  (% of control)	Mitotic indices  (% of control)	Aberrant cells (%)		
					incl. gaps*	excl. gaps*	with exchanges
Experiment IIA: Exposure period 18 hrs without S9 mix; preparation interval 18 hrs							
Solvent control <sup>1</sup>	1.7	0.0	100.0	100.0	2.5	2.0	0.5
Positive control (EMS) 600.0	n.d.	n.d.	n.d.	64.6	28.5	28.5 <sup>S</sup>	20.0
FOE 5043-trifluoroethanesulfonic acid Na-salt							
465.0	2.1	0.0	102.4	117.4	1.5	1.5	0.0
930.0	1.5	0.0	80.1	112.8	3.5	2.5	0.5
1860.0	1.7	0.0	103.0	92.4	3.0	2.5	0.0
Experiment IB: Exposure period 4 hrs with S9 mix; preparation interval 18 hrs							
Solvent control <sup>1</sup>	2.8	0.4	100.0	100.0	2.5	2.5	1.0
Positive control (CPA) 2.0#	n.d.	n.d.	n.d.	78.2	14.5	14.5 <sup>S</sup>	6.0
465.0	3.5	0.7	84.3	119.7	2.0	1.5	0.5
930.0	3.4	0.5	104.4	105.5	2.5	2.0	0.0
1860.0	3.3	0.4	68.5	126.3	1.0	1.0	0.5
Experiment IIA: Exposure period 4 hrs with S9 mix; preparation interval 18 hrs							
Solvent control <sup>1</sup>	1.7	0.1	100.0	100.0	2.5	2.0	1.0
Positive control (CPA) 2.0	n.d.	n.d.	n.d.	51.8	42.0	40.0 <sup>S</sup>	17.0
465.0	1.1	0.0	84.3	113.8	0.5	0.5	0.0
930.0	1.4	0.2	104.4	104.8	3.5	2.5	2.0
1860.0##	1.5	0.1	68.5	108.7	5.3	5.3 <sup>S</sup>	1.3
Experiment IIB: Exposure period 4 hrs with S9 mix; preparation interval 18 hrs							
Solvent control <sup>1</sup>	4.1	1.3	100.0	100.0	0.5	0.5	0.0
Positive control (CPA) 2.0	n.d.	n.d.	n.d.	63.3	36.0	36.0 <sup>S</sup>	11.0
930.0	2.6	0.2	65.4	116.7	4.0	3.5 <sup>S</sup>	1.5
1395.0	4.4	1.2	72.9	110.0	4.0	3.0 <sup>S</sup>	1.0
1627.5	4.2	0.9	51.6	119.1	2.0	2.0	1.0
1743.8	2.7	0.5	54.9	110.4	3.5	3.5 <sup>S</sup>	2.0
1860.0	2.7	0.2	54.2	96.8	1.5	1.0	0.0

\* Including cells carrying exchanges; n.d. Not determined

S Aberration frequency statistically significant higher than corresponding control values

# 50 metaphases per culture were evaluated ## 200 metaphases per culture were evaluated

<sup>1</sup> Deionised water 10.0 % (v/v)

<b>Conclusion</b>	Under the experimental conditions reported, the test item FOE 5043-trifluoroethanesulfonic acid Na-salt did not induce structural chromosomal aberrations in V79 cells of the Chinese hamster in vitro, when tested up to the highest required concentration.
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**Trifluoroacetate (TFA) (M45)**

<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /21; Johnson, M.; 2005</b>
<b>Title:</b>	Trifluoroacetate (TFA): reverse mutation in five histidine-requiring strains of <i>Salmonella typhimurium</i>
<b>Document No:</b>	M-256628-01-1
<b>Report No:</b>	2014/82
<b>Guidelines:</b>	OECD 471; EEC Annex V, B13/14; UKEMS Guidelines; Japanese MOHW; JMAFF; ICH Harmonised Tripartite Guideline; US-EPA OPPTS 870.5100; Deviations: none
<b>GLP</b>	Yes

**Materials and methods****A. Materials**

- 1. Test material:** Trifluoroacetate (TFA)  
 Description: white powder  
 Lot/Batch no: 016911/1  
 Purity: 99.1%  
 Stability of test compound: guaranteed for study duration; expiry date: 2007-03-14
- 2. Vehicle and/or positive control:** water  
 2-Nitrofluorene (2NF), Sodium azide (NaN<sub>3</sub>), 9-Aminoacridine (AAC), Mitomycin C (MMC), Benzo[a]pyrene (B[a]P), 2-Aminoanthracene (AAN)
- 3. Test system:** *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA102  
 Metabolic activation: S9 mix

**B. Study design and methods****1. Treatment**

- Dose: Experiment 1: 0, 1.6 - 5000 µg TFA/plate  
 Experiment 2: 0, 156.25 - 5000 µg TFA/plate  
 positive controls:  
 2NF: 5.0 µg/plate  
 NaN<sub>3</sub>: 2.0 µg/plate  
 AAC: 50 µg/plate  
 MMC: 0.2 µg/plate  
 B[a]P: 10.0 µg/plate  
 AAN: 5.0 and 20.0 µg/plate
- Application volume: 0.1 mL/plate  
 Incubation time: 1 hour

## Results and discussion

Following treatments of all the tester strains in the absence and in the presence of S-9, only in Experiment 2 treatment of strain TA98 in the absence of S-9 resulted in an increase in revertant numbers that was statistically significant when the data were analysed at the 1% level using Dunnett's test. This increase in revertant numbers showed no evidence of a dose-response and was not observed following comparable Experiment 1 treatments. Accordingly, this increase in revertant numbers was considered to have been a chance occurrence, and not a compound related effect. As no other treatments provided any statistically significant increases in revertant numbers, this study was considered to have provided no evidence of any mutagenic activity of trifluoroacetate (TFA).

**Table 5.8.1/21- 1: Summary of mean revertant colonies**

		Salmonella typhimurium strains				
	S-9 mix	TA98	TA100	TA1535	TA1537	TA102
Dose (µg/plate)	(-/+)	mean ± SD				
Experiment 1						
Solvent control	–	27 ± 6	105 ± 14	15 ± 6	19 ± 1	221 ± 8
TFA 1.6	–	27 ± 8	103 ± 15	14 ± 2	15 ± 8	214 ± 20
8	–	25 ± 7	101 ± 7	17 ± 6	18 ± 3	213 ± 27
40	–	28 ± 4	91 ± 26	11 ± 3	22 ± 1	219 ± 16
200	–	36 ± 3	101 ± 1	13 ± 3	16 ± 6	216 ± 10
1000	–	34 ± 4	103 ± 6	12 ± 5	20 ± 1	223 ± 6
5000	–	25 ± 5	104 ± 5	15 ± 2	17 ± 7	211 ± 27
Positive controls						
2NF: 5.0	–	1192 ± 121				
NaN3: 2.0	–		666 ± 26	643 ± 17		
AAC: 50.0	–				204 ± 30	
MMC: 0.2	–					643 ± 34
Experiment 2						
Solvent control	–	20 ± 4	97 ± 5	14 ± 5	19 ± 2	249 ±17
TFA 156.25	–	28 ± 7	103 ± 6	15 ± 4	16 ± 4	271 ± 29
312.5	–	25 ± 3	104 ± 9	16 ± 4	16 ± 7	234 ± 33
625	–	33 ± 8*	103 ± 2	18 ± 4	23 ± 4	208 ± 27
1250	–	23 ± 3	95 ± 10	13 ± 5	17 ± 4	219 ± 37
2500	–	21 ± 3	95 ± 2	10 ± 3	20 ± 1	248 ± 40
5000	–	19 ± 2	88 ± 9	14 ± 4	18 ± 2	232 ± 27
Positive controls						
2NF: 5.0	–	577 ± 20				
NaN3: 2.0	–		438 ± 30	438 ± 30		
AAC: 50.0	–				75 ± 12	
MMC: 0.2	–					620 ± 8



		Salmonella typhimurium strains				
	S-9 mix	TA98	TA100	TA1535	TA1537	TA102
Dose (µg/plate)	(-/+)	mean ± SD				
Experiment 1						
Solvent control	+	30 ± 7	107 ± 16	17 ± 3	20 ± 5	202 ± 30
TFA 1.6	+	36 ± 8	92 ± 12	13 ± 3	17 ± 3	193 ± 16
8	+	36 ± 10	99 ± 19	14± 7	20 ± 1	162 ± 10
40	+	49 ± 7	99 ± 5	19 ± 3	13 ± 3	180 ± 14
200	+	33 ± 8	107 ± 6	16 ± 2	18 ± 4	185 ± 20
1000	+	31 ± 2	88 ± 8	20 ± 2	17 ± 8	189 ± 7
5000	+	36 ± 15	93 ± 8	15 ± 5	22 ± 8	177 ± 45
Positive controls						
B[a]P: 10.0	+	245 ± 32				
AAN: 5.0	+		977 ± 35	196 ± 24	97 ± 3	
AAN: 20.0	+					492 ± 13
Experiment 2						
Solvent control	+	41 ± 5	112 ± 12	17 ± 3	22 ± 2	196 ± 34
TFA 156.25	+	32 ± 10	76 ± 5	12 ± 1	24 ± 7	176 ± 29
312.5	+	29 ± 6	94 ± 5	20 ± 3	23 ± 1	208 ± 3
625	+	38 ± 10	69 ± 5	21 ± 6	18 ± 3	178 ± 29
1250	+	41 ± 6	75 ± 6	11 ± 3	20 ± 4	212 ± 35
2500	+	26 ± 5	93 ± 9	10 ± 3	21 ± 2	246 ± 6
5000	+	34 ± 3	90 ± 4	15 ± 2	20 ± 4	232 ± 18
Positive controls						
B[a]P: 10.0	+	399 ± 62				
AAN: 5.0	+		1058 ± 107	290 ± 72	124 ± 16	
AAN: 20.0	+					796 ± 246

\* Dunnett's test, significant at 1% level

TFA = trifluoro acetate; 2NF = 2-Nitrofluorene (2NF); NaN<sub>3</sub> = sodium azide; AAC = 9-Aminoacridine; MMC = mitomycin C; B[a]P = Benzo[a]pyrene; AAN = 2-Aminoanthracene

<b>Conclusion</b>	Trifluoroacetate (TFA) did not induce mutation in five histidine-requiring strains of <i>Salmonella typhimurium</i> (TA98, TA100, TA1535, TA1537, TA 102) when tested under the conditions of this study.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /22; Ballantyne, M.; 2005</b>
<b>Title:</b>	Trifluoroacetate (TFA) - Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique
<b>Document No:</b>	<b>M-260699-01</b>
<b>Report No:</b>	2014/84-D6173
<b>Guidelines:</b>	OECD 476; UKEMS Guidelines; US-EPA OPPTS 870.5300; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** Trifluoroacetate (TFA)
  - Description: white powder
  - Lot/Batch no: 016911/1
  - Purity: 99.1%
  - Stability of test compound: guaranteed for study duration; expiry date: 2007-03-14
- 2. Vehicle and/or positive control:** sterile water for injection  
4-nitroquinoline 1-oxide (NQO), benzo(a)pyrene (BP)
- 3. Test system:** mouse lymphoma L5178Y TK <sup>+/+</sup> mouse cells
  - metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose: 0-360-560-760-960-1160-1360 µg TFA/mL (1360 µg/mL is equivalent to 10 mM TFA)  
positive control: 0.15, 0.20 µg/mL NQO Experiment 1  
0.05, 0.1 µg/mL NQO Experiment 2  
2.0-3.0 µg/mL BP
- Incubation time: 37±1 °C, 24h

## Results and discussion

No statistically significant increases in mutant frequency were observed at any dose tested in the absence of S9. A very small but statistically significant increase in mutant frequency was observed at the intermediate dose of 960 µg/mL in the presence of S9 in Experiment 1. This increase was sufficiently small in magnitude that it is not considered a biologically relevant response, and furthermore, provided no evidence of any dose-relationship or reproducibility, as it occurred at a single intermediate dose with no significant linear trend in only one experiment.

**Table 5.8.1/22-1: Summary of results**

Dose (µg/mL)		-S9		+S9	
		% rel. total growth	mutant frequency <sup>§</sup>	% rel. total growth	mutant frequency <sup>§</sup>
<b>Experiment 1</b> (3 hour treatment +/-S9)					
TFA	0	100	58.86	100	61.07
	360	88	45.14	94	91.37
	560	100	45.25	90	91.29
	760	119	44.31	78	94.48
	960	122	51.85	83	101.77*
	1160	120	59.43	103	75.57
	1360	112	55.90	93	85.10
NQO	0.15	57	314.40		
	3	42	435.99		
BP	2			46	648.58
	3			30	975.59
<b>Experiment 2</b> (24 hour treatment - S9, 3 hour treatment + S9)					
TFA	0	100	56.49	100	50.34
	360	81	44.44	100	74.18
	560	82	58.47	106	63.84
	760	82	41.65	81	58.07
	960	93	52.71	83	57.83
	1160	90	47.63	81	70.88
	1360	76	51.89	104	56.79
NQO	0.05	34	294.33		
NQO	0.1	14	398.07		
BP	2			63	270.86
BP	3			25	542.41

<sup>§</sup> 5-TFT (5-trifluorothymidine) resistant mutants 10<sup>6</sup> viable cells 2 days after treatment

\* Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level

<b>Conclusion</b>	Trifluoroacetate (TFA) did not induce mutation at the <i>tk</i> locus of L5178Y mouse lymphoma cells in the absence and presence of a rat liver metabolic activation system.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /23; Clare, G.; 2005</b>
<b>Title:</b>	Trifluoroacetate (TFA) - Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
<b>Document No:</b>	M-260807-01-1
<b>Report No:</b>	2014/83-D6172
<b>Guidelines:</b>	OECD 473; EEC Annex V, B.10; Japanese MOHW (1999); JMAFF; ICH Harmonised Tripartite Guideline; US-EPA-OPPTS Guideline 870.5375; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** Trifluoroacetate (TFA)  
 Description: white powder  
 Lot/Batch no: 016911/1  
 Purity: 99.3%  
 Stability of test compound: guaranteed for study duration; expiry date: 2007-03-14
- 2. Vehicle and/or positive control:** sterile water for injection  
 4-Nitroquinoline 1-oxide (NQO); cyclophosphamide (CPA)
- 3. Test system:** human blood lymphocytes prepared from pooled blood of three male donors  
 metabolic activation: S9 mix

### B. Study design and methods

#### 1. Treatment

- Dose: 0-85-170-340-1360 µg TFA/mL (20h treatment)  
 0-340-680-1360 µg TFA/mL (3h treatment)  
 positive controls:  
 NQO: 2.5 - 5 µg/mL  
 CPA: 6.25 - 12.5 µg/mL
- Treatment and recovery hours: Experiment 1: 3 + 17 hours (+/- S9)  
 Experiment 2: 20 + 0 hours (-S9)  
 3 + 17 hours (+S9)

## Results and discussion

### Structural aberrations

Exposure to TFA resulted in percentages of chromosome aberrations that were mostly similar to the concurrent vehicle controls in the presence and absence of S9. There was one exception after a 20-hour exposure to TFA in the absence of S9 in Experiment 2. There was a small increase in the percentage of cells with structural chromosome aberrations (excluding gaps) exposed at 1360 µg/mL, the highest concentrations of TFA assessed for chromosome damage. The aberrations included two

chromosome exchanges in one cell. However, the percentages of cells with aberrations fell within the historical vehicle control frequencies. Also exposure at 1360 µg/mL was associated with 61% mitotic inhibition in Experiment 2. Numbers of aberrant cells (excluding gaps) in all treated cultures fell within historical negative control ranges. For the reasons mentioned above the small increase mentioned above was not judged to represent a positive response.

**Table 6.8.1/23-1: Summary of cells with structural aberrations**

Substance Dose (µg/mL)	+/- S9	Cells scored	Cells with aberrations		Mitotic Index (mean)
Experiment 1 (3 hour treatment + 17 hour recovery, +/-S9)					
Solvent	+	200	5	1	7.1
TFA 340	+	200	0	0	7.6
680	+	200	3	1	7.6
1360	+	200	4	4	7.9
CPA 6.25	+	168	53	49 <sup>a</sup>	
Solvent	–	200	2	1	8.3
TFA 340	–	200	3	2	8.2
680	–	200	6	3	7.2
1360	–	200	0	0	7.0
NQO 2.50	–	186	45	44 <sup>a</sup>	
Experiment 2 (3 hour treatment + 17 hour recovery, +S9)					
Solvent	+	200	1	1	7.6
TFA 340	+	200	3	1	7.7
680	+	200	1	0	6.1
1360	+	200	1	1	6.2
CPA 6.25	+	97	48	40 <sup>a</sup>	
Experiment 2 (20 hour treatment + 0 hour recovery, -S9)					
Solvent	–	200	1	0	6.1
TFA 85	–	200	0	0	5.3
170	–	200	0	0	3.8
340	–	200	1	1	3.7
1360	–	200	6	4	2.4
NQO 2.50	–	102	40	34 <sup>a</sup>	

<sup>a</sup> statistical significance  $p \leq 0.001$

### Numerical aberrations

No increases in the frequency of cells with numerical aberrations, that exceeded the historical negative control range, were generally observed in cultures treated with TFA in the absence and presence of S9. The only exception to this was observed in Experiment 1 in a single culture at the lowest concentration analysed following 3+17 hour treatment in the presence of S9. In this culture the numerical aberration frequency marginally exceeded the historical control range. In isolation, this increase is not considered to be of any biological relevance.

**Table 5.8.1/23-2: Summary of numbers and types of numerical aberrations**

Substance Dose (µg/mL)	+/- S9	Cells scored	Numerical aberrations			Total abs	% with num abs
hyperdiploid    endoreduplicated    polyploid							
<b>Experiment 1</b> (3 hour treatment + 17 hour recovery, +/-S9)							
Solvent	+	200	0	0	0	0	0
TFA     340	+	203	0	1	2	3	1.5
680	+	200	0	0	0	0	0
1360	+	202	0	0	2	2	1.0
CPA    6.25	+	168	0	0	0	0	0
Solvent	-	200	0	0	0	0	0
TFA     340	-	200	0	0	0	0	0
680	-	202	2	0	0	2	1.0
1360	-	200	0	0	0	0	0
NQO    2.5	-	186	0	0	0	0	0
<b>Experiment 2</b> (3 hour treatment + 17 hour recovery, +S9)							
Solvent	+	200	0	0	0	0	0
TFA     340	+	202	0	0	2	2	1.0
680	+	200	0	0	0	0	0
1360	+	201	0	0	1	1	0.5
CPA    6.25	+	97	0	0	0	0	0
<b>Experiment 2</b> (20 hour treatment + 0 hour recovery, -S9)							
Solvent	-	201	0	0	1	1	1.0
TFA     85	-	200	0	0	0	0	0
170	-	201	0	1	0	1	0.5
340	-	203	2	0	1	3	1.5
1360	-	200	0	0	0	0	0
NQO    2.5	-	102	0	0	0	0	0

abs = aberrations, num = numerical

<b>Conclusion</b>	Trifluoroacetate (TFA) did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested up to 1360 µg/mL in either the absence or the presence of a rat liver metabolic activation system (S9).
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package</b>
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<b>Report:</b>	<b>KCA 5.8.1 /24; [REDACTED] 2013</b>
<b>Title:</b>	Sodium Trifluoroacetate - Acute oral toxicity study in rats
<b>Document No:</b>	M-444479-01-1
<b>Report No:</b>	12/333-001P
<b>Guidelines:</b>	OECD 425; Commission Regulation (EC) No 440/2008; B.1.TRIS; US-EPA 712-C-98-190 , OPPTS 870.1100; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

<b>1. Test material:</b>	Sodium Trifluoroacetate
Description:	solid white
Lot/Batch no:	SES 11755-1-1
Purity:	95.1%
Stability of test compound:	guaranteed for study duration; expiry date: 2013-01-24
<b>2. Vehicle:</b>	distilled water
<b>3. Test animals</b>	
Species:	Wistar rat
Strain:	CRL:(WI)
Age:	8 - 9 weeks
Weight at dosing:	190 g - 220 g
Source:	[REDACTED]
Acclimatisation period:	at least 6 days
Diet:	ssniff® SM R/M "Autoclavable complete diet for rats and mice - breeding and maintenance" (ssniff Spezialdiaeten GmbH, Soest, Germany) <i>ad libitum</i>
Water:	tap water <i>ad libitum</i>
Housing:	individually in Type II polypropylene/polycarbonate cages; Lignocel Bedding for Laboratory Animals

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	2000 mg/kg bw
Application route:	oral
Application volume:	10 mL/kg bw
Fasting time:	before administration: overnight after administration: 3 hours
Group size:	5 females
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, body weight, gross necropsy

**Results and discussion****A. Mortality**

Sodium trifluoroacetate did not cause mortality at the limit dose level of 2000 mg/kg bw.

**B. Clinical observations**

Treatment with sodium trifluoroacetate at the dose level of 2000 mg/kg bw did not cause any test item related adverse effects during the 14 days observation period.

**C. Body weight**

Body weight and body weight gain of sodium trifluoroacetate treated animals showed no indication of a treatment-related effect.

**D. Necropsy**

There was no evidence of observations at a dose level of 2000 mg/kg bw at necropsy.

<b>Conclusion</b>	Sodium trifluoroacetate is non-toxic after acute oral administration with an LD <sub>50</sub> value above 2000 mg/kg bw in female rats.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package. <u>Required to test the toxicological relevance of groundwater metabolite</u></b>
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<b>Report:</b>	<b>KCA 5.8.1 /25; [REDACTED]; 2001;</b>
<b>Title:</b>	Trifluoroacetate - Exploratory 14-day toxicity study in the rat by dietary administration
<b>Document No:</b>	M-202165-01-1
<b>Report No:</b>	C016316
<b>Guidelines:</b>	not applicable
<b>GLP</b>	no

## Materials and methods

### A. Materials

- 1. Test material:** Trifluoroacetate  
Description: white powder  
Lot/Batch no: 129H3458  
Purity: 98.7% (Sigma-Aldrich)
- 2. Positive control:** clofibric acid (positive control for peroxisomal proliferation)

### 3. Test animals

- Species: Wistar (HAN) rats  
Strain: RJ: W1 (TOPS HAN)  
Age: 8 weeks  
Weight at dosing: males: 304 g - 355 g; females: 212 g - 231 g  
Source: [REDACTED]  
Acclimatisation period: at least 6 days  
Diet: certified and irradiated rodent powder diet A04C-10 PI (U.A.R. (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France)) *ad libitum*  
Water: tap water (filtered and softened) *ad libitum*  
Housing: individually in suspended stainless steel wire mesh cages

## B. Study design and methods

### 1. Animal assignment and treatment

- Dose: Trifluoroacetate: 0 - 600 - 1200 - 2400 ppm  
males: 0 - 43 - 85 - 170 mg/kg bw/day  
females: 0 - 45 - 91 - 190 mg/kg bw/day  
Positive control (peroxisome proliferation)  
Chlofibric acid: 5000 ppm  
males/females: 291/359 mg/kg bw/day
- Duration: 14 days  
Application route: oral (dietary)  
Group size: 5 rats/sex/group

Observations: mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, hepatotoxicity testing, gross necropsy, organ weight, histopathology

## Results and discussion

### A. Mortality

There were no treatment-related mortalities during the study.

### B. Clinical observations

There were no treatment-related clinical signs during the study.

### C. Body weight

Trifluoroacetate: Body weight and body weight development was not changed.

Positive control: During the first treatment week the rats lost weight (males: 14 g; females: 1 g).

Lower body weight gain resulted in lower body weights (males: 19%; females: 10%,  $p < 0.01$ ), when compared with control mean values on Day 14 of the study.

**Table 6.8.1/25-1: Summary of mean body weights**

Dose (ppm)	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
	0	600	1200	2400	Contr.	0	600	1200	2400	Contr.
Body weight (g)	males					females				
Day 1	336	335	341	336	336	221	224	223	223	223
Day 7	376	373	383	368	322*	239	240	237	235	222*
Day 14	406	410	421	408	327*	251	249	248	246	227*

Pos. = positive; Contr. = control

\* statistically different from control  $p \leq 0.01$

**Table 6.8.1/25-2: Summary of mean body weight gain**

Dose (ppm)	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
	0	600	1200	2400	Contr.	0	600	1200	2400	Contr.
	males					females				
Body weight gain (g)										
Day 7	35	38	43	36	-14*	17	16	14	12	-1*
Day 14	66	75	80	76	-9*	29	25	26	23	4*

Pos. = positive; Contr. = control

\* statistically different from control  $p \leq 0.01$

### D. Food consumption

Trifluoroacetate: Food consumption was not affected.

Positive control: Mean food consumption was significantly decreased, more pronounced in males than in females (26 and 36% in males and 13 and 23% in females on weeks 1 and 2, respectively). Lower food consumption correlated with the observed body weight loss in both sexes.

**Table 6.8.1/25-3: Summary of mean food consumption**

Dose (ppm)	Trifluoroacetate				Pos. Contr.	Trifluoroacetate				Pos. Contr.
	0	600	1200	2400		0	600	1200	2400	
Food consumption (g)	males					females				
Day 7	27.3	27.9	27.9	26.6	20.1*	19.5	18.5	18.2	18.5	16.9
Day 14	27.4	27.6	28.9	28.2	17.6*	19.8	18.5	18.6	19.5	15.3*

\* statistically different from control  $p \leq 0.01$

## E. Laboratory investigations

### Haematology

Trifluoroacetate: A tendency towards lower total white blood cell counts was noted in females at 2400 ppm (30% compared to controls, statistically significant  $p \leq 0.05$ ). This slight change was associated with lower mean absolute lymphocyte count (38% compared to controls, statistically significant  $p \leq 0.01$ ). In the absence of relevant change in absolute neutrophil count, the statistically significant change in neutrophil percentage observed in females at 2400 ppm was considered not to be toxicologically relevant.

Positive control: No toxicologically relevant changes observed.

**Table 6.8.1/25-4: Summary of haematology**

Dose (ppm)	Trifluoroacetate				Pos. Contr.	Trifluoroacetate				Pos. Contr.
	0	600	1200	2400		0	600	1200	2400	
Parameter (unit)	males					females				
White blood cell count (10 <sup>9</sup> /L)	15.1	12.8	13.9	14.4	17.3	11.9	10.9	9.9	8.3*	11.7
Neutrophil count (10 <sup>9</sup> /L)	3.0	2.5	3.3	3.7	3.2	1.9	2.0	2.3	2.0	2.0
Neutrophils (%)	20	20	23	26	19	15	18	23	24*	17
Lymphocyte count (10 <sup>9</sup> /L)	11.4	9.7	9.9	9.9	13.1	9.3	8.3	7.1	5.8**	9.0

\* statistically different from control  $p \leq 0.05$

\*\* statistically different from control  $p \leq 0.01$

### Clinical chemistry

Trifluoroacetate: No treatment-related variation was observed.

Positive control: Treatment-related variations (increased aspartate aminotransferase activity, alkaline phosphatase activity, urea concentration and decreased total protein and cholesterol concentrations) were predominantly observed in males. In females, the only noticeable change was a tendency towards higher aspartate aminotransferase activity which was considered not to be toxicologically relevant.

**Table 6.8.1/25-5: Summary of clinical chemistry**

Dose (ppm)	Trifluoroacetate				Pos. Contr.	Trifluoroacetate				Pos. Contr.
	0	600	1200	2400		0	600	1200	2400	
Parameter (unit)	males					females				
Aspartate amino transferase (IU/L)	50	55	57	57	97	53	57	58	58	64*
Alkaline phosphatase (IU/L)	99	112	109	113	214*	63	60	77	63	67
Urea (mmol/L)	4.71	4.63	4.69	5.09	7.09**	5.32	5.74	5.06	5.20	4.84
Protein (g/L)	63	63	64	63	58**	63	62	62	65	60
Cholesterol (mmol/L)	1.89	1.26	1.46	1.44	0.95**	1.69	1.79	1.55	1.74	1.62

\* statistically different from control  $p \leq 0.05$ \*\* statistically different from control  $p \leq 0.01$ **Hepatotoxicity testing**Cytochrom P-450

Trifluoroacetate: At 2400 ppm a slightly increased total cytochrome P-450 content reaching 19% and 14% in males and females, respectively, occurred.

Positive control: The increase in total cytochrome P-450 content was pronounced after clofibric acid administration, especially in the males (35% increase compared to control mean).

Enzymatic activities

Trifluoroacetate: No significant changes occurred in BROD, EROD and PROD activities, whereas a significant dose-related increase in lauric acid hydroxylation was observed in males reaching 159% increase at 2400 ppm, when compared to controls. In the absence of other significant changes in the related parameters (liver weight, histology and peroxisomal activity), the increase in lauric acid hydroxylation observed at 600 ppm in males was considered not toxicologically relevant.

Positive control: BROD, EROD and PROD activities were not affected by the clofibric acid administration, whereas a significant increase in lauric acid hydroxylation was observed in males and females (+363% and +118%, respectively).

Cell cycling assessment

Trifluoroacetate: After 3 days of treatment, the labelling index was higher in males and females at 2400 ppm, when compared to controls. At terminal sacrifice, no effect of treatment on hepatocellular proliferation was noted at 2400 ppm.

Positive control: At terminal sacrifice, the labelling index was higher in comparison to control groups in males and females.

Palmitoyl-CoA oxidation activity

Trifluoroacetate: The hepatic whole protein content was not affected in either sex. Specific and total palmitoyl-CoA oxidation activities were increased in male rat  $\geq 1200$  ppm up to 184% and 192% of control, respectively. No statistically significant effects were observed in female rats.

Positive control: The whole homogenate protein content was statistically significantly increased to 112% of control in both male and female rats. Hepatic palmitoyl-CoA oxidation activity was statistically significantly induced in both sexes. The specific palmitoyl-CoA oxidation activity was increased by clofibric acid in both sex to 1029 and 503% of control, respectively. For total palmitoyl-CoA oxidation activity the increases were to 1144 and 564% of control, respectively.

**Table 6.8.1/25-6: Summary of hepatotoxicity assessment**

Dose (ppm)	Trifluoroacetate				Pos. Contr.	Trifluoroacetate				Pos. Contr.
	0	600	1200	2400		0	600	1200	2400	
Parameter (unit)	males					females				
Cytochrome P-450 activity										
Cytochrome P-450 (nmol)	1.40	1.51	1.64	1.66	1.89	0.95	1.05	1.03	1.08	1.12
Enzymatic activities										
BROD (pmol/min/mg protein)	14.71	20.20	22.58	17.51	42.86	2.99	3.65	4.35	4.45	13.31
EROD (pmol/min/mg protein)	54.75	71.24	23.90	24.98	11.34	55.95	38.14	41.34	29.63	29.99
PROD (pmol/min/mg protein)	8.31	8.47	7.85	5.94	12.32	4.74	3.72	3.77	4.32	5.26
Lauric acid hydroxylation (nmol/min/mg protein)	3.20	5.85	7.20	8.28	14.82	2.56	2.10	2.05	2.26	5.59
Cell cycling										
PCNA positive cells /1000 (day 3)	9.2	--	--	20.8	--	8.4	--	--	17.4	--
PCNA positive cells /1000 (day 14)	2.8	--	--	3.7	7.7	3.2	--	--	3.3	5.8
Palmitoyl-CoA oxidation activity										
Whole protein content (mg/protein/g liver)	234	238	247 *	246	261 *	224	244 **	236	237	250 ***
Palmitoyl-CoA oxidation (nmol/min/mg homogenate protein)	4.38	5.39	6.37 **	8.06 **	45.05 ***	4.50	4.18	4.50	4.24	22.64 ***
Palmitoyl-CoA oxidation (μmol/min/g liver)	1.03	1.29	1.57 **	1.98 **	11.78 ***	1.00	1.02	1.06	1.01	5.64 ***

\*, \*\*, \*\*\* statistically different from control  $p \leq 0.05$ ,  $p \leq 0.01$ ,  $p \leq 0.001$

-- no data

## F. Organ weight

**Trifluoroacetate:** Body weights in treated animals were not affected at interim as well as at terminal sacrifice. Absolute and relative liver weights were statistically significantly increased in male rat  $\geq 1200$  ppm. There was no difference of the liver weight in females. Other statistically significant changes were considered incidental and not treatment related since they were lacking dose-response and were not associated with any microscopic finding.

**Positive control:** Mean terminal body weight was statistically significantly lower in males and females. Absolute and relative liver weights were higher in males and females. The increased relative thyroid weight was not considered treatment-related since it was not associated with any histopathological finding and the absolute weight was not increased.

**Table 6.8.1/25-7: Summary of organ weights**

Dose (ppm)	Trifluoroacetate				Pos. Contr.	Trifluoroacetate				Pos. Contr.
	0	600	1200	2400		0	600	1200	2400	
Parameter (unit)	males					females				
Interim sacrifice day 3										
Body weight (g)	306	--	--	315	--	203	--	--	196	
Liver weight (g) - abs.	9.2	--	--	10.2	--	5.9	--	--	5.7	
Liver weight (g) - rel.	3.0	--	--	3.2		2.9	--	--	2.9	
Terminal sacrifice day 14										
Body weight (g)	373	374	381	367	304**	231	228	226	223	213**

Dose (ppm)	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
	0	600	1200	2400	Contr.	0	600	1200	2400	Contr.
Parameter (unit)	males					females				
Liver weight (g) - abs.	9.9	10.7	11.7	11.7	14.7**	6.3	6.1	6.2	6.4	8.6**
Liver weight (g) - rel.	2.6	2.9	3.1**	3.2**	4.8**	2.7	2.7	2.7	2.9	4.1**
Thyroid weight (g) - abs.	0.016	0.019	0.016	0.020	0.019	0.015	0.012	0.014	0.013	0.015
Thyroid weight (g) - rel.	0.004	0.005	0.004	0.005	0.006**	0.006	0.005	0.006	0.006	0.007

Pos. Contr. = positive control

\*\* statistically different from control  $p \leq 0.01$ , -- no data

### G. Gross necropsy

Trifluoroacetate: Only few gross pathology changes were noted and considered as incidental findings.

Positive control: At terminal sacrifice obviously larger livers were observed in 2/5 males.

### H. Micropathology

Trifluoroacetate: At interim sacrifice a slight increase of hepatocellular mitoses was observed in all males and 2/3 females at 2400 ppm. At terminal sacrifice slight diffuse centrilobular hepatocellular hypertrophy was observed in 1/5 and 2/5 males at 2400 and 1200 ppm, respectively.

All other changes were considered to be incidental in origin and unrelated to the treatment.

<b>Conclusion</b>	The NOAEL is 600 ppm (43/45 mg/kg bw/day males/ females) based on liver findings (increased organ weight in correlation with hepatocellular hypertrophy, increased cytochrome P-450, lauric acid hydroxylation activity, specific and total palmitoyl-CoA oxidation activities) in male rats. Trifluoroacetate is a very weak peroxisome proliferator in male rats at doses $\geq 1200$ ppm (85 mg/kg bw/day).
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package. Required to test the toxicological relevance of groundwater metabolite</b>
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<b>Report:</b>	<b>KCA 5.8.1 /26; [REDACTED]; 2005;</b>
<b>Title:</b>	Sodium trifluoroacetate (TFA) - 28-day toxicity study in the rat by dietary administration
<b>Document No:</b>	<b>M-259106-01</b>
<b>Report No:</b>	SA05054
<b>Guidelines:</b>	OECD 407; Directive 96/54/EC, Method B.7; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

<b>1. Test material:</b>	Trifluoroacetate (TFA)
Description:	white crystals
Lot/Batch no:	016911/1
Purity:	99.1%
Stability of test compound:	guaranteed for study duration; expiry date: 2007-03-14
<b>2. Vehicle:</b>	none
<b>3. Test animals</b>	
Species:	Wistar rat
Strain:	Rj:WI (IOPS HAN)
Age:	6 weeks
Weight at dosing:	males: 204 g – 207 g; 168 g – 172 g (means)
Source:	[REDACTED]
Acclimatisation period:	6 days
Diet:	certified rodent powdered and irradiated diet A04C 10 P1 (S.A.F.E. (Scientific Animal Food and Engineering, Augy, France) <i>ad libitum</i>
Water:	tap water <i>ad libitum</i>
Housing:	in suspended stainless steel wire mesh cages

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0 - 600 - 1800 - 5400 - 16000 ppm males: 0-50-149-436-1315 mg/kg bw/day females: 0-52-157-457-1344 mg/kg bw/day
Duration:	28 days
Application route:	Oral (dietary)
Group size:	5 rats/sex/group
Observations:	mortality, clinical signs, body weight, food consumption, ophthalmology, haematology, clinical chemistry, urinalysis, gross necropsy, organs weight, histopathology

## Results and discussion

### A. Mortality

No mortalities were noted during the study.

### B. In life observations

No clinical signs were observed during the study in either sex.

### C. Body weight

Body weight and body weight gain were not affected by treatment.

### D. Food consumption

No effect on mean food consumption was noted in either sex.

### E. Ophthalmology

There were no treatment related ophthalmological findings during the study in either sex.

### F. Laboratory investigations

#### Hematology

No treatment related effects.

#### Clinical chemistry

Slightly higher alanine aminotransferase activity (ALAT) was observed at 16000 ppm in both sexes (+37% in males and +23% in females). Decreased lower cholesterol concentration (CHOL) was noted in males  $\geq 5400$  ppm (-30% and -29%, respectively). Increased concentration of glucose (GLUC) was noted in all treated groups in both sexes.

However, in the absence of associated histopathological findings these changes are not considered to be adverse.

**Table 6.8.1/26-1: Summary of clinical chemistry**

Parameter means (unit)	Dose group (ppm) males					Dose group (ppm) females				
	0	600	1800	5400	16000	0	600	1800	5400	16000
ALAT (IU/L)	38	44	45	43	52**	35	36	41	40	43*
CHOL (mmol/L)	2.14	1.69	1.65	1.51*	1.50*	1.75	1.84	1.60	1.86	2.00
GLUC (mmol/L)	5.77	4.11**	3.70**	4.25**	4.09**	6.17	4.32**	5.19	4.18**	4.32**

\*, \*\* statistically different from control  $p \leq 0.05$ ,  $p \leq 0.01$

### Urinalysis

A dose-related increase of the ketone concentration was noted in all dose groups in both sexes. Higher mean urinary volume was noted at 16000 ppm in males (+65%). However, based on the variability of individual values in the control group, this isolated difference was not considered toxicologically relevant.



**Table 6.8.1/26-2: Urinalysis summary**

Parameter means (unit)	Dose group (ppm) males					Dose group (ppm) females				
	0	600	1800	5400	16000	0	600	1800	5400	16000
Ketones										
0.0 g/L	1	0	0	0	0	0	1	0	0	0
0.05 g/L	3	0	0	0	0	0	2	1	2	0
0.15 g/L	1	1	0	0	0	0	2	3	2	3
0.04 g/L	0	1	0	0	0	0	0	0	1	1
≥ 0.8 g/L	0	3	5	5	5	0	0	0	0	0
Volume mL	7.1	9.9	9.6	8.5	11.7*	2.0	4.4	2.5	3.2	5.3

\* statistically different from control  $p \leq 0.05$

### G. Organ weight

At 16000 ppm, mean absolute and relative liver weights were higher and statistically different in both sexes, when compared to controls. At 5400 ppm in both sexes and at 1800 ppm in males, mean liver to body weight ratios were higher and statistically different, when compared to controls. As these differences were not associated with relevant histopathological findings, they were considered not to be toxicologically relevant.

**Table 6.8.1/26-3: Liver weight changes at terminal sacrifice (% change when compared to controls)**

Dose (ppm)	Male				Female			
	600	1800	5400	16000	600	1800	5400	16000
Mean absolute liver weight	NC	+6% NS	+9% NS	+24% $p \leq 0.05$	NC	NC	+7% NS	+15% $p \leq 0.05$
Mean liver to body weight ratio	NC	+15% $p \leq 0.01$	+19% $p \leq 0.01$	+33% $p \leq 0.01$	NC	+7% NS	+13% $p \leq 0.05$	+18% $p \leq 0.01$
Mean liver to brain weight ratio	NC	NC	+12% NS	+27% $p \leq 0.01$	NC	+10% NS	+12% NS	+24% $p \leq 0.01$

NC: no relevant change      NS: not statistically significant

The other organ weight differences, even if statistically significant were judged to be incidental and not treatment related.

### H. Gross necropsy

A higher incidence of enlarged liver was observed in both sexes at 16000 and 5400 ppm when compared to controls. As this finding was not correlated with any histopathological finding at the microscopic examination, it was considered to be without toxicological significance.

All other gross pathology changes were considered as incidental and not treatment related.

### I. Micropathology

There were no treatment related histopathological changes. All histopathological findings encountered were considered to have arisen spontaneously.

<b>Conclusion</b>	The NOAEL of the present study was established at 16000 ppm in both sexes after 28-day exposure to sodium trifluoroacetate (TFA) which is equivalent to 1315/1344 mg/kg bw/day in males and females.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package. <u>Required to test the toxicological relevance of groundwater metabolite</u></b>
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<b>Report:</b>	<b>KCA 5.8.1 /27; [REDACTED] 2007</b>
<b>Title:</b>	Sodium trifluoroacetate (TFA) 90-day toxicity study in the rat by dietary administration
<b>Document No:</b>	<b>M-283994-01</b>
<b>Report No:</b>	SA06080
<b>Guidelines:</b>	OECD 408; Directive 2001/59/EC , Method B.26; US-EPA OPPTS 870.3100; JMAFF 12 Nousan n°8147; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

<b>1. Test material:</b>	Sodium trifluoroacetate (TFA)
Description:	white solid
Lot/Batch no:	KTS 10279-1-1
Purity:	99%
Stability of test compound:	guaranteed for study duration; expiry date: 2006-10-05
<b>2. Vehicle:</b>	none
<b>3. Test animals</b>	
Species:	Wistar rat
Strain:	Rj:WI (IOPS HAN)
Age:	7 weeks
Weight at dosing:	males: 225 g - 259 g, females: 165 g - 200 g
Source:	[REDACTED]
Acclimatisation period:	at least 12 days
Diet:	certified rodent powdered and irradiated diet A04CP1 10 (S.A.F.E. (Scientific Animal Food and Engineering, Augy, France))
Water:	tap water
Housing:	suspended, stainless steel, wire-mesh cages

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0-160-1600-16000 ppm equivalent to: 0-9.9-98-1043 mg/kg bw/day (males) 0-12.2-123-1216 mg/kg bw/day (females)
Duration:	90 days
Application route:	oral
Group size:	10 rat/sex/group
Observations:	mortality, clinical signs, body weight, food consumption, neurotoxicity ophthalmology, urinalysis, haematology, clinical chemistry, gross necropsy, organs weight, histopathology

## Results and discussion

### A. Mortality

One male from the 1600 ppm group was found dead on study day 15.

### B. In life observations

No treatment-related clinical signs were observed.

One male from the 16000 ppm group was noted to have ocular discharge in both eyes from study days 78 to 85. As this sign was transient and disappeared before the end of the study, it was considered not to be treatment-related.

### C. Body weight

At 16000 ppm, mean body weight of males was reduced by 5 to 11% from study day 15 onwards, resulting in an overall reduction in mean body weight gain of 17% on day 92, when compared to controls. The effect was statistically significant at most time points ( $p \leq 0.01$  or  $0.05$ ). In females, mean body weight was reduced by up to 6% during the course of the study, resulting in an overall reduction in mean body weight gain of 14% on Day 92, when compared to controls. The effect was statistically significant on a number of occasions for cumulative body weight gain ( $p \leq 0.01$  or  $0.05$ ).

Body weight parameters were not affected in either sex at 1600 ppm and at 160 ppm.

**Table 6.8.1/27-1: Summary of mean body weights (g)**

Dose (ppm)	Males													
	Mean body weight (g) on study day													
	1	8	15	22	29	36	43	50	57	64	71	78	85	92
0	245	299	348	384	412	442	466	485	503	516	524	535	543	550
160	246	298	348	384	410	438	461	480	498	509	516	530	536	544
1600	244	294	342	376	401	431	449	470	483	496	499	514	522	529
16000	243	291	332*	359 <sup>+</sup>	378 <sup>+</sup>	404 <sup>+</sup>	421 <sup>+</sup>	439 <sup>+</sup>	450 <sup>+</sup>	465 <sup>+</sup>	471 <sup>+</sup>	482 <sup>+</sup>	490 <sup>+</sup>	496 <sup>+</sup>

Dose (ppm)	Females													
	Mean body weight (g) on study day													
	1	8	15	22	29	36	43	50	57	64	71	78	85	92
0	182	204	220	228	239	249	260	264	269	271	274	278	280	282
160	181	203	222	230	238	248	256	264	271	276	276	277	280	282
1600	183	200	217	228	237	244	251	257	262	268	271	275	277	284
16000	183	199	214	223	231	239	245	253	255	258	260	264	267	270

\* Statistically significant different from control ( $p < 0.05$ )

<sup>+</sup> Statistically significant different from control ( $p < 0.01$ )

In males, there was a dose-related trend towards lower terminal body weight when compared to controls, the effect being statistically significant at 16000 ppm (11 %,  $p \leq 0.01$ ). In females, the mean terminal body weight was slightly lower at 16000 ppm (not statistically significant).

### D. Food consumption

Up to the highest dose level tested food consumption was not affected in either sex.

### E. Ophthalmology

There was no evidence of treatment-related effects up to the highest dose level tested of 16000 ppm.

One male from the 16000 ppm group had a corneal opacity in the left eye and another male had anterior synechia in the iris of the left eye.

**F. Neurotoxicological investigations****Locomotor activity**

At 16000, 1600 and 160 ppm in both sexes, overall mean exploratory locomotor activity was comparable to control values. In addition, the pattern of the locomotor activity over time was similar to controls.

**Open field observation**

No treatment-related changes were recorded during the open field observation at any dose level in either sex. The few changes noted were observed in isolation and/or with no dose-relationship and were considered not to be treatment-related

**Sensory reactivity**

All reflexes and responses evaluated were unaffected by the treatment at any dose level in either sex. The increased incidence of exaggerated flexor reflex for both hind paws observed in the high dose females was considered not to be treatment-related, due to the limited magnitude of the change and inter-individual variation of this parameter.

**Grip strength**

The fore- and hind-limb grip strength were unaffected by treatment at any dose level in either sex. A slight decrease in forelimb grip strength was observed in high dose females in comparison to controls ( $-17\%$ ,  $p \leq 0.01$ ), but it was considered to be fortuitous and due to a particularly high mean value in the control group. Furthermore, the mean value observed in the high dose females for this parameter was within the in-house historical control range

**G. Laboratory investigations****Haematology**

Treatment-related changes were noted only in females at 16000 and 1600 ppm.

When compared to the controls, lower mean haemoglobin concentration ( $-8\%$ ,  $p \leq 0.01$ ) was noted at 16000 ppm in females only. This slight change was associated with lower mean corpuscular volume ( $-6\%$ ,  $p \leq 0.01$ ), mean corpuscular haemoglobin ( $-7\%$ ,  $p \leq 0.01$ ) and haematocrit ( $-6\%$ ,  $p \leq 0.01$ ).

At 1600 ppm, lower mean haemoglobin concentration ( $-4\%$ ,  $p \leq 0.05$ ), essentially due to low values noted in two animals, and lower mean corpuscular haemoglobin ( $-3\%$ ,  $p \leq 0.01$ ) were also noted.

No treatment-related change was noted in males at any dose level and in females at 160 ppm.

The few other statistically significant differences were considered to be incidental in view of their occurrence at the lowest dose and/or their low magnitude.

**Table 6.8.1/27-2: Summary of haematology parameter changes in females**

Parameter Dose (ppm)	Mean ± SD (% change when compared to control)			
	Hb (g/dL)	MCV (fl)	Hct (L/L)	MCH (pg)
0	15.6 ± 0.7 (–)	52 ± 1 (–)	0.462 ± 0.019 (–)	17.4 ± 0.4 (–)
160	15.6 ± 0.4 (±0%)	51 ± 2 (–2%)	0.467 ± 0.010 (+1%)	17.1 ± 0.5 (–2%)
1600	14.9 ± 0.6* (–4%)	50 ± 1 (–4%)	0.448 ± 0.018 (–3%)	16.8 ± 0.3** (–3%)
16000	14.4 ± 0.4** (–8%)	49 ± 1** (–6%)	0.435 ± 0.010** (–6%)	16.2 ± 0.5** (–7%)

Hb = haemoglobin concentration; MCV = mean corpuscular volume; Hct = haematocrit;

MVH = mean corpuscular haemoglobin

\* = statistically significant different from control ( $p \leq 0.05$ ); \*\* = statistically significant different from control ( $p \leq 0.01$ )

### Clinical chemistry

Treatment-related changes were observed at 16000 and 1600 ppm in both sexes. Mean total bilirubin and glucose concentrations were lower in both sexes and mean alkaline phosphatase; alanine aminotransferase and aspartate aminotransferase activities were higher in males only.

The slightly lower mean total bilirubin concentration noted at 160 ppm in both sexes was considered not to be treatment-related as the difference to controls was not statistically significant and all individual values were within the in-house historical control data.

**Table 6.8.1/27-3: Summary of clinical chemistry parameter changes in males and females**

Parameter Dose (ppm)	Mean ± SD (% change when compared to control)				
	Bili (mmol/L)	Gluc (mmol/L)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
<b>males</b>					
0	1.6 ± 0.4 (–)	5.87 ± 0.53 (–)	80 ± 9 (–)	89 ± 37 (–)	47 ± 25 (–)
160	1.1 ± 0.2 (–31%)	5.40 ± 0.64 (–8%)	68 ± 11 (–15%)	83 ± 21 (–7%)	47 ± 20 (±0%)
1600	0.5 ± 0.1** (–69%)	4.21 ± 0.44** (–28%)	106 ± 18 (+33%)	146 ± 118 (+64%)	87 ± 84 (+85%)
16000	0.3 ± 0.2** (–81%)	4.14 ± 0.84** (–29%)	156 ± 39** (+95%)	111 ± 24 (+25%)	65 ± 19* (+38%)
<b>females</b>					
0	2.1 ± 0.5 (–)	5.57 ± 0.86 (–)	50 ± 12 (–)	73 ± 12 (–)	38 ± 9 (–)
160	1.8 ± 0.4 (–14%)	5.13 ± 0.56 (–8%)	45 ± 10 (–10%)	82 ± 17 (+12%)	40 ± 10 (+5%)
1600	1.0 ± 0.6** (–52%)	4.19 ± 0.45** (–25%)	53 ± 15 (+6%)	87 ± 16 (+19%)	47 ± 17 (+26%)
16000	0.5 ± 0.3** (–76%)	4.62 ± 1.11** (–17%)	50 ± 12 (±0%)	85 ± 12 (+16%)	45 ± 5 (+18%)

Bili = total bilirubin; Gluc = glucose; ALP = alkaline phosphatase; AST = aspartate amino transferase

ALP = alanine amino transferase

\* = statistically significant different from control ( $p \leq 0.05$ ); \*\* = statistically significant different from control ( $p \leq 0.01$ )

Several males from all treated and control groups had elevated aspartate aminotransferase and alanine aminotransferase activities. These effects were considered to be treatment-related at 16000 and 1600 ppm in males as they were of high magnitude and/or outside the in-house historical control data. There was no effect on these parameters in females at any dose level.

The other statistically significant differences were considered not to be treatment-related in view of the variation of the individual values and/or their low magnitude.

### Urine analysis

When compared to the control groups, higher ketone levels were noted at 16000 and 1600 ppm in both sexes.

No other treatment-related change was noted for the parameters assayed. The few other statistically significant differences were considered to be incidental.

**Table 6.8.1/27-4: Semi-quantitative urinalysis- incidence summary table**

Dose (ppm) Grade n° samples examined		0	160	1600	16000	0	160	1600	16000
		males				females			
		10	10	8	10	9	10	10	10
Glucose	0	10	10	8	10	9	10	10	10
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
Bilirubin	0	10	10	8	10	9	10	10	10
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
Ketones	0	0	0	0	0	6	10	3	0
	1	0	1	0	0	3	0	1	0
	2	9	7	0	0	0	0	6	3
	3	1	2	0	0	0	0	0	4
	4	0	0	8	10	0	0	0	5
Occult blood	0	7	10	8	9	9	10	10	10
	1	1	0	0	0	0	0	0	0
	2	1	0	0	0	0	0	0	0
	3	1	0	0	1	0	0	0	0
	4	0	0	0	0	0	0	0	0
Protein	0	0	0	0	0	9	10	10	10
	1	0	1	0	2	0	0	0	0
	2	9	8	8	8	0	0	0	0
	3	1	0	0	0	0	0	0	0
	4	0	1	0	0	0	0	0	0
Urobilinogen	0	10	10	8	10	9	10	10	10
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0

### H. Organ weight

Mean absolute and relative liver weight were statistically significantly higher in male and female rats at 16000 and 1600 ppm when compared to controls. These changes were dose- and treatment related and associated with hepatocellular hypertrophy.

All other statistically significant organ weight differences were judged to be incidental in view of their individual variation and in the absence of any correlated histopathological finding.

**Table 6.8.1/27-5: Summary of liver weight data at terminal sacrifice**

Parameter Dose (ppm)	Mean (% change when compared to control)					
	Absolute liver weight (g)		Liver to body weight ratio		Liver to brain weight ratio	
	males					
0	12.15	(--)	2.327	(--)	566.930	(--)
160	11.61	(−4%)	2.258	(−3%)	546.177	(−4%)
1600	13.25*	(+9%)	2.657**	(+14%)	613.081	(+8%)
16000	14.48	(+19%)	3.102**	(+33%)	701.329**	(+24%)
Dose (ppm)	females					
0	5.96	(--)	2.243	(--)	307.108	(--)
160	6.25	(+5%)	2.343	(+4%)	316.173	(+3%)
1600	6.71*	(+13%)	2.520**	(+12%)	334.508	(+9%)
16000	7.36**	(+23%)	2.880**	(+28%)	382.160**	(+24%)

\* = statistically significant different from control ( $p \leq 0.05$ ); \*\* = statistically significant different from control ( $p \leq 0.01$ )

## J. Gross necropsy

### Unscheduled death

One male was found dead on study day 15. This animal was noted to have torsion and a dark content within the ileum and jejunum. This intestinal torsion was considered to be the cause of death and was therefore incidental. All other macroscopic findings were related to agonal changes found at the histopathology examination and were considered not to be treatment-related.

### Terminal sacrifice

With the exception of the higher incidence of foci (red or white) within the liver observed in males at 16000 ppm, all the other changes were considered to be incidental and not treatment-related.

## K. Micropathology

### Unscheduled death

In addition to agonal changes, degenerative cardiomyopathy was noted. This change is a common spontaneous finding observed in the Wistar rat of this strain and age, it was considered not to be treatment-related. The cause of death was considered to be the intestinal torsion noted at necropsy.

### Terminal sacrifice

Treatment-related histopathological changes were observed in the liver.

In all male and most females at 16000 ppm, as well as in a proportion of males at 1600 ppm, a minimal to moderate diffuse centrilobular to panlobular hepatocellular hypertrophy with ground-glass appearance of the hepatocellular cytoplasm was observed. This latter observation is usually induced by peroxisome proliferators. This change was associated with a loss of the periportal hepatocellular vacuolation observed at 16000 ppm in both sexes and at 1600 ppm in males. The effect was dose-related and correlated with the higher mean liver weight noted in these groups.

There was also a higher incidence of hepatocellular necrotic foci in males at 16000 ppm when compared to controls, which was considered to be adverse. This finding was correlated with higher individual values of aspartate aminotransferase and alanine aminotransferase activities observed in clinical chemistry evaluation.

A higher incidence of minimal to slight degenerative cardiomyopathy was noted in males at 16000 ppm. As this change is a common spontaneous finding observed in the Wistar rat of this strain and age, including in untreated control animals, with a similar severity and incidence, it was considered not to be treatment-related.

No effect of treatment was seen in any other organ examined microscopically. Some other histopathological findings were noted in animals of all groups but they were considered to be



incidental, as they were within the range of expected changes for rats of this age and strain kept under laboratory conditions.

**Table 6.8.1/27-6: Incidence and severity of microscopic changes in the liver, all animals, terminal sacrifice**

Dose (ppm)	0	160	1600	16000	0	160	1600	16000
Sex	Males				Females			
Number of animals examined	10	10	9	10	10	10	10	10
<b>Centrilobular to panlobular hepatocellular hypertrophy, diffuse</b>								
Minimal	1	0	3	1	0	0	0	5
Slight	0	0	2	6	0	0	0	4
Moderate	0	0	0	3	0	0	0	0
Total	1	0	5	10	0	0	0	9
<b>Periportal hepatocellular vacuolation, diffuse</b>								
Minimal	4	3	0	0	5	6	7	0
Total	4	3	0	0	5	6	7	0
<b>Hepatocellular necrotic focus (i), focal/multifocal</b>								
Minimal	1	0	1	5	1	0	1	1
Slight	1	1	1	1	0	1	0	0
Moderate	1	1	1	1	0	0	0	0
Total	3	2	3	7	1	1	1	1

<b>Conclusion</b>	Based on the study results (changes in haematological and clinical chemistry parameters, organ weights and histopathological liver findings) the NOAEL of the present study was established at 160 ppm in both sexes after 90-day exposure to sodium trifluoroacetate (TFA) which is equivalent to 10/12 mg/kg bw/day in males and females.
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<b>New studies; not evaluated</b>	Toxicity studies of metabolites. <b>Completion of metabolite data package. <u>Required to test the toxicological relevance of groundwater metabolite</u></b>
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<b>Report:</b>	<b>KCA 5.8.1 /28; ██████████ 2010;</b>
<b>Title:</b>	Trifluoroacetic acid: Embryo-fetal oral gavage toxicity study in rats
<b>Document No:</b>	09-4352
<b>Report No:</b>	<b>M-411209-01</b>
<b>Guidelines:</b>	US-EPA OPPTS 870.3700; OECD 414; Deviations: none
<b>GLP</b>	Yes

This study was designed to assess any maternal and/or embryo-fetal toxicity of the test article, Trifluoroacetic Acid (TFA), in the rat, following daily oral (gavage) administration during the period of major organogenesis, from implantation to the day of closure of the hard palate, Gestation Days (GD) 6 to 19, with Cesarean section on GD 20. Eighty-eight time-mated female Sprague-Dawley rats were distributed into 4 groups, each containing 22 rats. The animals were administered TFA at 0 (Control Article – distilled water), 37.5, 75 and 150 mg/kg bw/day TFA at a dose volume of 5 mL/kg by oral gavage once daily from GD 6 to 19. The following parameters were evaluated for all animals: viability, detailed physical examinations (immediately prior to dosing [within approximately 30 minutes] and approximately 2 hours after dosing), body weights, including gravid uterine adjusted body weights, food consumption and organ weights. A macroscopic postmortem evaluation was performed on GD 20, during which corpora lutea, implantation data and uterine weights were recorded. The fetuses were removed, weighed (live and recently dead fetuses), sexed and examined externally for defects. Approximately half of the fetuses were examined for soft tissue abnormalities. The other half were examined for skeletal abnormalities and ossification state. Placentas were examined and weighed. All females survived until termination.

One female each in the control and high-dose groups was not pregnant, although this was not attributed to the administration of the test article. Dosing was well-tolerated by all females, and doses up to 150 mg/kg bw/day had no adverse effect on body weight, body weight gain, food consumption, pregnancy, c-section parameters, fetal, placental, and uterine weights, organ weights, or fetal abnormalities, variations or ossification parameters. In conclusion, under the conditions of this study, the maternal and the embryo-fetal no-observed-adverse-effect-level (NOAEL) were established at 150 mg/kg bw/day TFA. Due to the non-adverse, test article-related organ weight increases, the maternal and embryo-fetal no-observed-effect-levels (NOEL) were established at 75 mg/kg bw/day (maternal) and 150 mg/kg bw/day (embryo-fetal).

## MATERIALS AND METHODS

Twenty-two time-mated females per group were dosed once daily by oral gavage on GD 6 to 19. Terminal cesarean sections occurred on GD 20 with evaluation of pregnancies for pre- and post-implantation loss, enumerating corpora lutea, live and dead/resorbed implantations. The fetuses were examined externally, weighed and preserved. Approximately half of the fetuses from each litter were examined subsequently for soft-tissue abnormalities, the remaining half for skeletal abnormalities.

Group		Dose (once daily, oral gavage)			Number of females
		Dose <sup>a</sup> (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Main Animals
1	Control	0	0	5	22
2	Low	37.5	7.5	5	22
3	Intermediate	75	15	5	22
4	High	150	30	5	22

<sup>a</sup>Doses represent active ingredient.

### 1. TEST GUIDELINES:

This study complies with the following: •Pre-natal Development Toxicity Study ref. OECD TG 414

### 2. TEST ARTICLE:

Trifluoroacetic Acid (TFA)

### 3. SUPPLIER:

Aldrich Chemical Company; 6950 Ambassador Drive; Allentown, Pennsylvania 18106

### 4. LOT NUMBER:

61496MK

### 5. PURITY:

99% (assumed 100% for purposes of preparation of dose formulations)

### 6. DESCRIPTION:

Colorless to very faint yellow liquid

### 7. CONTROL (VEHICLE):

Distilled Water

Lot Number DDW0007

### 8. TEST ANIMALS

Albino Rats (Outbred) VAF/Plus CD (Sprague-Dawley derived) [CrI:CD<sub>v</sub> (SD) IGS BR]

- supplier

████████████████████  
████████████████████

-number of animals

Received/Placed on test: 88 time-mated females (22 per group)

**-age at receipt**

The animals were approximately 10 to 12 weeks of age at receipt on GD 1 to 3, where GD 0 was the day of detection of a copulatory plug *in situ* and/or sperm in a vaginal smear.

**- weight at initiation of dosing**

The weight range was 245 to 299 grams at commencement of dosing.

**-stabilization period**

A 3 to 5 day stabilization period was completed prior to commencement of dosing as dictated by the mating and receipt

**10. EXPERIMENTAL EVALUATIONS****- viability checks**

Animals were observed in their cages twice daily (once in the morning and once in the afternoon) for mortality, morbidity and signs of severe toxic or pharmacologic effects.

**11. DETAILED PHYSICAL EXAMINATIONS**

All animals on study were examined daily from GD 4 through terminal euthanasia (GD 20). Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration. During the stabilization period, and during the post-dosing phase, these evaluations were performed once daily. During the treatment period, these examinations were performed prior to dosing (within approximately 30 minutes) and at approximately 2 hours after dosing. Observations after dosing were limited to recording transient signs directly associated with dosing.

**12. BODY WEIGHTS**

Body weights were recorded for all animals on GD 4 and daily from GD 6 through 20.

**13. FOOD CONSUMPTION**

Feed was available without restriction 7 days/week. A weighed quantity of feed was presented to each animal daily from GD 4 to GD 18. After 2 or 3 days, any feed remaining was weighed

**14. STATISTICAL EVALUATIONS**

All statistical analyses were performed using the individual animal (or litter) as the basic experimental unit. For litter/fetal findings, the litter was the treated experimental unit and the basis for statistical analysis and biological significance was assessed with relevance to the severity of the anomaly and the incidence of the finding within the background control population.

**15. Continuous Data:**

- Maternal body weights, using absolute weights and body weight changes over appropriate study periods
- Food consumption, over appropriate study periods
- Fetal and placental weights per litter

## 16. Discrete Data (Count, Binomial or Incidence)

- Litter size
- Corpora lutea, implantation sites, percent pre- and post-implantation loss (resorptions and early/late fetal deaths), sex ratio per litter
- Incidence of fetal external, visceral and skeletal abnormalities per litter

## Results And Discussion

- **maternal data:** All females survived to termination of the study.

- **clinical observations:** There were no test article-related clinical observations

- **gestation body weights:**

There were no test article-related effects on gestation body weight or body weight change.

Slight decreases in body weight gain pretreatment occurred at 37.5 and 75 mg/kg bw/day. After the initiation of treatment, there was a slight, non-statistically-significant decrease in body weight gain at  $\geq 75$  mg/kg/day (7% and 18%, respectively). There was a statistically-significant increase in body weight gains during GD 12 to 15 (+17%, +26% and +21%, respectively) at  $\geq 37.5$  mg/kg bw/day. These body weight changes were transitory and were not considered adverse

## 17. Gestation food consumption

There were no test article-related effects on food consumption. Food consumption was slightly reduced during GD 6 to 9 at  $\geq 75$  mg/kg bw/day, and GD 18 to 20 at 37.5 and 75 mg/kg bw/day, although these decreases were 6% or less that of control means. These food consumption changes were transitory and were not considered adverse.

Females	Mean Gestation Food Consumption (g/animal/day)								Table 5
Phase Day	GES 4-6	6-9	9-12	12-15	15-18	18-20	6-20		
Group 1 - 0.0 mg/kg/day									
Mean	29.8	29.0	31.1	32.4	33.6	28.0	31.0		
SD	4.06	3.61	3.81	3.90	4.09	5.56	3.69		
N	21	21	21	21	21	21	21		
Group 2 - 37.5 mg/kg/day									
Mean	29.4	28.9	30.5	31.6	32.4	26.4	30.2		
SD	2.67	2.14	2.98	2.97	3.25	4.50	2.58		
N	22	22	22	22	22	22	22		
Group 3 - 75.0 mg/kg/day									
Mean	28.5	27.5	30.0	31.8	32.1	26.6	29.8		
SD	2.63	2.16	3.16	2.78	2.92	3.82	2.46		
N	22	22	22	22	22	22	22		
Group 4 - 150.0 mg/kg/day									
Mean	29.2	27.7	30.4	32.1	33.2	28.6	30.5		
SD	3.56	2.16	3.10	2.51	2.90	3.00	2.28		
N	21	21	21	21	21	21	21		

No statistically significant differences from control mean

## 18. Organ weights

There were no adverse test article-related effects on liver and kidney weights. Slight, test article-related, statistically-significant increases in liver and kidney weights were observed at 150 mg/kg bw/day (+9.6% and 5.6%, respectively). These increased liver and kidney weights were not considered adverse.

Females	Mean Organ Weights		Table 10
	Kidneys (g)	Liver (g)	
Group 1 – 0.0 mg/kg/day			
Mean	1.9683	15.5760	
S.D.	0.19102	2.29569	
N	22	22	
Group 2 – 37.5 mg/kg/day			
Mean	2.0497	15.6756	
S.D.	0.15422	2.32189	
N	22	22	
Group 3 – 75.0 mg/kg/day			
Mean	2.0573	15.7141	
S.D.	0.15592	2.08299	
N	22	22	
Group 4 – 150.0 mg/kg/day			
Mean	2.0778*	17.0649*	
S.D.	0.15989	2.00985	
N	22	22	
* = p<0.05			

**Maternal macroscopic postmortem****Evaluations**

There were no test article-related macroscopic observations.

	Incidence Summary Report for Gross Necropsy Observations Preface	Table 12
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Corresponding dose levels for each group were as follows:

Group 1	-	0 mg/kg/day
Group 2	-	37.5 mg/kg/day
Group 3	-	75 mg/kg/day
Group 4	-	150 mg/kg/day

**Pregnancy outcome and cesarean section****Data**

One female each in the control group and high dose group (150 mg/kg bw/day) was not pregnant at terminal sacrifice. This was not considered to be related to administration of Trifluoroacetic Acid. There was no difference between the groups pertaining to the number of corpora lutea, number of implantations, resorptions, dead, or live fetuses, pre- or post-implantation loss, or sex ratio (% male). The statistically significant decrease in post-implantation loss at 75 mg/kg bw/day was not considered dose-related or toxicologically relevant.

Females	Pregnancy Summary								Table 6
Dose Group Dose Level (mg/kg/day)	1 0		2 37.5		3 75		4 150		
	n	%	n	%	n	%	n	%	
Number of females entered on study (mated)	22		22		22		22		
- Non pregnant	1	(5)	0	(0)	0	(0)	1	(5)	
- Pregnant	21	(95)	22	(100)	22	(100)	21	(95)	
Pregnant with termination before scheduled date	0		0		0		0		
Pregnant at scheduled termination	21		22		22		21		
- With total implantation loss	0	(0)	0	(0)	0	(0)	0	(0)	
- With viable fetuses	21	(100)	22	(100)	22	(100)	21	(100)	

Females	Summary of Cesarean Section Data									Table 7		
	Corpora Lutea	Implantations	Early	Resorptions Late	Total	Dead Fetuses	Male	Live Fetuses Female	Total	Sex Ratio (% Male)	Pre Impl. Loss (%)	Post Impl. Loss (%)
Group 1 - 0.0 mg/kg/day												
Mean	13.2	12.7	1.0	0.0	1.0	0.0	5.5	6.1	11.6	46.4	4.6	9.1
SD	2.57	2.80	1.16	0.00	1.16	0.00	2.56	1.97	3.02	13.49	7.97	10.52
N	21	21	21	21	21	21	21	21	21	21	21	21
Group 2 - 37.5 mg/kg/day												
Mean	13.9	13.4	0.7	0.0	0.7	0.0	6.7	6.0	12.7	51.9	3.6	5.0
SD	1.60	1.33	1.39	0.00	1.39	0.00	2.06	1.54	1.81	14.15	5.34	10.08
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 3 - 75.0 mg/kg/day												
Mean	14.6	13.7	0.4	0.0	0.4	0.0	6.5	6.9	13.4	48.1	5.7	2.6*
SD	2.17	1.98	0.58	0.00	0.58	0.00	2.46	2.08	1.94	15.88	6.27	4.12
N	22	22	22	22	22	22	22	22	22	22	22	22
Group 4 - 150.0 mg/kg/day												
Mean	14.2	13.3	1.3	0.0	1.4	0.0	5.8	6.1	11.9	48.8	4.7	10.0
SD	3.51	2.22	2.20	0.22	2.20	0.00	2.02	2.35	2.41	15.08	8.52	13.57
N	21	21	21	21	21	21	21	21	21	21	21	21

\* = p < 0.05, Impl. = Implantation

## Fetal data

### Gravid uterine, placental and fetal weights

There were no test article-related effects on gravid uterine, placental, or fetal weight

Females	Summary of Gravid Uterine Adjusted Body Weights (grams)						Table 8
	Bodyweight on Day 6	Bodyweight on Day 20	Bwt change Days 6-20	Gravid Uterine Weight	Adj Bwt Day 20	Adj bwt change Days 6-20	
Group 1 - 0.0 mg/kg/day							
Mean	275	383	109	74.43	309	34	
SD	15	31	20	17.813	27	20	
N	21	21	21	21	21	21	
Group 2 - 37.5 mg/kg/day							
Mean	275	386	112	81.87	304	30	
SD	12	20	13	9.386	21	15	
N	22	22	22	22	22	22	
Group 3 - 75.0 mg/kg/day							
Mean	269	384	115	85.27	299	30	
SD	9	21	15	11.391	16	12	
N	22	22	22	22	22	22	
Group 4 - 150.0 mg/kg/day							
Mean	276	386	111	78.64	308	32	
SD	10	20	16	13.848	18	12	
N	21	21	21	21	21	21	

No statistically significant differences from Control mean, Adj Bwt = adjusted body weight.



Females	Mean Litter Placental and Fetal Weights (grams)					Table 9
	Placental Weight	Total Litter Weight	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight	
Group 1 – 0.0 mg/kg/day						
Mean	0.6	46.0	4.0	3.9	4.0	
SD	0.06	12.19	0.39	0.29	0.30	
N	21	21	21	21	21	
Group 2 – 37.5 mg/kg/day						
Mean	0.6	51.3	4.2	3.9	4.1	
SD	0.09	6.40	0.25	0.22	0.23	
N	22	22	22	22	22	
Group 3 – 75.0 mg/kg/day						
Mean	0.5	54.1	4.2	4.0	4.0	
SD	0.06	8.34	0.20	0.18	0.18	
N	22	22	22	22	22	
Group 4 – 150.0 mg/kg/day						
Mean	0.6	47.5	4.1	3.9	4.0	
SD	0.05	9.38	0.26	0.25	0.26	
N	21	21	21	21	21	

No statistically significant differences from control mean

### Fetal observations: external, visceral and skeletal findings

There were no test article-related effects on the incidence of external, visceral, skeletal, or ossification malformations or variations. Most of the malformations observed during this study were from one high dose litter (#4501). The litter incidence of malformations observed were not considered test article-related because the incidences fell within published Historical Control Data<sup>1</sup>. All other findings were considered to be infrequent, not dose-related, and consistent with normal background variation. External Evaluations Administration of TFA up to 150 mg/kg bw/day did not result in any test article-related external malformations or variations. Malformations consisted of gastroschisis, omphaloceles, and limb flexures, although all occurred within the same litter (# 4501). MARTA HCD indicates the following expected malformation incidences: gastroschisis is expected to occur in at least 1 litter out of 21 litters (maximum incidence: 6.7%), limb flexure in at least 2 litters out of 21 (maximum incidence: 10%), and omphaloceles in at least 1 litter out of 21 (maximum incidence: 8%).

#### Visceral malformations:

The presence of one retroesophageal subclavian artery at 37.5 mg/kg bw/day was not considered a test article-related effect as it did not occur in a dose related manner and there were no associated findings. This malformation is expected to occur in 2 litters for a group size of 22 litters evaluated (maximum incidence: 9.1%). The omphaloceles at 150 mg/kg/day were visceral confirmations of the external findings of omphalocele. Additionally, the folded folded retina of 6.3% (or 1 finding for a group size of 21 litters evaluated).

#### Skeletal malformations

Findings of partially-fused ribs (4 fetuses) and thoracic vertebral arches (2 fetuses), as well as a cleft/split sternum (1 fetus) were considered non-test article-related as they were present in one litter at 150 mg/kg bw/day and the incidences were within historical control ranges. The maximum incidence for fused ribs is 5.6% (or 1 litter for a group size of 21 litters evaluated), for fused thoracic vertebral arches is 4% (or 1 litter for a group size of 21 litters evaluated), and for a cleft/split sternum is 13% (or 2 litters for a group size of 21 litters evaluated).

**Table-1 Major malformations**

Dose level (mg/kg/day)	Dam No.- Fetus No.	External	Visceral	Skeletal
0	-	-	-	-
37.5	2504-3	-	<u>Subclavian artery-</u> <u>retroesophageal</u>	-
75	-	-	-	-
150	4501-1	<u>Umbilicus-</u> <u>omphalocele</u> ; <u>Hindlimbs-</u> flexure, malrotated;	-	<u>Ribs-</u> partially fused
	4501-2	<u>Umbilicus-</u> <u>omphalocele</u>	<u>Abdomen-</u> omphalocele; <u>Eye-</u> retina, bilateral, folded	-
	4501-3	<u>Umbilicus-</u> <u>omphalocele</u>	-	<u>Ribs-</u> partially fused; <u>Sternum-</u> partially cleft/split; <u>Thoracic vertebral</u> <u>arches-</u> partially fused
	4501-4	<u>Abdomen-</u> <u>gastroschisis</u> ; <u>Hindlimbs-</u> flexure, malrotated	-	<u>Ribs-</u> partially fused; <u>Thoracic vertebral</u> <u>arches-</u> partially fused
	4501-6	<u>Umbilicus-</u> <u>omphalocele</u>	<u>Abdomen-</u> omphalocele	-
	4501-9	<u>Umbilicus-</u> <u>omphalocele</u>	-	<u>Ribs-</u> partially fused

<b>Conclusion</b>	<p>All females survived until termination.</p> <p>One female each in the control and high-dose groups was not pregnant, although this was not attributed to the administration of the test article. Dosing was welltolerated by all females, and doses up to 150 mg/kg bw/day had no adverse effect on body weight, body weight gain, food consumption, pregnancy, cesarean section parameters, fetal, placental, and uterine weights, organ weights, or fetal abnormalities, variations, or ossification parameters.</p> <p>In conclusion, under the conditions of this study, the maternal and the embryo-fetal NOAEL were established at 150 mg/kg bw/day TFA. Due to the non-adverse, test article-related organ weight increases, the maternal and embryo-fetal no-observed-effect-levels (NOEL) were established at 75 mg/kg bw/day (maternal) and 150 mg/kg bw/day (embryo-fetal).</p>
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### B.6.8.2 Supplementary studies on the active substance

#### Summary of supplementary studies

##### Flufenacet

In a mechanistic study, male rats were provided thyroid hormone replacement therapy via osmotic minipumps and then fed diets of FOE 5043. The data suggested that FOE 5043-induced alterations in serum thyroid hormone levels, most notably serum thyroxine ( $T_4$ ), are being mediated indirectly. Specifically, a chemically-induced increase in hepatic  $T_4$  metabolism, implied by the gross and histopathologic changes in the liver, rather than through a mechanism of direct chemical interference with the synthesizing/secretory functions of the thyroid gland is strongly suggested. This study was conducted to investigate the hypothesis that the effects of FOE 5043 on the thyroidal economy of the male rat were secondary to chemical induction of the liver's capacity to clear from the circulation, metabolize, and excrete thyroxine.

In this mechanistic study, the structural and/or functional integrity of each potential target site or sites comprising the hypothalamic-pituitary-thyroid-hepatic axis was examined in male rats (CDF[F-344]/BR) following exposure to FOE 5043. Twenty one days of treatment with FOE 5043 (purity ~97.2%) at a rate of 1000 ppm as a dietary admixture was found to significantly increase the clearance of [ $^{125}$ I] $T_4$  from the serum, suggesting an enhanced excretion of the hormone. In the liver, the activity of hepatic uridine glucuronosyl transferase (UDP-GT), a major pathway of thyroid hormone biotransformation in the rat, increased in a statistically significant and dose-dependent manner; conversely, hepatic 5' monodeiodinase activity trended downward with dose. Bile flow as well as the hepatic uptake and biliary excretion of [ $^{125}$ I] $T_4$  were increased following exposure to FOE 5043. Thyroidal function, as measured by the discharge of iodide ion in response to perchlorate, and pituitary function, as measured by the capacity of the pituitary to secrete thyrotropin (TSH) in response to an exogenous challenge by hypothalamic thyrotropin releasing hormone (TRH), were both unchanged from the controlled response.

These data suggest that the functional status of the thyroid and pituitary glands have not been altered by treatment with FOE 5043, and that reductions in circulating levels of  $T_4$  are being mediated indirectly through an increase in the biotransformation and excretion of thyroid hormone in the liver. Please refer to the Monograph and baseline dossier KCA 5.8.2, M-004982-03-1, M-012231-01-2, M-012226-01-1)

A mechanistic study was conducted with FOE-thiadone (M09) in order to test the hypothesis that the neurotoxicity in high-dose dogs given parent compound was likely caused by metabolic limitations. The study provides a preponderance of scientific support for the conclusion that limitations in glutathione interdependent pathways and antioxidant stress resulted in metabolic lesions in the brain and heart of dogs. (M-004978-01-1)

**For registration of flufenacet in the United States (US), a developmental neurotoxicity study was conducted based on thyroid-related findings and therefore, the potential for affecting development of the nervous system. In this study dietary exposure to flufenacet did not cause any neurotoxic effect in parental and offspring animals. Treatment-related findings consisted of reduced food consumption and a reduction in maternal body weights during gestation and in males at the mid- and high-dose. Body weights were also reduced in mid- and high-dose F1-males and high-dose F1-females. F1 offspring of these dose groups exhibited also a delay in development (eye opening, preputial separation), for details please refer to supplemental**

**dossier MCA 5.7.1.**

Furthermore, the US EPA required a special comparative thyroid sensitivity assay with flufenacet in neonatal and adult (pregnant and lactating) female rats in order to investigate potential neonatal susceptibility to thyroid-related neurodevelopmental effects. Besides the range-finding study, two dietary studies were conducted to evaluate the effects of flufenacet on thyroid endpoints in pregnant and lactating rats and their offspring during fetal and post-natal development.

Dietary exposure to flufenacet during pregnancy from gestation day 6 to 20 revealed no adverse effects up to the top dose tested in dams and foetuses. Slight (non-statistical) decrease in T4 showed no compensatory thyroid response.

Dietary exposure to flufenacet during pregnancy and lactation from gestation day 6 to lactation/post natal day 21 induced a slight decrease in maternal body weight gain resulting in lower body weight and decreases in T4 and T3 with thyroid follicular cell hypertrophy in two dams. In post natal day (PND) 21 pups, the highest dose tested (500 ppm) reduced body weight and weight gain, with slightly lower T3 in males and females. Thus, these results support 16.7 mg/kg bw/day (100 ppm) flufenacet as a NOAEL and 84.2 mg/kg bw/day (500 ppm) as a LOAEL in the dam and offspring with dietary exposure during pregnancy/gestation and lactation.

Flufenacet administration once daily by gavage from PND 10 to 20 to male and female pups at 1.7 mg/kg bw/day had no effect on the thyroid or any other endpoint measured. Thus, 1.7 mg/kg bw/day is a NOAEL in pre-weaning rats.

**Table 6.8.2-1: Summary of additional studies on the active substance**

Study	Sex	NOAEL (mg/kg bw/d)	LOAEL	Main effects seen at LOAEL	Reference
Rat developmental neurotoxicity diet	Dam	1.7/3.0	8.3/15	Dam: BW ↓, food intake ↓ (gestation)	██████████, 2000 M-026105-01-1 also cited MCA 5.7.1
	Pup	(DG 6-21/DL 1-12)		Pup: BW/BWgain ↓, rel. food intake ↑, delayed development (eye opening, preputial separation)	
Rat range-finder diet	Dam	Na	Na	500 ppm maternal and pup toxicity	██████████, 2012 M-434509-01-1
	Pup	(DG 6-DL 10 or 16)			
Rat mechanistic study thyroid effects, diet	Dam	35 (500 ppm)	--	Slight changes in T4 without correlating changes in T3 and TSH, as well as histopathological changes in the thyroid	██████████, 2012 M-435619-01-1
	Fet	(DG 6-20)			
Rat mechanistic study thyroid effects, diet	Dam	13 (100 ppm)	65 (500 ppm)	Dams: BW ↓, T4 ↓ (-70%), T3 ↓ (-19%), rel. liver weight ↑, thyroid follicular cell hypertrophy 2 cases Pups: BW/gain ↓, T3 ↓ (-24 %)	██████████, 2012 M-435313-01-1
	Pup	(DG 6-DL21)			
Rat mechanistic study thyroid effects, gavage	Pup	1.7	--	No adverse effects.	██████████, 2012 M-435126-01-1
		(DL 10-21)			

n.a. = not applicable, BW = body weight, Fet = foetuses, DG = Day gestation, DL = Day lactation

**Toxicological studies conducted with FOE-hydroxy, FOE-(TDA)-sulfone and FOE-acetate are considered supportive to justify the limits of specified impurities.**

### **FOE 5043-hydroxy**

FOE 5043-hydroxy showed no genotoxicity potential in the bacterial reverse mutation assay. The substance was moderately toxic after acute oral, and slightly toxic after acute inhalation exposure. FOE 5043-hydroxy was not irritating to the skin and irritating to eyes of rabbits, and showed no skin sensitizing potential under the conditions of the Magnusson-Kligman test.

**Table 6.8.2- 2: Summary of studies with FOE 5043-hydroxy**

Study	Dose	Result	Reference
<b>Bacterial reverse mutation assay</b>	8-5000 mg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Herbold, 1993 M-004586-01-1
<b>Acute oral, rat</b>	500-800-1000 mg/kg bw (m) 200-400-500 mg/kg bw (f)	LD <sub>50</sub> approx. 726 mg/kg bw (m) LD <sub>50</sub> approx. 474 mg/kg bw (f)	██████████, 1992 M-004579-01-1
<b>Acute inhalation, rat (4 hours)</b>	0-301-6802 mg/m <sup>3</sup>	LC <sub>50</sub> > 6802 mg/m <sup>3</sup> (males) LC <sub>50</sub> ≈ 6800 mg/m <sup>3</sup> (females)	██████████, 1993 M-004589-01-2
<b>Skin irritation, rabbit</b>	0.5 g/patch (undiluted)	Not irritating	██████████, 1992 M-004564-01-1
<b>Eye irritation, rabbit</b>	0.1 mL/animal (undiluted)	Irritating According to CLP criteria	██████████, 1992 M-004564-01-1
<b>Skin sensitization, Guinea pig (MKT*)</b>	Intradermal: 5% Topical: 50% Challenge: 25%	Not sensitizing	██████████, 1994 M-004614-01-2

\*MKT = Magnusson Kligman maximisation test

### **FOE 5043-TDA sulfone (synonym FOE 5043-sulfone)**

FOE 5043-TDA sulfone showed no genotoxic potential in the bacterial reverse mutation assay. The substance was moderately toxic after acute oral, and highly toxic after acute inhalation exposure. The substance was irritating to the skin and severely irritating to eyes of rabbits, and showed also a skin sensitizing potential under the conditions of the Magnusson-Kligman test. After inhalation a severe sensory irritation potential with a non-irritant threshold concentration of 0.3 mg/m<sup>3</sup> was observed. Signs of respiratory tract irritation were observed at concentrations of ≥0.5 mg/m<sup>3</sup>.

**Table 6.8.2- 3: Summary of studies with FOE 5043-Sulfon**

Study	Dose	Result	Reference
Bacterial reverse mutation assay	8-5000 mg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Herbold, 1993 M-004606-01-1
Acute oral, rat	50-100-150-170-200-300-1000 mg/kg bw	LD <sub>50</sub> > 150 - < 2000 mg/kg bw	██████████, 1992 M-004578-01-1
Acute inhalation, rat (4 hours)	Dust: 0-35.3-122.7 mg/m <sup>3</sup> Aerosol: 0-8.2-52.6-89.8-146.3 mg/m <sup>3</sup>	LC <sub>50</sub> ≈ 69 mg/m <sup>3</sup> (males) LC <sub>50</sub> > 146.3 mg/m <sup>3</sup> (females)	██████████, 1992 M-004576-01-1
Skin irritation, rabbit	0.5 g/patch (undiluted)	Irritating	██████████, 1992 M-004522-01-1
Eye irritation, rabbit	0.1 mL/animal (undiluted)	Severely irritating	██████████, 1992 M-004522-01-1
Skin sensitization, Guinea pig (MKT**)	Intradermal: 5% Topical: 6% Challenge: 1 and 0.5%	Sensitizing	██████████, 1994 M-004673-01-1
Sensory irritation, mice (45 min)	0-4.3-9.8-13.3 mg/m <sup>3</sup>	Severe sensory irritation potential non-irritant threshold concentration 0.3 mg/m <sup>3</sup>	██████████, 1993 M-004601-01-1
Sub-acute inhalation, range finder, rat (5 x 6h/day)	0-0.5-3.5-16.3 mg/m <sup>3</sup>	NOAEC: 0.5 mg/m <sup>3</sup> LOAEC: 3.5 mg/m <sup>3</sup> (slight body weight changes, respiratory tract irritation, hypothermia caused by irritation) Mortality occurred at 16.3 mg/m <sup>3</sup>	██████████, 1992 M-004571-01-2
Sub-acute inhalation, rat (5 x 6h/day) 28-days	0-0.47-2.04-7.63 mg/m <sup>3</sup>	LOAEC: 0.47 mg/m <sup>3</sup> Inflammatory changes in the upper respiratory tract, sensory irritation, effect on body weight, clinical signs	██████████, 1994 M-004779-01-1

\*\*MKT = Magnusson Kligman maximisation test

### **FOE 5043-acetate**

FOE 5043-acetate was moderately toxic after acute oral and non-toxic after acute inhalation exposure. It was not irritating to the skin and eyes of rabbits.

**Table 6.8.2- 4: Summary of studies with FOE 5043-acetate**

Study	Concentration range Dose level tested	Result	Author / Reference
Acute oral, rat	50-200-1000 mg/kg bw	LD <sub>50</sub> > 1000 mg/kg bw (m) LD <sub>50</sub> > 200 < 1000 mg/kg bw (f)	██████████, 1994 M-004640-01-1
Acute inhalation, rat (4 hours)	0-2350 mg/m <sup>3</sup>	LC <sub>50</sub> > 2350 mg/m <sup>3</sup> (maximum technically attainable concentration)	██████████, 1996 M-004734-01-1
Skin irritation, rabbit	0.5 g/patch	Not irritating	██████████, 1994 M-004662-01-1
Eye irritation, rabbit	0.1 mL/animal	Not irritating	██████████, 1994 M-004662-01-1

**Flufenacet- supplementary studies**

<b>New studies; not evaluated</b>	This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. (2010). <b>Requested by non-EU authorities, relevant for reference dose derivation.</b>
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<b>Report:</b>	<b>KCA 5.8.2 /06; [REDACTED] 2012;</b>
<b>Title:</b>	FOE 5043 (flufenacet) - A tolerability and pilot study to verify the exposure of offspring during lactation when administered via the diet to Sprague-Dawley rats
<b>Document No:</b>	<b>M-434509-01</b>
<b>Report No:</b>	SA 10153
<b>Guidelines:</b>	not applicable; Deviations: not applicable
<b>GLP</b>	Yes

**Materials and methods****A. Materials****1. Test material:**

Description:	Flufenacet
Lot/Batch no:	Beige solid
Purity:	NK61AX0177
Stability of test compound:	96.8%
	guaranteed for study duration; expiry date: 2012-09-03

**2. Vehicle:**

Plain diet

**3. Test animals**

Species:	Rat
Strain:	Sprague-Dawley, Crl:CD (SD)
Age:	11 to 13 weeks
Weight at dosing:	285 – 334 g
Source:	[REDACTED]
Acclimatisation period:	At least 3 days
Diet:	A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), <i>ad libitum</i>
Water:	Filtered and softened tap water from municipal water supply, <i>ad libitum</i> .
Housing:	Individual housing of pregnant females in suspended stainless steel wire mesh cages.

**B. Study design and methods****1. Animal assignment and treatment**

Dose	0-500 ppm corresponding to 0-35.7 mg/kg bw/day (gestation) and 67.7 mg/kg bw (lactation)
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Duration:	Gestation day (GD) 6 through lactation (LD)/ postnatal day (PND) 10 or LD/PND 16
Application route:	Oral (diet)
Group size:	10 females
Observations:	Diet analyses, mortality, clinical signs, body weight, food consumption, TSH, T4, flufenacet and thiadone concentrations in liver tissue, gross pathology, litter weight

## Results and discussion

### A. Dose formulations analysis

Homogeneity analysis revealed concentrations were within the range of 94 to 103% of nominal. Achieved concentrations were 98% of nominal. The results were within the in-house target range of 85 to 115% of nominal and therefore within the acceptable range.

At 500 ppm, flufenacet was found to be stable in the diet over a 10-day period at room temperature or a 24-day freezing period followed by 14 days at room temperature. Results were within the in-house target range of 85 to 115% of nominal concentration, with the exception of the mean value measured after 24 days frozen storage and 14 days at room temperature, which was very close to the lowest target value of acceptability (83%). Therefore, the stability of test item in diet was considered to be acceptable under the study conditions.

### B. Maternal data

#### Mortality

There were no mortalities in dams.

#### Clinical signs

There were no treatment-related clinical signs observed in any dam.

#### Body weight

In dams, there was a slightly reduced mean cumulative body weight gain between gestation day (GD) 13 and 20 (12%,  $p \leq 0.05$ ), when compared to controls. During lactation, maternal body weight gain parameters were unaffected by treatment.

**Table 6.8.2/06-1: Summary of maternal body weight and body weight changes (mean  $\pm$  standard deviation)**

Dose (ppm)	Body weight (g)		Body weight change (g) GD 13-20
	GD 13	GD 20 (mean $\pm$ standard deviation)	
0	35.5 $\pm$ 9.14	125.3 $\pm$ 16.01	89.9 $\pm$ 9.20
500	32.9 $\pm$ 11.99	111.7 $\pm$ 15.33	78.8 $\pm$ 9.09*T
Dose (ppm)	Body weight (g)		
	LD 4	LD 7 (mean $\pm$ standard deviation)	LD 14
0	13.7 $\pm$ 13.21	28.2 $\pm$ 13.06	34.9 $\pm$ 19.46
500	14.8 $\pm$ 11.01	24.4 $\pm$ 10.77	38.2 $\pm$ 18.44

\* Significantly different from the vehicle control group value ( $p \leq 0.05$ ).

T Student T test

GD = gestation day



Food consumption and compound intake

There were no treatment-related effects on food consumption noted.

The mean achieved dose levels in mg/kg bw/day received by the animals during the study are summarised in the following table.

**Table 6.8.2/06-2: Summary of maternal food consumption**

Dose	Mean achieved dietary intake of flufenacet (mg/kg bw/day)						Mean lactation phase
	GD 6-13	GD 13-20	Mean gestation phase	LD 0-4	LD 4-7	LD 7-14	
500 ppm	35.2	36.2	<b>35.7</b>	50.2	69.1	77.2	<b>67.7</b>

Terminal body weight

There was no relevant change in mean terminal body weight in treated dams, compared to the controls.

Gross pathology

At necropsy, enlarged liver was noted in 4/10 treated dams.

**C. Fetal data**Mortality

An increased number of dead pups was noted in the flufenacet-treated group at parturition (live-birth index of 93.8%, compared to 99.5% in the control group) and between PND 0 and 2 (viability index of 88.3%, compared to 92% in the control group).

Pup and litter weights

Pup weights in the 500 ppm group were slightly lower.

When compared to controls, the litter weights in the treated group were lower by between 10 and 16% over the lactation period.

**Table 6.8.2/06-3: Summary of pup body weights and litter weights**

Dose (ppm)	Pup weight (g)		
	PND 4	PND 7 (mean ± standard deviation)	PND 14
0	10.24 ± 1.27	15.70 ± 2.92	34.26 ± 5.29
500	8.94 ± 1.23	13.48 ± 1.61	30.99 ± 3.35
Dose (ppm)	Litter weight (g)		
	PND 4	PND 7 (mean ± standard deviation)	PND 14
0	98.14 ± 14.06	149.51 ± 37.73	186.99 ± 55.05
500	84.94 ± 19.62	125.43 ± 31.50	167.40 ± 45.28

**Table 6.8.2/06-4: Summary of observations at caesarean section**

Parameter	Dose (ppm)	0	500
Number of rats tested (n)		10	10
Number of pregnant rats (n)		10	10
Mean gestation length (days)		23.1	23.3
Total number of fetuses at parturition		146	142
Live fetuses (n)		145	132
Dead fetuses (n)		0	0
Fetus viability uncertain (n)		1	10
Sex male / female (n)		65 / 68	56 / 59
Number of implantations (mean ± SD)		15.0 ± 2.67	15.4 ± 2.22
Number of pups (mean ± SD)		14.6 ± 2.84	14.2 ± 2.39
Live pub at PND 0 (mean ± SD)		14.5 ± 2.68	13.2 ± 2.25
Live pub at PND 0 (mean ± SD)		13.2 ± 3.16	11.5 ± 2.80
Live birth index (%) (mean ± SD)		99.5 ± 1.66	93.8 ± 11.79
Viability index (%) (mean ± SD)		92.0 ± 17.19	88.3 ± 19.41

Hormone analyses

In PND 10 pups, mean TSH concentration was moderately higher when compared to the controls:

- +71% (not statistically significant) for male pups,
- +104% (not statistically significant) for female pups,
- +83% (statistically significant at  $p \leq 0.01$ ) for combined sexes.

On PND 16, mean TSH concentration was slightly higher in females only, +38% (not statistically significant) relative to the control group. There were no statistically significant differences in T<sub>4</sub>-levels.

Hormone analyses data are summarized in the following tables.

**Table 6.8.2/06-5: Summary of TSH hormone analyses in pups**

Dose (ppm)	TSH concentration (ng/mL) mean ± standard deviation (% change when compared to controls)			
	PND 10 pups		PND 16 pups	
	0	500	0	500
Males	0.31 ± 0.31	0.53 ± 0.22 (+71%)	0.72 ± 0.25	0.75 ± 0.32 (+4%)
Females	0.26 ± 0.24	0.53 ± 0.34 (+104%)	0.56 ± 0.11	0.77 ± 0.31 (+38%)
Males + females	0.29 ± 0.27	0.53 ± 0.28** (+83%)	0.64 ± 0.20	0.76 ± 0.31 (+19%)

\*\* Significantly different from the vehicle control group value ( $p \leq 0.01$ ).

**Table 6.8.2/06-6: Summary of T<sub>4</sub> hormone analyses in pups**

Dose (ppm)	T <sub>4</sub> concentration (µg/mL) mean ± standard deviation			
	PND 10 pups		PND 16 pups	
	0	500	0	500
Males	1.7 ± 0.4	1.5 ± 0.3	3.4 ± 0.4	3.3 ± 0.7
Females	2.0 ± 0.4	1.7 ± 0.5	3.4 ± 0.4	3.3 ± 0.7
Males + females	1.8 ± 0.4	1.6 ± 0.4	3.4 ± 0.6	3.4 ± 0.6

Gross pathology

7/37 pups necropsied on PND 10 and 6/37 pups necropsied on PND 16 had prominent lobulation of the liver, compared to 0/40 and 1/38 pups in the control group, respectively.

Test substance / metabolite analysis in liver tissues

Analyses of liver tissues collected from pups at PND 10 and 16 revealed presence of thiadone, a main metabolite of flufenacet. The parent compound was below the limit of detection (LOD).

**Table 6.8.2/06-7: Summary of analyses in liver tissue**

Flufenacet dose (ppm)	Concentration in liver (mean ± standard deviation)			
	Flufenacet		Thiadone	
	Extract (µg/L)	Tissue (µg/g)	Extract (µg/L)	Tissue (µg/g)
<b>PND 10</b>				
0	ND	ND	<50	--
500	ND	ND	0.56 ± 0.11	1.82 ± 0.42
<b>PND 16</b>				
0	<10	--	<50	
500	<10	--	137 ± 41	1.13 ± 0.31

<b>Conclusion</b>	<p>Based on the study results flufenacet at dietary exposure of 500 ppm induced maternal and pup toxicity. <u>Thus, 500 ppm was considered to be an appropriate dose level for subsequent toxicity assays.</u></p> <p>The study results showed clear evidence that pups were exposed during lactation to flufenacet and/or its major metabolite when administered to dams via the diet. Dietary administration is therefore an appropriate route of administration to ensure pups' exposure during lactation.</p>
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<b>New studies; not evaluated</b>	This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. (2010). <b>Requested by non-EU authorities, relevant for reference dose derivation.</b>
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<b>Report:</b>	<b>KCA 5.8.2 /07; [REDACTED] 2012a</b>
<b>Title:</b>	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat (gestational exposure phase)
<b>Document No:</b>	<b>M-435619-01</b>
<b>Report No:</b>	SA 10154
<b>Guidelines:</b>	US E.P.A. OCSPP 870.SUPP; Deviations: not specified
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	Flufenacet
Lot/Batch no:	Beige solid
Purity:	NK61AX0177
Stability of test compound:	96.8%
	guaranteed for study duration; expiry date: 2012-09-03

#### 2. Vehicle / positive control:

Vehicle: Plain diet  
Positive control: 6-propyl-2-thiouracil (PTU)

#### 3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, CrI:CD (SD)
Age:	12 to 13 weeks
Weight at dosing:	232 – 375 g
Source:	[REDACTED]
Acclimatisation period:	At least 3 days
Diet:	A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), <i>ad libitum</i>
Water:	Filtered and softened tap water from municipal water supply, <i>ad libitum</i> .
Housing:	Individual housing of pregnant females in suspended stainless steel wire mesh cages.

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose	Flufenacet: 0, 20, 100, 500 ppm corresponding to : 0-1.3-6.8-34.8 mg/kg bw/day (gestation) Positive control PTU: 15 ppm corresponding to approx. 0.9 mg/kg bw/day (gestation)
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Duration:	Gestation day 6 to 20 (GD 6 to GD 20)
Application route:	Oral (diet)
Group size:	10 presumed pregnant dams
Observations:	Dams: mortality, clinical signs, detailed clinical examinations, body weight (GD 3, 6, 13, 20; LD 0, 4, 7, 14, 21), food consumption, compound intake, necropsy, organ weights (thyroid, liver), hormone analyses (T4, TSH, T3), histopathology(thyroid) Fetuses: terminal body weight, organ weights (thyroid, liver), hormone analyses (T3, T4, TSH), histopathology (thyroid)

## Results and discussion

### A. Dose formulations analysis

#### Flufenacet

With the exception of the first day of administration at 20 ppm, results were within the in-house target range of 85 to 115% of nominal concentration and were therefore considered to be acceptable for use on the current study.

At 20 ppm, the homogeneity results of the first formulation were (71-179% of nominal concentration), which is outside the in-house target range of nominal concentration. Due to time constraints, this formulation was administered to animals on the first day only. It was replaced immediately by a new formulation on the next day (89 - 99% of nominal concentration), which was within the in-house target range.

Stability analyses showed that flufenacet is stable at 20, 100, and 500 ppm in the diet for 49 days in the freezer followed by 14 days at room temperature.

#### Positive control: PTU

Homogeneity and concentration analysis revealed concentrations were within the range of 91-93% of nominal. The results were within the in-house target range of 85-115% of nominal, and were therefore considered to be acceptable for use on the current study.

Stability analyses revealed PTU, at 15 ppm, was stable in the diet after an 11-day period in the freezer followed by 14 days at room temperature

### B. Maternal data

#### Mortality

There were no mortalities in dams up to and including flufenacet doses of 500 ppm.

There were no mortalities in the positive control group.

#### Clinical signs and pregnancy status

The pregnancy rate and the number of dead and live fetuses were unaffected by treatment. There were no treatment-related clinical signs recorded throughout the study at any dose level tested.

#### Body weight

There was no treatment-related effect on mean body weight or on mean body weight gain at any flufenacet dose level. Positive control (PTU) animals gained 9% less weight than controls from GD 6 to 20, but this difference was not statistically significant and the resulting difference in body weight on GD 20 was minimal (3%).

**Table 6.8.2/07-1: Summary of maternal body weight**

Dose (ppm)	Mean body weight $\pm$ standard deviation (g)			
	Gestation day			
	3	6	13	20
0	278.2 $\pm$ 27.7	293.3 $\pm$ 26.0	340.2 $\pm$ 33.2	420.3 $\pm$ 43.7
20	276.0 $\pm$ 36.4	293.5 $\pm$ 35.6	336.7 $\pm$ 41.8	421.7 $\pm$ 47.3
100	272.8 $\pm$ 36.3	289.9 $\pm$ 37.5	336.9 $\pm$ 40.7	426.9 $\pm$ 46.3
500	272.7 $\pm$ 31.0	294.2 $\pm$ 30.99	337.1 $\pm$ 33.8	413.9 $\pm$ 38.3
15 (PTU)	276.1 $\pm$ 33.0	291.0 $\pm$ 34.0	333.7 $\pm$ 38.9	406.0 $\pm$ 46.7

Food consumption and compound-intake

Food consumption was not affected by treatment with flufenacet up to the highest dose of 500 ppm.

There were no effects on food consumption in the positive control group.

**Table 5.8.2/07-2: Summary of test compound intake**

Dose (ppm)	Mean achieved dietary intake (mg/kg bw/day)			
	PTU	Flufenacet		
	15	20	100	500
From GD 6 to GD 20	0.89	1.3	6.8	34.8

Terminal body and organ weights

There were no treatment-related effects on terminal body weights noted in any flufenacet dose group. Organ weights were also unaffected by treatment with flufenacet.

In the positive control group there was no relevant change in mean terminal body weight in treated dams when compared to the controls.

At 15 ppm PTU, mean absolute and relative thyroid weights were higher in dams when compared to the controls (+91% and +100%,  $p \leq 0.01$ , respectively).

**Table 5.8.2/07-3: Summary of dam organ weights**

Dose (ppm)	Terminal Body weight (g)	Organ weights (mean $\pm$ standard deviation) (% change when compared to controls)			
		Liver weight		Thyroid weight	
		Absolute (g)	Relative (%)	Absolute (mg)	Relative (%)
0	418.4 $\pm$ 43.3	14.85 $\pm$ 1.49	3.553 $\pm$ 0.155	19.5 $\pm$ 6.0	0.0046 $\pm$ 0.0011
20	420.9 $\pm$ 47.4	14.87 $\pm$ 1.85	3.534 $\pm$ 0.202	17.1 $\pm$ 3.7	0.0040 $\pm$ 0.0006
100	426.6 $\pm$ 45.4	15.22 $\pm$ 1.75	3.573 $\pm$ 0.264	18.7 $\pm$ 6.8	0.0043 $\pm$ 0.0011
500	413.5 $\pm$ 38.0	15.46 $\pm$ 1.57	3.741 $\pm$ 0.206	15.46 $\pm$ 1.57	0.0043 $\pm$ 0.0009
15 (PTU)	405.9 $\pm$ 46.8	13.58 $\pm$ 1.71	3.343 $\pm$ 0.152**	37.2 $\pm$ 3.9**	0.0092 $\pm$ 0.0010**

\*\* Significantly different from control group value ( $p \leq 0.01$ )

PTU = positive control

Gross pathology

There were no treatment-related macroscopic findings.

At 100 and 20 ppm flufenacet, an atrophic/small thyroid gland was noted in some treated dams (4/10 and 5/10, respectively). As this gross morphological alteration was neither dose-related nor associated to any histopathological change at 500 ppm, it was considered not to be treatment-related.

At 15 ppm PTU, enlarged and/or congested/red thyroid glands were noted in treated dams (9/10 and 7/10, respectively). As these changes were associated with increased absolute and relative thyroid weights and microscopic findings, they were considered to be treatment-related.

**Table 5.8.2/07-4: Summary of gross pathological thyroid findings in dams (incidences)**

Dose (ppm)	Dams examined (n)	Thyroid glands			
		congested / red	enlarged	atrophic / small	pale
0	10	0	0	0	0
20	10	0	0	5	0
100	10	0	1	4	1
500	10	2	0	0	0
15 (PTU)	10	7	9	0	0

#### Histopathology

There were no treatment-related findings observed at any flufenacet dose level.

In the positive control group PTU moderate follicular cell hypertrophy / hyperplasia in thyroids was noted in all dams. This change was considered to be treatment-related.

#### Hormone analyses

At 500 ppm flufenacet mean T4 concentration was slightly lower (37%, not statistically significant) when compared to control. This slight difference from control was not associated with any relevant change in T3 or TSH concentration at 500 ppm. There was no relevant change involving T3, T4 or TSH at 20 or 100 ppm. The slightly lower mean TSH concentrations in the treated groups, compared to controls, were due to the high concentration (6.25 ng/mL) noted in one control dam. When this value is excluded, the mean TSH concentration of the control group is 1.749 ng/mL.

Treatment with the positive control PTU caused a statistically significant decreases of T3 and T4 concentrations (42 %,  $p \leq 0.01$ , and (82 %,  $p \leq 0.01$ ), and a statistical significant increase of TSH (+331 %,  $p \leq 0.01$ ).

The results are summarised in the following table.

**Table 5.8.2/07-5: Summary of hormone analyses in dams**

Dose (ppm)	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)		
	T3 (ng/ml)	T4 ( $\mu$ g/dL)	TSH (ng/mL)
0	1.08 $\pm$ 0.111	1.90 $\pm$ 0.837	2.200 $\pm$ 1.6260
20	1.07 $\pm$ 0.120 (-1%)	1.62 $\pm$ 0.593 (-15%)	1.926 $\pm$ 0.5525 (-12%)
100	1.05 $\pm$ 0.224 (-3%)	1.66 $\pm$ 0.529 (-13%)	1.823 $\pm$ 0.6124 (-17%)
500	0.98 $\pm$ 0.088 (-9%)	1.20 $\pm$ 0.653 (-37%)	1.691 $\pm$ 0.5493 (-23%)
15 (PTU)	0.63 $\pm$ 0.13** (-42%)	0.34 $\pm$ 0.24** (-82%)	9.49 $\pm$ 2.71** (+331%)

\* Statistically significantly different from controls ( $p \leq 0.05$ )

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

### C. Fetal data

#### Terminal body and organ weights

In the flufenacet dose groups there were no relevant change in mean terminal body or organ weights when compared to controls.

Fetuses of the positive control group PTU had significantly increased absolute and relative thyroid weights. These changes were considered to be related to treatment.

**Table 5.8.2/07-6: Summary of organ weights in fetuses**

Dose (ppm)	Terminal Body weight (g)	Organ weights (mean per litter per group ± standard deviation) (% change when compared to controls)			
		Liver weight		Thyroid weight	
		Absolute (g)	Relative (%)	Absolute (mg)	Relative (%)
Male fetuses					
0	3.94 ± 0.21	0.3375 ± 0.0284	8.5648 ± 0.3785	1.3 ± 0.3	0.0341 ± 0.0096
20	4.05 ± 0.31	0.3291 ± 0.0307	8.1198 ± 0.4485	1.0 ± 0.2	0.0260 ± 0.0046
100	4.09 ± 0.21	0.3271 ± 0.0315	7.9954 ± 0.5678	1.3 ± 0.1	0.0309± 0.0027
500	3.83 ± 0.25	0.3271 ± 0.0385	8.5381 ± 0.5787	1.2 ± 0.2	0.0310 ± 0.0054
15 (PTU)	3.80 ± 0.32	0.3057 ± 0.0310	8.0521 ± 0.6787	1.8 ± 0.4* (+38%)	0.0461 ± 0.0087** (+35%)
Female fetuses					
0	3.80 ± 0.20	0.3169 ± 0.0254	8.3338 ± 0.3573	1.2 ± 0.2	0.0318 ± 0.0044
20	3.85 ± 0.29	0.3179 ± 0.0485	8.2393 ± 0.6372	1.1 ± 0.2	0.0297 ± 0.0044
100	3.94 ± 0.28	0.3271 ± 0.0334	8.2980 ± 0.5410	1.1 ± 0.2	0.0273± 0.0059
500	3.68 ± 0.26	0.3164 ± 0.0368	8.5762 ± 0.5486	1.1 ± 0.2	0.0308 ± 0.0035
15 (PTU)	3.60 ± 0.31	0.2912 ± 0.0386	8.0839 ± 0.6228	1.5 ± 0.4* (+25%)	0.0425 ± 0.0100** (+34%)

#### Histopathology

There were no treatment-related findings observed at any dietary level of flufenacet.

In the positive control group PTU a higher incidence of minimal follicular cell hypertrophy / hyperplasia, associated with a loss of follicular organization and a solid appearance of the thyroid gland, was noted in treated fetuses (32/40 animals in 9/10 litters). In addition, minimal increased number of mitoses was recorded with a higher incidence in the treated group (5/40 animals in 4/10 litters). Both changes were considered to be treatment-related.

#### Hormone analyses

There were no changes observed in T3 concentrations at any dose level of flufenacet.

T4 and TSH concentrations of flufenacet dose groups and control showed high variations in the individual values. A tendency toward slightly lower mean T4 value and TSH was noted at 500 ppm.



However a relatively-lower TSH value does not support compensation for a decrease in T4 and has no known biological significance.

Tendencies towards lower mean TSH concentration were noted at 100 ppm. As there were no associated differences in T3, T4 or other thyroid parameter, this minimal difference from control is not considered as biologically relevant. Individual values at 20 ppm were within the range of controls (with the exception of one TSH value).

In the fetuses of the positive control group PTU mean T4 concentration was lower (-79%,  $p \leq 0.01$ ) and mean TSH concentration was higher (+160%,  $p \leq 0.01$ ) when compared to controls. T3 concentrations were not affected.

Hormone analyses data are summarized in the following tables.

**Table 5.8.2/07-7: Summary of hormone analyses in fetuses**

Dose (ppm)	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)		
	T3	T4	TSH
0	$0.55 \pm 0.058$	$0.70 \pm 0.397$	$4.493 \pm 1.2687$
20	$0.57 \pm 0.051$ (+4%)	$0.55 \pm 0.308$ (-21%)	$3.645 \pm 0.4770$ (-19%)
100	$0.55 \pm 0.057$ (0%)	$0.48 \pm 0.186$ (-31%)	$3.174 \pm 0.6148^{**}$ (-29%)
500	$0.54 \pm 0.040$ (-2%)	$0.36 \pm 0.259$ (-49%)	$2.474 \pm 0.5043^{**}$ (-45%)
15 (PTU)	$0.51 \pm 0.04$	$0.15 \pm 0.15^{**}$ (-79%)	$11.67 \pm 1.90^{**}$ (+160%)

\* Statistically significantly different from controls ( $p \leq 0.05$ )

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

<b>Conclusion</b>	<p>Dietary administration of 500 ppm flufenacet induced slight changes in T4 concentrations in dams and fetuses. A high individual variability was noted in the hormonal parameters measured in fetuses, including controls. The effects were much lower in magnitude compared to the effects of the direct thyroid-acting compound PTU. In addition, there was no corresponding TSH concentration increase, as noted in PTU-treated animals, but rather TSH levels were slightly decreased in fetuses only, relative to the controls. No thyroid weight or histopathological changes were observed and no general toxicity parameters were affected by flufenacet at any dietary level.</p> <p><b><u>Dose level of 500 ppm (equating to 34.8 mg/kg bw/day) is considered a No Observed Adverse Effect Level (NOAEL) for both dams and fetuses.</u></b></p>
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<b>New studies; not evaluated</b>	<p>This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Requested by non-EU authorities, relevant for reference dose derivation.</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /08; [REDACTED] 2012b</b>
<b>Title:</b>	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat by dietary exposure (gestational and lactational exposure phase)
<b>Document No:</b>	<b>M-435313-01</b>
<b>Report No:</b>	SA 11052
<b>Guidelines:</b>	<b>US E.P.A. OCSPP 870.SUPP; Deviations: not specified</b>
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	Flufenacet
Lot/Batch no:	Beige solid
Purity:	NK61AX0177
Stability of test compound:	96.8%
	guaranteed for study duration; expiry date: 2012-09-03

#### 2. Vehicle / positive control:

Vehicle:	Plain diet
Positive control:	6-propyl-2-thiouracil (PTU)

#### 3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, Crl:CD (SD)
Age:	12 to 13 weeks
Weight at dosing:	Dams on GD 6: 245 – 348 g.
Source:	[REDACTED]
Acclimatisation period:	At least 4 days
Diet:	A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), <i>ad libitum</i>
Water:	Filtered and softened tap water from municipal water supply, <i>ad libitum</i> .
Housing:	Individual housing of dams with litters in suspended polycarbonate cages with bedding material.

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose	<p>Flufenacet: 0, 20, 100, 500 ppm</p> <p>corresponding to : 0-1.3-6.6-35.2 mg/kg bw/day (gestation)</p> <p>corresponding to : 0-3.4-16.7-84.2 mg/kg bw/day (lactation)</p> <p>Positive control PTU: 15 ppm</p> <p>corresponding to approx. 0.9 and 1.9 mg/kg bw/day during gestation and lactation, respectively</p>
Duration:	Gestation day 6 (GD 6) through postnatal or lactation day 4

	or 21 (PND/LD 4 or 21)
Application route:	Oral (diet)
Group size:	20 presumed pregnant dams
Observations:	Dams: mortality, clinical signs, detailed clinical examinations, body weight (GD 3, 6, 13, 20; LD 0, 4, 7, 14, 21), food consumption, compound intake, necropsy, organ weights (thyroid, liver), hormone analyses (T4, TSH, T3), histopathology (thyroid) Pups: mortality, clinical signs, body weight (PND 4, 7, 14, 21), detailed clinical examination, hormone analyses (TSH, T4, T3), necropsy, thyroid weights, histopathology (thyroid)

## Results and discussion

### A. Dose formulations analysis

#### Flufenacet

Homogeneity and concentration analysis revealed concentrations were within the range of 88-118% of nominal. The results were within the in-house target range of 85-115% of nominal, with the exception of one value measured at 118% (i.e. slightly outside the target range) at 500 ppm. In view of all results, preparations were considered to be acceptable for use on the current study.

#### Positive control: PTU

Homogeneity and concentration analysis revealed concentrations were within the range of 90-95% of nominal. The results were within the in-house target range of 85-115% of nominal, and were therefore considered to be acceptable for use on the current study.

### B. Maternal data

#### Mortality

There were no mortalities in dams up to and including flufenacet doses of 500 ppm and in the positive control group PTU.

**Table 6.8.2/08-1: Summary of mortality, exclusion and sacrifice throughout the study**

Number of females	Control	PTU 15 ppm	Flufenacet 20 ppm	Flufenacet 100 ppm	Flufenacet 500 ppm
On GD 3	20	20	20	20	20
Not delivered or excluded	0	1 (2_1768)	1 (3_1779)	0	0
At scheduled sacrifice on LD 4	5	5	5	5	5
Killed for humane reason	0	2 2_1760 (LD 6) 2_1758 (LD 4)	0	1 4_1808 (LD 4)	2 5_1817 (LD 4) 5_1822 (LD 4)
At scheduled sacrifice on LD 21	15	12	14	14	13

#### Clinical signs

There were no treatment-related clinical signs observed in any dam in any dose group.

Body weightFlufenacet

There were no effects on body weights noted up to and including 100 ppm flufenacet.

At 500 ppm (corresponding to 64.6 mg/kg bw/day from GD 6 to LD<sub>21</sub>) body weights of dams were reduced by 28% between GD 6 and 13, when compared to controls (not statistically significant).

Thereafter mean body weight was comparable to controls.

Positive control: PTU

In the positive control group PTU the mean body weight gain/day was reduced between 14 and 17% (not statistically significant) throughout the gestation period. The mean body weight was reduced by 6 and 7% on LD 0 and 4, respectively, when compared to the controls ( $p \leq 0.05$ ). Following culling on LD 4, animals recovered and the mean body weight was comparable to the control group towards the end of the study.

Maternal body weights are summarized in the following table.

**Table 6.8.2/08-2: Summary of maternal body weight**

Dose (ppm)	Mean body weight (g)							
	Gestations GD6	Gestations GD13	Gestations GD20	Lactation LD0	Lactation LD4	Lactation LD7	Lactation LD14	Lactation LD21
0	291.4	330.7	415.2	316.9	337.0	340.8	358.7	346.8
20	288.9	328.6	402.9	309.3	331.4	332.2	346.5	338.4
100	288.4	323.5	409.4	310.8	329.1	334.8	354.1	344.9
500	288.1	315.8	401.5	304.4	320.2	317.7	341.2	332.8
15 (PTU)	294	328	399	299*	315*	328	355	351

GD = gestation day; LD = lactation day

\* Statistically significant different from control ( $p \leq 0.05$ )

\*\* Statistically significant different from control ( $p \leq 0.01$ )

Food consumption and compound-intakeFlufenacet

Food consumption was not affected by treatment up to the highest dose of 500 ppm flufenacet.

Positive control: PTU

The mean food consumption was reduced by 12 to 29% from GD 13 to LD 21, compared to the controls ( $p \leq 0.01$ ).

The mean achieved dose levels of PTU or flufenacet expressed in mg/kg bw/day received by the animals during the study are summarized in the following table.

**Table 6.8.2/08-3: Summary of test compound intake**

Dose level	Mean achieved test compound intake (mg/kg bw/day)			
	PTU 15 ppm	20 ppm	Flufenacet 100 ppm	500 ppm
From GD 6 to GD 20	0.9	1.3	6.6	35.2
From LD 0 to LD 21	1.9	3.4	16.7	84.2
From GD 6 to LD 21	1.5	2.5	12.7	64.6

Hormone analysesFlufenacet

At 500 ppm T3 and T4-levels were reduced by 19% ( $p \leq 0.01$ ) and 70% ( $p \leq 0.01$ ), respectively. At 100 ppm there were no treatment-related effects on T3 or TSH concentrations noted. The mean T4-level was slightly (26%,  $p \leq 0.01$ ) less than controls. Hormone concentrations were unaffected by treatment at 20 ppm flufenacet.

Positive control: PTU

Treatment with the positive control PTU caused statistically significant decreases in T3 and T4-levels, and a statistical significant increase of TSH.

Thyroid hormone data are summarised in the following table.

**Table 6.8.2/08-4: Summary of hormone analyses in dams on LD 21**

Dose group (ppm)	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)		
	T3 (ng/mL)	T4 ( $\mu$ g/dL)	TSH (ng/mL)
0	$0.73 \pm 0.088$	$2.29 \pm 0.428$	$1.365 \pm 1.0147$
20	$0.71 \pm 0.159$ (-3%)	$2.36 \pm 0.442$ (+3%)	$2.277 \pm 1.7483$ (+67%)
100	$0.67 \pm 0.144$ (-8%)	$1.69 \pm 0.395^{**}$ (-26%)	$1.890 \pm 1.1473$ (+38%)
500	$0.59 \pm 0.112^*$ (-19%)	$0.68 \pm 0.245^{**}$ (-70%)	$2.669 \pm 2.6633$ (+96%)
15 (PTU)	$0.44 \pm 0.090^{\#T}$	$0.38 \pm 0.103^{\#T}$	$18.530 \pm 5.7364^{\#W}$

\* Statistically significantly different from controls ( $p \leq 0.05$ )

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

#T Statistically significantly different from controls ( $p \leq 0.001$ ) Student T test

#W Statistically significantly different from controls ( $p \leq 0.001$ ) adjusted Welch test

Gross pathology and terminal body and organ weightsFlufenacet

There were no differences in terminal body weight between controls and treated animals at any dietary level. At 500 ppm, mean liver-to-body weight ratio was statistically-increased, relative to controls. There was no treatment-related change in mean thyroid gland weight at any dose level.

At terminal sacrifice all macroscopic findings were considered as incidental and not treatment-related.

Positive control: PTU

There were no differences in terminal body weight between controls and treated animals. The mean absolute and relative thyroid gland weights were statistically significantly higher, when compared to controls (+175% and +171%, respectively;  $p \leq 0.01$ ). There was no treatment-related change in mean absolute or relative liver weight.

At terminal sacrifice enlarged thyroid gland was noted in 10/12 females. Dark thyroid gland was noted in 3/12 females. Other changes were considered as incidental and not treatment-related.

Dam organ weights are summarized in the following table.

**Table 6.8.2/08-5: Summary of dam organ weights**

Dose group (ppm)	Terminal Body weight (g)	Organ weights (mean ± standard deviation) (% change when compared to controls)			
		Liver weight		Thyroid weight	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
0	346.8 ± 30.73	15.08 ± 1.230	4.361 ± 0.2899	0.01751 ± 0.0026	0.0051 ± 0.00102
20	338.4 ± 30.18	15.18 ± 2.197 (+1%)	4.494 ± 0.4137 (+3%)	0.01966 ± 0.0061	0.00576 ± 0.0015
100	344.9 ± 33.91	15.66 ± 2.294 (+4%)	4.531 ± 0.3765 (+4%)	0.02022 ± 0.0063	0.00589 ± 0.0018
500	332.8 ± 20.10	16.57 ± 1.217 (+10%)	4.987 ± 0.3754** (+14%)	0.01867 ± 0.0053	0.00561 ± 0.0015
15 (PTU)	351 ± 17.3	15.7 ± 1.4	4.36 ± 0.290	0.00081 ± 0.0095**	0.0138 ± 0.003**

\*\* Significantly different from control group value ( $p \leq 0.01$ )

PTU = positive control

### Histopathology

#### Flufenacet

In the high-dose group follicular cell hypertrophy in the thyroid gland was observed in 2 out of 13 dams, while there were no cases in control animals. The single incidence at 100 ppm is not attributed to flufenacet, based on low incidence and lack of corresponding effect on thyroid hormones.

#### Positive control: PTU

In dams of the positive control group at scheduled sacrifice, diffuse follicular cell hypertrophy/hyperplasia (12/12), focal follicular cell hyperplasia (1/12) and colloid depletion (12/12) were noted in the thyroid gland, compared to only one case of colloid depletion in the control group.

Histopathological findings in dams are summarised in the following table.

**Table 6.8.2/08-6: Summary of histopathology in dams on LD21 (terminal sacrifice)**

Dose group (ppm)	Dams examined (n)	Follicular cell hypertrophy		
		Minimal	Slight	Total
0	15	0	0	<b>0</b>
20	14	0	0	<b>0</b>
100	14	0	1	<b>1</b>
500	13	2	0	<b>2</b>
15 (PTU)	12	No data	No data	<b>12#</b>

# diffuse follicular cell hypertrophy / hyperplasia

**C. Fetal data**Mortality

There were treatment-related effects on mortality, clinical signs or organ weights noted in any dose group.

**Table 6.8.2/08-7: Summary of mortality, culling and sacrifice throughout the study**

Number of pups	Control	PTU 15ppm	Flufenacet 20 ppm	Flufenacet 100 ppm	Flufenacet 500 ppm
At delivery (PND 0)	267	252	270	262	258
Culled at PND 4 or 21	232	193	225	227	208
Dead on PND 0 (during delivery)	2	3	3	4	5
Dead from PND 0 through PND 4 (prior to culling)*	3	22	14	2	19
Dead after PND 4 (post culling)*	0	10	0	1	0
Final sacrifice	30	24	28	28	26

\*Include found dead, cannibalized or killed for humane reason

Clinical signs

There were no treatment-related clinical signs observed in any pup of any dose group.

Body weightsFlufenacet

At 500 ppm the mean body weight was reduced during lactation between 13 and 22%, compared to the controls ( $p \leq 0.01$ ). Overall, the mean cumulative body weight gain from PND 4 to 21 was reduced by 15% in males ( $p \leq 0.01$ ) and by 12% in females ( $p \leq 0.05$ ), compared to the controls. This effect was mainly attributed to a reduced body weight gain between PND 4 and 7 (-25% in males and -22% in females) ( $p \leq 0.01$ ).

Body weights were unaffected by treatment in the low- and mid-dose groups.

Positive control: PTU

In the positive control group (PTU) the mean body weight was reduced throughout the study period between 16 and 43%, compared to the controls ( $p \leq 0.01$ ). The mean cumulative body weight gain was reduced by 36% in both sexes between PND 4 and PND 14. Thereafter, the effect was more pronounced when pups were exposed directly via dietary intake, reaching a body weight gain reduction of 64% in males and 69% in females between PND 14 and 21, compared to controls. Overall, the mean cumulative body weight gain was reduced by 50% in males and by 47% in females between PND 4 and 21, compared to the controls ( $p \leq 0.01$ ).

Pup body weights are summarised in the following tables.

**Table 6.8.2/08-8: Summary of pup body weight**

Dose (ppm)	Mean body weight (g)							
	Males				Females			
	PND4	PND7	PND14	PND21	PND4	PND7	PND14	PND21
0	11.6	18.9	37.5	57.7	11.1	18.0	36.5	54.0
20	11.1	18.1	36.8	55.3	10.3	17.4	35.6	52.7
100	11.2	18.1	36.6	57.5	10.5	16.9	34.9	54.4
500	9.3**	14.8**	31.4*	48.5**	9.0**	14.4**	31.2**	46.8**
15 (PTU)	9.7**	15.0**	26.6**	32.9**	9.3**	14.3**	25.8**	32.1**

PND = postnatal day

\*\* Statistically significant different from control ( $p \leq 0.01$ )**Table 6.8.2/08-9: Summary of pup body weight gain**

Dose (ppm)	Male pups – mean body weight gain (g)				
	PND4-PND7	PND7-PND14	PND14-PND21	PND4-PND14	PND4-PND21
0	7.3	18.6	20.2	25.9	46.1
20	7.0	18.7	18.5	25.7	44.2
100	6.9	18.5	20.9	25.4	46.3
500	5.5**	16.6	17.1	22.1**	39.2**
15 (PTU)	5.1**	11.6**	6.3**	16.7**	23.0**

Dose (ppm)	Female pups – mean body weight gain (g)				
	PND4-PND7	PND7-PND14	PND14-PND21	PND4-PND14	PND4-PND21
0	6.9	18.4	17.6	25.3	42.9
20	7.1	18.2	17.1	25.3	42.3
100	6.4	18.0	19.4	24.4	43.9
500	5.4**	16.8	15.6	22.2*	37.8*
15 (PTU)	4.9**	11.4**	6.3**	16.3**	22.6**

\* Statistically significant different from control ( $p \leq 0.05$ )\*\* Statistically significant different from control ( $p \leq 0.01$ )

### Hormone analyses

#### Flufenacet

There were no treatment-related changes observed on T3, T4 or TSH at PND 4 and on T4 or TSH concentrations at PND 21. The higher TSH-level observed at 100 ppm on PND 4 is considered to be incidental and unrelated to treatment, as this variation was mainly due to only 3 values out of 18 and was not associated with a decrease in T4 or T3, and as TSH was not increased at 500 ppm.

At 500 ppm T3-levels in PND 21 pups were slightly lower than controls in males (-24%;  $p \leq 0.01$ ) and females (-16%;  $p \leq 0.05$ ). The biological significance of this finding is unclear since only one value was below the control range and there was no associated change in T4, TSH, thyroid weight, or thyroid histopathology.

At 100 ppm T3-levels in PND 21 pups was slightly reduced, relative to controls, in males (-16%;  $p \leq 0.05$ ) but not their female littermates. It is unclear whether this finding represents a treatment-related effect, since all but one value was within the control range and there were no associated differences in T4, TSH or other thyroid parameter. Therefore, this minimal difference from control is not considered to be biologically significant or adverse.



**Positive control: PTU**

In PND 4 pups, mean T3 and T4 concentrations were markedly lower (-38% and -88%, respectively;  $p \leq 0.001$ ) and mean TSH concentration was markedly higher (+721%;  $p \leq 0.001$ ), when compared to the controls.

In PND 21 pups, mean T3 concentrations were markedly lower (-87% in males and -83% in females;  $p \leq 0.001$ ), mean T4 concentrations were markedly lower (-93% in males and -94% in females;  $p \leq 0.001$ ) and mean TSH concentrations were markedly higher (+697% in males;  $p \leq 0.001$  and +429% in females;  $p \leq 0.001$ ), when compared to the controls.

Thyroid hormone data are summarized in the following tables.

**Table 6.8.2/08-10: Summary of hormone analyses in pups on PND 4 (pooled per litter)**

Dose group (ppm)	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)		
	T3 (ng/mL)	T4 ( $\mu$ g/dL)	TSH (ng/mL)
0	0.71 $\pm$ 0.141	1.00 $\pm$ 0.226	1.505 $\pm$ 0.5547
20	0.72 $\pm$ 0.137 (+1%)	1.00 $\pm$ 0.217 (0%)	1.521 $\pm$ 0.7668 (+1%)
100	0.76 $\pm$ 0.135 (+7%)	0.97 $\pm$ 0.223 (-3%)	2.963 $\pm$ 4.1301 (+97%)
500	0.72 $\pm$ 0.157 (+1%)	1.05 $\pm$ 0.363 (+5%)	1.237 $\pm$ 0.4397 (-18%)
15 (PTU)	0.44 $\pm$ 0.070#T	0.12 $\pm$ 0.120#W	12.361 $\pm$ 3.2925#T

\* Statistically significantly different from controls ( $p \leq 0.05$ )

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

#T Statistically significantly different from controls ( $p \leq 0.001$ ) Student T test

#W Statistically significantly different from controls ( $p \leq 0.001$ ) adjusted Welch test

**Table 6.8.2/08-11: Summary of hormone analyses in pups on PND 21 (1 pup/sex/litter)**

Dose group (ppm)	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)					
	T3 (ng/mL)	Males T4 ( $\mu$ g/dL)	TSH (ng/mL)	Females T3 (ng/mL)	Females T4 ( $\mu$ g/dL)	TSH (ng/mL)
0	1.10 $\pm$ 0.196	3.18 $\pm$ 0.458	0.779 $\pm$ 0.506	1.24 $\pm$ 0.179	3.09 $\pm$ 0.667	1.236 $\pm$ 0.423
20	0.94 $\pm$ 0.217 (-15%)	3.30 $\pm$ 0.975 (+4%)	0.856 $\pm$ 0.423 (+10%)	1.14 $\pm$ 0.191 (-8%)	3.36 $\pm$ 1.091 (+9%)	1.395 $\pm$ 0.763 (+13%)
100	0.92 $\pm$ 0.152* (-16%)	3.16 $\pm$ 0.673 (-1%)	1.172 $\pm$ 1.208 (+50%)	1.16 $\pm$ 0.145 (-6%)	3.03 $\pm$ 0.554 (-2%)	1.624 $\pm$ 1.086 (+31%)
500	0.84 $\pm$ 0.139** (-24%)	3.41 $\pm$ 0.864 (+7%)	0.790 $\pm$ 0.559 (+1%)	1.04 $\pm$ 0.178* (-16%)	3.33 $\pm$ 0.535 (+8%)	1.218 $\pm$ 0.589 (-1%)
15 (PTU)	0.14 $\pm$ 0.053#W	0.23 $\pm$ 0.166#W	6.208 $\pm$ 1.9850#W	0.21 $\pm$ 0.078#W	0.18 $\pm$ 0.136#W	6.541 $\pm$ 2.2392#T

\* Statistically significantly different from controls ( $p \leq 0.05$ )

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

#T Statistically significantly different from controls ( $p \leq 0.001$ ) Student T test

#W Statistically significantly different from controls ( $p \leq 0.001$ ) adjusted Welch test

Gross pathology, terminal body and organ weightsFlufenacet

At 500 ppm Terminal body weight was reduced by 9% in males (not statistically significant) and 19% in females ( $p \leq 0.01$ ) on PND 4 and by 14% in both sexes on PND 21 ( $p \leq 0.01$ ).

Terminal body weights were unaffected at lower doses.

There were no treatment-related changes in mean thyroid gland weights at any dose level on PND 4 and PND 21.

Furthermore, at scheduled sacrifice, all macroscopic changes in PND4 and PND 21 pups were considered as incidental and not treatment-related.

Positive control: PTU

PND 4 pups of the positive control group had statistically significant lower mean terminal body weights (-14%,  $p \leq 0.01$  in males, -21%,  $p \leq 0.01$  in females), when compared to the controls. Mean absolute and relative thyroid gland weights were statistically significantly higher, when compared to controls (+65% and +92% in males and +72% and +116% in females, respectively;  $p \leq 0.01$ ).

On PND 21 mean terminal body weight (-44%,  $p \leq 0.01$  in males, -41%,  $p \leq 0.01$  in females) was statistically significantly lower, when compared to the controls.

Mean thyroid gland-to-body weight ratio was statistically significantly higher, when compared to controls (+104% in males and +82% in females;  $p \leq 0.01$ ).

In PND 4 pups at terminal sacrifice congested/red thyroid gland was observed in 4/13 females. Other changes were considered as incidental and not treatment-related.

In PND 21 pups at terminal sacrifice enlarged thyroid gland was noted in 4/12 males and 5/12 females. Congested/red thyroid gland was observed in 3/12 males and 3/12 females.

Terminal pup body weights and thyroid weights are summarised in the following table.

**Table 6.8.2/08-12: Summary of terminal pup body weights and thyroid weights**

Dose group (ppm)	Terminal body weight (g)	Thyroid weight (mean) absolute (g)	Thyroid weight (mean) relative (%)	Terminal body weight (g)	Thyroid weight (mean) absolute (g)	Thyroid weight (mean) relative (%)
	<b>PND 4 - Males</b>			<b>PND 4 - Females</b>		
0	10.7	0.00200	0.01857	10.6	0.00182	0.01708
20	10.9	0.00178	0.01640	10.7	0.00203	0.01905
100	10.5	0.00188	0.01825	10.4	0.00184	0.01810
500	9.7	0.00161	0.01737	8.6+D	0.00163	0.01896
15 (PTU)	9.2**	0.0033**	0.0358**	8.4**	0.0031	0.0369**
	<b>PND 21 - Males</b>			<b>PND 21 - Females</b>		
0	57.8	0.00653	0.01136	54.9	0.00664	0.01206
20	55.4	0.00669	0.01199	52.1	0.00663	0.01272
100	56.9	0.00695	0.01230	53.6	0.00651	0.01191
500	49.8+D	0.00549	0.01104	47.2+D	0.00582	0.01234
15 (PTU)	32.1**	0.0075	0.0232**	32.3**	0.0071	0.0220**

+D Statistically significantly different from controls ( $p \leq 0.01$ ) Dunnetts LSD test

\*\* Statistically significantly different from controls ( $p \leq 0.01$ )

Microscopic pathologyFlufenacet

There were no treatment-related findings observed in PND4 and PND 21 pups that were considered to be related to treatment with flufenacet.

Positive control: PTU

At scheduled sacrifice on PND 4 diffuse follicular cell hypertrophy (12/13 in males and 10/13 in females) and colloid depletion (11/13 in males and 11/13 in females) were noted in the thyroid gland, compared to only one case of colloid depletion in the control group.

In PND 21 pups, diffuse follicular cell hypertrophy (11/12 in males and 11/12 in females) and colloid depletion (10/12 in males and 11/12 in females) were noted in the thyroid gland, compared to no case in the control group.

<b>Conclusion</b>	The No-Observed-Adverse-effect Level (NOAEL) for dams and pups is 100 ppm (13 mg/kg bw/day) based on decreased maternal body weights, reduction in T4 and T3, increased relative liver weight and two cases of thyroid follicular cell hypertrophy, observed in dams after dietary exposure to 500 ppm (65 mg/kg bw/day) flufenacet during gestation and lactation and slightly decreased body weight/body weight gain and slightly decrease in T3 values in pups at the same dose.
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<b>New studies; not evaluated</b>	This study was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. <b>Requested by non-EU authorities, relevant for reference dose derivation.</b>
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<b>Report:</b>	<b>KCA 5.8.2 /12; [REDACTED] 2012c</b>
<b>Title:</b>	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat complementary assay (gavage exposure of pups)
<b>Document No:</b>	<b>M-435126-01</b>
<b>Report No:</b>	SA 11167
<b>Guidelines:</b>	US-EPA OCSPP 870.SUPP;none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

- 1. Test material:** Flufenacet
- Description: Beige solid
- Lot/Batch no: NK61AX0177
- Purity: 96.8%
- Stability of test compound: guaranteed for study duration; expiry date: 2012-09-03
- 2. Vehicle:** 0.5 % aqueous methylcellulose 400

### 3. Test animals

- Species: Rat
- Strain: Sprague-Dawley, Crl:CD (SD)
- Age: 11 to 13 weeks
- Weight at dosing: Pups: males: 18.4 – 27.3 g; females: 19.3 – 26.0 g.
- Source: [REDACTED]
- Acclimatisation period: Dams: during gestation; pups: from birth through postnatal day 9 (PND/LD 9)
- Diet: A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum*
- Water: Filtered and softened tap water from municipal water supply, *ad libitum*.
- Housing: Individual housing of dams with litters in suspended polycarbonate cages with bedding material.

## B. Study design and methods

### 1. Animal assignment and treatment

- Dose: 1.7 mg/kg bw/day to pups only
- Duration: postnatal day (PND) 10 through PND 20
- Application route: Oral (gavage)
- Group size: 15 male and female pups

**Observations:**

Dams: mortality, clinical signs, body weight (LD 0 – LD 21), births, necropsy (only dams found dead or selected for study)

Pups: mortality, clinical signs, body weight (PND 10 – PND 20 and PND 21), TSH, T4, T3, thyroid weights, thyroid histopathology

**Results and discussion****A. Dose formulations analysis**

The stability of flufenacet formulations in aqueous 0.5% methylcellulose 400 suspensions has been demonstrated in a previous study (SA11177) at 0.2 and 20 g/L over a time period of 28 days that covers the period of storage and usage for the current study.

Homogeneity and concentration analysis revealed concentrations were within the range of 91-92% of nominal. The results were within the in-house target range of 90-110% of nominal and therefore within the acceptable range.

**B. Maternal data**Mortality

There were no mortalities in dams.

Clinical signs

There were no treatment-related clinical signs observed in any dam.

Body weight

With the exception of one dam (1\_1693) all maternal animals gained body weight over the course of the study. There were no clinical signs which may affect the results of the study.

The one control dam (1\_1693) had a body weight loss of 41g between Lactation Day (LD) 15 and 21 and was observed wasted on LD 20 and 21.

In addition, two other dams (1\_1688 and 1\_1690) presented a body weight loss of respectively 32 and 45g between Lactation Day (LD) 15 and 21 without any clinical signs. Their pups showed no evidence of an adverse effect during this week of maternal weight loss.

**Table 6.8.2/12-1: Summary of maternal body weight (mean ± standard deviation)**

	Study day						
	GD4	GD8	GD15	LD0	LD8	LD15	LD21
Mean (g)	265.7	291.9	335.7	310.5	333.4	343.1	335.7
SD	24.81	26.89	26.53	26.71	26.27	24.13	19.22

GD = gestation day; LD = lactation day

**C. Fetal data**Mortality

There were no mortalities during the course of the study.

Clinical signs

There were no treatment-related clinical signs observed in any pup of any dose group.

Body weights

There was no treatment-related effect on mean body weight or on mean body weight gain throughout the study.

Animals from the litter 1\_1693 (R1M1630, R2M1660, R1F1645, R2F1675) presented a reduced body weight gain on Study Day 9 (PND/LD 19) and body weight loss on Study Days 10 and 11 (PND/LD 20 and 21). Since this finding was associated with maternal weight loss and observed in both control and treated pups, it was considered not to be related to flufenacet treatment.

**Table 5.8.2/12-2: Summary of pup body weights (mean)**

Study day	1	2	3	4	5	6	7	8	9	10	11
Dose											
(mg/kg bw/day)	<b>Males</b>										
0	23.01	25.25	27.45	30.15	32.48	34.99	37.13	39.31	41.11	43.13	46.13
1.7	24.19	26.39	28.90	31.77	34.10	36.65	38.92	41.15	43.07	45.52	48.25
	<b>Females</b>										
0	22.95	26.37	27.67	30.48	32.97	35.37	37.61	39.54	41.24	43.35	45.88
1.7	22.36	24.74	27.07	29.61	32.17	34.71	36.74	38.94	40.72	42.93	45.74

#### Hormone analyses

When compared to the control group, no relevant change was noted in TSH, T4 and T3 concentrations in either sex.

**Table 5.8.2/12-3: Summary of hormone analyses in pups**

	Hormone analysis mean $\pm$ standard deviation (% change when compared to controls)			
	Males		Females	
	0	1.7	0	1.7
Dose (mg/kg bw/day)				
<b>T3 (ng/mL)</b>	1.14 $\pm$ 0.16	1.07 $\pm$ 0.16 (-6%)	1.09 $\pm$ 0.16	1.05 $\pm$ 0.15 (-4%)
<b>T4 (<math>\mu</math>g/dL)</b>	3.40 $\pm$ 0.51	3.19 $\pm$ 0.42 (-6%)	3.13 $\pm$ 0.74	3.24 $\pm$ 0.51 (+4%)
<b>TSH (ng/mL)</b>	1.66 $\pm$ 0.80	1.30 $\pm$ 0.79 (-22%)	1.65 $\pm$ 0.59	1.63 $\pm$ 0.81 (-1%)

#### Terminal body weight and organ weight

There was no relevant change in mean terminal body weight in treated pups, when compared to the controls.

There was no relevant change in thyroid weights in treated pups, when compared to the controls.

**Table 5.8.2/12-4: Summary of mean pup body weights (g) and thyroid weights (g)**

Dose (mg /kg bw/day)	Terminal body weight	Thyroid weight (mean $\pm$ standard deviation)	Relative thyroid weight
<b>males</b>			
0	50.0 $\pm$ 4.8	0.0045 $\pm$ 0.00155	0.0087 $\pm$ 0.00269
1.7	52.0 $\pm$ 5.8	0.0052 $\pm$ 0.00120	0.0102 $\pm$ 0.00300
<b>females</b>			
0	49.0 $\pm$ 4.2	0.0047 $\pm$ 0.00118	0.0096 $\pm$ 0.00263
1.7	49.0 $\pm$ 4.6	0.0051 $\pm$ 0.00160	0.0105 $\pm$ 0.00371

#### Gross pathology

There were no treatment-related effects observed.

Microscopic pathology

No treatment-related effect on the thyroid was observed.

<b>Conclusion</b>	A dose level of 1.7 mg/kg/day flufenacet administered to male and female Sprague-Dawley rats from PND 10 through PND 20 by oral gavage is a No Observed Effect Level (NOEL).
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**FOE 5043-hydroxy**

<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /15; Herbold, B. A.; 1993</b>
<b>Title:</b>	FOE 5043-Hydroxy - Salmonella/microsome test plate incorporation and preincubation method
<b>Document No:</b>	<b>M-004586-01</b>
<b>Report No:</b>	22438
<b>Guidelines:</b>	OECD 471 (1983), EEC Directive 84/449/EEC Method B.14
<b>GLP</b>	Yes

**Materials and methods****A. Materials**

- 1. Test material:** FOE 5043-Hydroxy  
Description: Light brown crystals  
Lot/Batch no: 17001/93  
Purity: Not reported  
Stability of test compound: guaranteed for study duration; expiry date:
- 2. Vehicle and/or positive control:** DMSO  
without metabolic activation: Sodium azide (Na-azide), nitrofurantoin (NF), 4-nitro-1,2-phenylene diamine (4-NPDA),  
With metabolic activation: 2-aminoanthracene (2-AA)  
*Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98
- 3. Test system:** *Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98  
**Metabolic activation:** S9 mix prepared from Aroclor 1254 induced male Sprague-Dawley rats

**B. Study design and methods**

- Dose:** 0-8-40-200-1000-5000 µg/plate (plate incorporations and pre-incubation)  
positive controls:  
Na-azide: 10 µg/plate (only TA 1535)  
4-NPDA: 10 µg/plate (only TA 1537)  
0.5 µg/plate (only TA 98)  
NF: 0.2 µg/plate (only TA 100)  
2-AA: 3 µg/plate
- Application volume:** 0.1 mL
- Incubation time /temperature:** Pre-incubation: 20 minutes, 37 °C  
48 hours, 37 °C

**II. Results and discussion**

The potential of FOE 5043-Hydroxy to induce gene mutations was investigated according to the plate incorporation and the pre-incubation method in two independent experiments both with and without liver microsomal activation (S9 mix).



The plates incubated with the test item showed normal background growth up to concentrations of 1000 µg/plate. 5000 mg per plate had a weak, strain-specific bacteriotoxic effect.

In the plate incorporation test there were no dose-related and biologically relevant increases in mutant counts of any of the four tester strains observed following treatment with FOE 5043-hydroxy at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

In the pre-incubation test there was no indication of a bacteriotoxic effect of FOE 5043-Hydroxy at doses of up to and including 40 µg per tube. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had only a weak, strain-specific bacteriotoxic effect. None of the four strains concerned showed a dose-related and biologically relevant increase in mutant counts over those of the negative controls and thus confirmed the results of the plate incorporation method.

**Table 6.8.2/15-1: Summary of results**

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)			
			TA1535	TA1537	TA98	TA100
Summary of results – Plate incorporation method						
Without Activation	FOE 5043-Hydroxy	0	11 ± 3	9 ± 4	21 ± 2	70 ± 8
		8	10 ± 2	8 ± 3	24 ± 7	87 ± 13
		40	13 ± 3	8 ± 1	26 ± 1	79 ± 8
		200	8 ± 4	8 ± 3	19 ± 4	88 ± 12
		1000	14 ± 6	8 ± 2	23 ± 2	70 ± 11
		5000	13 ± 3#	3 ± 3	20 ± 5	74 ± 9#
	NaN <sub>3</sub>	10	825 ± 18*			
	NF	0.2				337 ± 53*
	4-NPDA	10		64 ± 13*		
4-NPDA	0.5			74 ± 8*		
With Activation	FOE 5043-Hydroxy	0	14 ± 2	7 ± 1	33 ± 6	108 ± 13
		8	14 ± 5	8 ± 2	33 ± 10	128 ± 8
		40	11 ± 2	9 ± 3	34 ± 4	120 ± 6
		200	11 ± 4	9 ± 2	25 ± 6	113 ± 14
		1000	11 ± 5	9 ± 2	23 ± 10	124 ± 17
		5000	14 ± 5#	7 ± 5	30 ± 2	108 ± 9#
	2-AA	3	59 ± 4*	56 ± 10*#	1286 ± 133*#	775 ± 95*#

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)			
			TA1535	TA1537	TA98	TA100
Summary of results – Pre-incubation method						
Without Activation	FOE 5043-Hydroxy	0	10 ± 3	7 ± 1	23 ± 4	97 ± 16
		8	10 ± 3	9 ± 2	22 ± 3	85 ± 3
		40	10 ± 4	7 ± 2	26 ± 7	98 ± 19
		200	7 ± 2	9 ± 2	28 ± 1	88 ± 15
		1000	9 ± 5	7 ± 2	28 ± 8	93 ± 13
		5000	8 ± 1#	4 ± 2#	24 ± 4#	73 ± 7#
	NaN <sub>3</sub>	10	564 ± 20*			
	NF	0.2				424 ± 15*
	4-NPDA	10		58 ± 4*		
	4-NPDA	0.5			70 ± 4*	
With Activation	FOE 5043-Hydroxy	0	13 ± 3	10 ± 3	34 ± 7	129 ± 17
		8	12 ± 6	10 ± 4	37 ± 8	80 ± 12
		40	13 ± 3	9 ± 1	28 ± 3	91 ± 10
		200	10 ± 6	9 ± 4	28 ± 9	64 ± 15
		1000	12 ± 3	5 ± 1	33 ± 12	68 ± 11
		5000	8 ± 4#	4 ± 2#	30 ± 7#	64 ± 4#
	2-AA	3	111 ± 15*#	231 ± 19*	1102 ± 162*	903 ± 32*

NaN<sub>3</sub> = sodium azide; NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene

# = bacteriotoxic effect; \* = mutagenic effect

<b>Conclusion</b>	FOE 5043-Hydroxy is considered to be non-mutagenic in this <i>Salmonella typhimurium</i> reverse mutation assay.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /16; [REDACTED] 1992a</b>
<b>Title:</b>	FOE 5043-Hydroxy - Study of the acute oral toxicity to rats
<b>Document No:</b>	<b>M-004579-01</b>
<b>Report No:</b>	21889
<b>Guidelines:</b>	OECD 401 (1987), US-EPA Pesticide assessment Guidelines, Series 81-1 (1984) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Name:	FOE 5043-Hydroxy
Description:	Brown crystal powder
Batch / Lot No.:	TE 90006, 17003/91
Purity:	99.2%
Stability of test compound:	guaranteed for study duration; expiry date: 1992-05-05

#### 2. Vehicle:

2% (v/v) Cremophor® EL in deionized water

#### 3. Test animals

Species:	Rat
Strain:	Wistar, Bor:WISW (SPF-Cpb)
Age:	Young adults, approx. 7 (males) and 10 (females) weeks
Weight at dosing:	males: 162 g - 178 g; females: 172 g - 186 g
Source:	████████████████████
Acclimatisation period:	at least 7 days
Diet:	Altromin® 1324 maintenance diet for rats and mice (Altromin GmbH & Co KG, Germany), <i>ad libitum</i> , except during a 17 hour fasting period prior to dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	During acclimatization 5 per sex in Makrolon® Type 3 cages. During the experimental period individually in Makrolon® Type 2 cages. Low-dust wood shavings were used as bedding material.

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	Males: 500-800-1000 mg/kg bw Females: 200-400-500 mg/kg bw mg/kg bw
Application route:	Oral, gavage
Application volume:	10 mL/kg bw
Fasting time:	before administration: 17 ± 1 hour
Group size:	5 rats/sex
Post-treatment observation period:	14 days
Observations:	clinical signs, mortality, body weight, gross necropsy

## Results and discussion

### A. Mortality

Mortalities occurred at 400 mg/kg bw and above for females and at 800 mg/kg bw and above for males. The results are summarised in the following table.

**Table 5.8.2/16-1: Result summary**

Animal Nos.	Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats							
1 - 5	500	0	5	5	4 min – 4 d	--	0
21 - 25	800	3	5	5	2 min – 3 d	6 min – 2.5 h	60
11 - 15	1000	4	5	5	1 min – 2 d	5 min – 2.25 h	80

Animal Nos.	Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Approximate LD <sub>50</sub> = 726 mg/kg bw							
Female rats							
16 – 20	200	0	5	5	0 min – 2 d	--	0
26 - 30	400	1	5	5	1 min – 2 d	2 d	20
6 - 10	500	3	5	5#	2 min – 5 d	2 d – 3 d	60
Approximate LD <sub>50</sub> = 474 mg/kg bw							

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

# One animal was sacrificed in moribund condition

### B. Clinical observations

The following signs were observed in males at 500 mg/kg bw and above and in females at 200 mg/kg bw and above: piloerection, reduced or increased activity, dyspnea, spasmodic state, lateral recumbency, and spastic or staggering gait.

In the males, lethargy, increased salivation, convulsions, and atony were also observed; in the females, no reflexes were observed. For males and females, these findings were first observed at the higher dose levels. For both sexes, extended limbs or extended hind limbs, extension spasms, head bent backward, temporary rolling over, sternal recumbency (females only), difficult breathing, and pallor were also observed in a few cases and some animals were cold. The following signs were observed in one animal each: reduced reflexes, soft feces, narrowed palpebral fissures, and self-mutilation (high-dose female). The signs, which were mostly of up to moderate severity, were observed in some cases immediately after administration and continued in the males until day 4 maximum and in the females until day 5 maximum.

### C. Body weight

There were no effects on body weight gain noted.

### D. Necropsy

*Animals that died during the study:* The three males of the 800 mg/kg bw dose group that died had dark livers, and pale spleens. In addition, one rat had pale, severely distended lungs. All four high-dose males that died had an empty intestinal tract, the stomach filled with yellowish or clear fluid and a pale spleen. In addition, two males had pale lungs. In three high-dose males the small intestine was reddened and one rat had severely injected mesenteric vessels.

The one females of the 400 mg/kg bw dose group that died had pale distended lungs; liver with lobular pattern, mottled; sporadic ulcer-like foci in the glandular stomach; the intestinal tract was partially reddened, and partially empty. The two high dose females that died had a pale liver with mottled, lobular pattern, One female had also pale mottled kidneys. The other abdominal organs were not assessable.

*Animals sacrificed moribund:* The one female of the 500 mg/kg bw dose group that was sacrificed moribund the following gross lesions were observed: liver pale, mottled, lobular pattern; stomach with brown fluid content; glandular stomach reddened; intestinal tract reddened, partially empty.

*Animals sacrificed at termination:*

There were no gross lesions observed in males of all three dose groups sacrificed at termination. In females of the 200 and 400 mg/kg bw dose group that were sacrificed at termination there were no gross lesions observed. In two females of the high dose group the lungs were slightly distended. In one female the liver showed also a slight lobular pattern.

<b>Conclusion</b>	FOE 5043-hydroxy is considered to be moderately toxic after acute oral administration. The estimated acute oral LD <sub>50</sub> values of male and female rats were approximately 726 mg/kg bw and approximately 474 mg/kg bw, respectively.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /17; ██████████; 1993</b>
<b>Title:</b>	FOE 5043-hydroxy (intermediate for the manufacture of FOE 5043 technical) - Study of the acute inhalation toxicity in rats in accordance with OECD guideline no. 403
<b>Document No:</b>	<b>M-004589-01</b>
<b>Report No:</b>	22155
<b>Guidelines:</b>	OECD 403 (1981), EC guideline 84/449/EEC B.2, US-EPA TSCA guideline 798.1150 (1985) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

FOE 5043-Hydroxy
Description: Brown, crystalline
Lot/Batch no: TE 90006, 17003/91
Purity: 99.2%
Stability of test compound: guaranteed for study duration; expiry date: 1992-05-05

#### 2. Vehicle:

Group 3: Acetone / Polyethylene glycol 400 (PEG 400) solution (1/1, v/v)  
Group 4: none

#### 3. Test animals

Species:	Wistar rat
Strain:	Bor WISW (SPF-Cpb)
Age:	2 to 3 months
Weight at dosing:	Mean weights: 170 to 210 g
Source:	████████████████████
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula diet (Altromin ® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	In groups of 5 in conventional Makrolon® Type III cages; bedding: type S8/15 low-dust wood shavings (Rettenmaier & Sons, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0 - 301 - 6802 mg/m³ air (actual concentration)
Application route:	Inhalation, head/nose-only

Exposure:	4 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	2 weeks
Observations:	mortality, clinical signs, body weights, body temperature, reflex measurements, gross necropsy

## 2. Generation of the test atmosphere / chamber description

### Generation and characterization of chamber atmosphere

	Group 1	Group 2	Group 3	Group 4
Target concentration (mg/m <sup>3</sup> )	Control (air)	Control (vehicle)	2500	--
Analytical concentration (mg/m <sup>3</sup> )	--	--	301	6802
Test substance concentration in vehicle (% w/v)				
Temperature (mean, °C)	20.8	23.2	20.9	21.3
Relative humidity (mean, %)	12.5	19.2	30.3	39.8
MMAD (µm)	--	1.63	1.44	2.25
GSD	--	1.51	1.83	1.78
Aerosol mass < 3 µm (%)	--	93	89	69

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; - = not applicable.

## Results and discussion

### A. Mortality

Three high-dose females died on study day 1. At lower concentrations and in high-dose males there were no mortalities.

**Table 6.8.2/17-1: Result summary**

Dose (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats						
0 (air)	0	0	5	--	--	0
0 (vehicle)	0	5	5	4 h – 5 h	--	0
301	0	0	5	--	--	0
6802	0	5	5	4 h – 11 d	--	0
LC <sub>50</sub> > 6802 mg/m <sup>3</sup>						
Female rats						
0 (air)	0	0	5	--	--	0
0 (vehicle)	0	5	5	4 h – 5 h	--	0
301	0	0	5	--	--	0
6802	3	5	5	4 h – 5 h	3 d	60
LC <sub>50</sub> approximate 6800 mg/m <sup>3</sup>						

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

**B. Clinical observations**

Group 1 (air control): There were no signs of toxicity observed in male and female rats during the study.

Group 2 (vehicle control): All rats exhibited clinical signs of toxicity. The signs consisted of piloerection and staggering gait. These signs were attributed to the high concentration of the acetone vehicle.

Group 3 (301 mg/m<sup>3</sup>): There were no signs of toxicity observed in males and females.

Group 4 (6802 mg/m<sup>3</sup>): All rats exhibited clinical signs of toxicity. The signs consisted of piloerection and unpreened fur, reduced motility, staggering gait, vocalization, atony, sternal recumbancy, comatose-like state, bradypnea and labored breathing, gasping, rales, serous nasal discharge, bloody incrustations around nose and corneal opacity.

**C. Reflex measurements**

The battery of reflex measurements revealed no changes of reflexes in any animal of dose groups 1 to 3 (i.e. air control, vehicle control and 301 mg/m<sup>3</sup>).

On day 1 of the recovery period, pinna reflex, reaction to noises and the righting reflex were impaired for one moribund high-dose female. This animal died on the same day. On day 4 of the recovery period, temporary impairment of the reaction to noises and of the righting reflex was observed for one high-dose male. By day 7 of the recovery period the observations were fully reversible.

**D. Body weight**

A temporary body weight loss was determined for the rats of groups 3 and 4 (i.e. test substance groups).

**E. Rectal temperature**

A reduction in the rectal temperature (hypothermia) was found for the rats of Group 4 and the female rats of Group 2; the latter was caused primarily by the high concentrations of the acetone vehicle component in the chamber atmosphere. The rectal temperatures determined for the two other groups were within the normal physiological range for rats. Thus, no exposure-related hyperthermia was found.

**D. Necropsy**

*Animals sacrificed moribund:* Lungs not completely collapsed, reddish and mottled; liver pale and with lobulation; spleen pale; glandular stomach with bloody ulcerative changes; duodenum reddish and with mucoid, yellowish-black and bloody content, kidneys pale; renal pelvis reddish.

*Animals sacrificed at termination:* There were no treatment-related gross-pathological findings observed in any animal examined at terminal sacrifice. The not completely collapsed lungs in two high-dose females are regarded as sacrifice-related findings.

<b>Conclusion</b>	FOE 5043-hydroxy is considered to be slightly toxic after acute inhalation exposure. The determined acute LC <sub>50</sub> values of male and female rats were > 6802 mg/m <sup>3</sup> and approximately 6800 mg/m <sup>3</sup> .
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /18; ██████████ 1992b</b>
<b>Title:</b>	FOE 5043-Hydroxy - Study for skin and eye irritation/corrosion in rabbits
<b>Document No:</b>	<b>M-004564-01</b>
<b>Report No:</b>	21257
<b>Guidelines:</b>	OECD 404 (1981), EEC Directive 84/449/EEC B.4 (1984), US-EPA TSCA Test guidelines 798.4470 (1985), US-EPA Pesticide assessment guidelines §81-5 (1984), OECD 405 (1987); EEC Directive 84/449/EEC B.5 (1984), US-EPA TSCA Test guidelines 798.4450 (1985), US-EPA Pesticide assessment guidelines §81-4 (1984) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Name:	FOE 5043-Hydroxy
Description:	Brown crystalline powder
Lot/Batch no:	TE 90006, 17003/91
Purity:	99.2%
Stability of test compound:	guaranteed for study duration;

#### 2. Vehicle:

None, the test item was used in its original form.

#### 3. Test animals

Species:	Rabbit
Strain:	New Zealand White, HC:NZW
Sex:	Males (skin irritation), females (eye irritation)
Age:	adult
Weight at dosing:	Males: 3.0 – 3.4 kg; females: 3.1 – 3.8 kg
Source:	████████████████████
Acclimatisation period:	At least 14 days
Diet:	Standard diet “Ssniff K4” (Ssniff Spezialdiaeten GmbH, Soest, Germany), 100 – 120 g per animal per day
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in stainless steel cages with flat rod bases or plastic cages with perforated bases

## B. Study design and methods

### 1. Animal assignment and treatment (skin irritation)

Dose:	0.5 g (moistened with water)
Application route:	Dermal (area: approx. 6 cm <sup>2</sup> )
Duration:	4 hours
Group size:	3 males
Observations:	Mortality, clinical signs, skin effects, body weight (at beginning of study)

### 2. Animal assignment and treatment (eye irritation)

Dose	0.1 mL/animal
Application route:	Single instillation to the conjunctival sac of one eye (eyes were rinsed with saline 24 h after application)
Group size:	3 females
Observations:	Mortality, clinical signs, eye effects, body weight (at beginning of study)

## Results and discussion

### A. Findings skin irritation

There were no mortalities or systemic intolerance reactions.

There were no sign of skin irritation observed in any animal at any observation time point.

The mean irritation scores for the individual animals were 0.0, 0.0 and 0.0 for erythema and for oedema.

The skin irritation observations are summarized in the Table 6.8.2/18-1.

**Table 6.8.2/18-1: Summary of irritant effects (Score)**

Time after patch removal	Animal #1		Animal #2		Animal #3	
	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema
60 min	0	0	0	0	0	0
24 h	0	0	0	0	0	0
48 h	0	0	0	0	0	0
72 h	0	0	0	0	0	0
<b>Mean 24-72 h</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>

### B. Findings eye irritation

There were no mortalities or systemic intolerance reactions.

Exposure of the test substance to the eye caused reactions of the mucous membranes and effects of the cornea and discharge in all animals. The iris was also transiently affected in one animal. These signs proved to be fully reversible within 7 days.

The eye observations are summarized in the Table 6.8.2/18-2.

**Table 6.8.2/18-2: Summary of irritant effects (Score)**

Animal No.	Observation	1 h	24 h	48 h	72 h	Mean scores	Response	Reversibility (days)
1	Corneal opacity	1	1	1	1	1.0	+	7
	Iris	0	0	0	0	0.0	–	na
	Redness conjunctivae	2	2	1	1	1.3	–	7
	Chemosis conjunctivae	3	1	1	1	1.0	–	7
2	Corneal opacity	1	1	1	1	1.0	+	7
	Iris	1	1	0	1	0.7	–	7
	Redness conjunctivae	2	2	2	2	2.0	+	7
	Chemosis conjunctivae	3	2	1	1	1.3	–	7
3	Corneal opacity	1	1	1	0	0.7	–	3
	Iris	0	0	0	0	0.0	–	na
	Redness conjunctivae	2	2	1	1	1.3	–	7
	Chemosis conjunctivae	3	2	1	1	1.3	–	7

Response for mean scores		Corneal opacity	Iritis	Conjunctival redness oedema		
–	= negative	<1	<1	<2	<2	(Regulation (EC) No 1272/2008 and GHS) (Directive 1999/45/EC as amended)
+	= irritant	≥1 - <3	≥1 - <2	≥2	≥2	(Regulation (EC) No 1272/2008 (GHS) category 2) (Directive 1999/45/EC as amended)
++	= irreversible effects	≥3	≥1.5			(Regulation (EC) No 1272/2008 and GHS category 1) (Directive 1999/45/EC as amended)
	serious damage	≥3	≥2			
na	not applicable					

<b>Conclusion</b>	The test item FOE 5043-hydroxy is not irritating to the skin. Based on the study results the test substance FOE 5043-hydroxy is irritating to eyes of rabbits.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /19; [REDACTED] 1994</b>
<b>Title:</b>	FOE 5043-Hydroxy - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman)
<b>Document No:</b>	<b>M-004614-01</b>
<b>Report No:</b>	22824
<b>Guidelines:</b>	OECD 406, Directive 84/449/EEC B.6 (1984) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test materials:

Name:	FOE 5043-Hydroxy
Description:	Brown crystalline powder
Lot/Batch no:	17001/93
Purity:	98.9%
Stability of test compound:	guaranteed for study duration; expiry date: 1995-03-29
<b>2. Vehicle:</b>	Cremophor EL in sterile physiological saline solution (2% v/v)

#### 3. Test animals:

Species:	Guinea pig
Strain:	Hsd/Win:DH (SPF-bred)
Age:	Approx. 5 to 8 weeks
Sex:	Males
Weight at dosing:	317 - 420 g
Source:	[REDACTED]
Acclimatisation period:	At least 7 days
Diet:	Altromin® 3020 maintenance diet for guinea pigs (ALTROMIN GmbH, Lage, Germany), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	In Makrolon® type 4 cages with low-dust wood shavings as bedding. During acclimatization 5 per cage, during experiment 2 to 3 animals per cage.

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose	
Intradermal induction:	5% (= 20 mg test substance/animal)

Irritation treatment:	Sodium lauryl sulfate prior to topical induction
Topical induction:	50% (= 250 mg test substance/animal)
Challenge:	50% (= 250 mg test substance/animal) and 25% (=125 mg test substance/animal)
Application route:	Intradermal, dermal
Application volume:	intradermal induction: 0.1 mL/injection topical induction: 0.5 mL/patch challenge: approx. 0.5 mL/patch
Duration:	topical induction: 48 hours, challenge: 24 hours
Group size:	20 in test item groups; 10 in control group
Observations::	mortality, clinical signs, skin effects, body weight (at beginning and termination of study)

## Results and discussion

### A. Findings

After the second induction, open wounds followed by incrustations or skin flaking in the treated areas were observed on a few animals of the control group; a few animals of the test substance group exhibited incrustations or skin flaking in the treated areas.

Neither the control animals nor those of the test substance group exhibited skin reactions after the challenge with a 50% and a 25% test substance concentration.

A summary of the skin reactions observed after challenge exposure are given in the Table 6.8.2/19-1.

**Table 6.8.2/19-1: Number of animals exhibiting skin effects**

	Test item group (20 animals)					Control group (10 animals)				
	Test item patch			Control patch		Test item patch			Control patch	
Hours	24	48	Total	24	48	24	48	Total	24	48
Challenge 50%	0/20	0/20	0/20	0/20	0/20	0/10	0/10	0/10	0/10	0/10
Challenge 25%	0/20	0/20	0/20	0/20	0/20	0/10	0/10	0/10	0/10	0/10

The last two reliability checks performed in the laboratory with 2-mercaptobenzothiazole confirmed the sensitivity and reliability of the test method.

<b>Conclusion</b>	Based on the study results FOE 5043-hydroxy does not possess a skin sensitizing potential.
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**FOE 5043-TDA sulfone**

<b>New studies; not evaluated</b>	This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.  <b>Supportive information for justification of the active substance specification</b>
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<b>Report:</b>	<b>KCA 5.8.2 /20; Herbold, B. A.; 1993</b>
<b>Title:</b>	FOE 5043-Sulfon - Salmonella/microsome test plate incorporation and preincubation method
<b>Document No:</b>	<b>M-004606-01</b>
<b>Report No:</b>	22629
<b>Guidelines:</b>	EEC Directive 84/449/EEC B.14; OECD 471 (1983); US-EPA New and revised health effects test guidelines (1984) Deviations: none
<b>GLP</b>	Yes

**Materials and methods****A. Materials**

- 1. Test material:** FOE 5043-Sulfon  
Description: Colourless crystals  
Lot/Batch no: 17007/92  
Purity: 99.1%  
Stability of test compound: guaranteed for study duration; expiry date: 1995-04-13
- 2. Vehicle and/or positive control:** DMSO  
without metabolic activation: Sodium azide (Na-azide), nitrofurantoin (NF), 4-nitro-1,2-phenylene diamine (4-NPDA),  
With metabolic activation: 2-aminoanthracene (2-AA)
- 3. Test system:** *Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98  
**Metabolic activation:** S9 mix prepared from Aroclor 1254 induced male Sprague-Dawley rats

**B. Study design and methods**

- Dose:** 0-8-40-200-1000-5000 µg/plate (plate incorporations and pre-incubation)  
positive controls:  
Na-azide: 10 µg/plate (only TA1535)  
4-NPDA: 10 µg/plate (only TA 1537)  
0.5 µg/plate (only TA 98)  
NF: 0.2 µg/plate (only TA 100)  
2-AA: 3 µg/plate
- Application volume:** 0.1 mL
- Incubation time /temperature:** Pre-incubation: 20 minutes, 37 °C  
48 hours, 37 °C

### Results and discussion

The potential of FOE 5043-Sulfon to induce gene mutations was investigated according to the plate incorporation and the pre-incubation method in two independent experiments both with and without liver microsomal activation (S9 mix).

In the plate incorporation test there was no indication of a bacteriotoxic effect of FOE 5043-Sulfon at doses of up to and including 4 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a strong, strain-specific bacteriotoxic effect, and could only partly be used for assessment up to and including 32 µg per plate. None of the four strains used showed a dose-related and biologically relevant increase in the mutant frequency over those of the negative controls. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

In the pre-incubation test there was no indication of a bacteriotoxic effect of FOE 5043-Sulfon at doses of up to and including 2 µg per tube. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a bacteriotoxic effect, and could only partly be used for assessment up to and including 16 µg per tube. None of the four strains concerned showed a dose-related and biologically relevant increase in mutant counts over those of the negative controls and thus confirmed the results of the plate incorporation method.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

**Table 6.8.2/20-1: Summary of results of plate incorporation experiment**

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)			
			TA1535	TA1537	TA98	TA100
Without Activation <sup>S</sup>	FOE 5043-Hydroxy	0	10 ± 2	8 ± 3	20 ± 4	66 ± 14
		8	4 ± 2	5 ± 2	13 ± 6	45 ± 8
		40	0 ± 1 <sup>B#</sup>	0 ± 0 <sup>B#</sup>	0 ± 0 <sup>B#</sup>	0 ± 0 <sup>B#</sup>
		200	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
		1000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
		5000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
	NaN <sub>3</sub>	10	445 ± 36*			
	NF	0.2				336 ± 16*
	4-NPDA	10		49 ± 7*		
	4-NPDA	0.5			105 ± 32*	
With Activation <sup>S</sup>	FOE 5043-Hydroxy	0	17 ± 5	9 ± 2	24 ± 7	72 ± 10
		8	11 ± 1	8 ± 2	24 ± 6	89 ± 9
		40	5 ± 1 <sup>B#</sup>	4 ± 3 <sup>B#</sup>	14 ± 6#	53 ± 17 <sup>B#</sup>
		200	0 ± 0#	0 ± 0#	0 ± 0 <sup>B#</sup>	0 ± 0#
		1000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
		5000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
	2-AA	3	149 ± 9*	391 ± 25*#	1256 ± 36*	871 ± 41*#
Without Activation	FOE 5043-Hydroxy	0	18 ± 5	8 ± 2	23 ± 2	106 ± 1
		1	16 ± 3	8 ± 3	18 ± 5	95 ± 5
		2	16 ± 4	7 ± 1	23 ± 3	90 ± 19

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)			
			TA1535	TA1537	TA98	TA100
		4	14 ± 5	5 ± 2	23 ± 6	99 ± 19
		8	12 ± 1	5 ± 3#	25 ± 7	100 ± 6
		16	10 ± 3	7 ± 2 <sup>B</sup> #	19 ± 3 <sup>B</sup>	103 ± 18
		32	8 ± 3 <sup>B</sup> #	1 ± 1 <sup>B</sup> #	3 ± 2 <sup>B</sup> #	76 ± 11 <sup>B</sup> #
	NaN <sub>3</sub>	10	567 ± 33*#			
	NF	0.2				450 ± 22*
	4-NPDA	10		96 ± 18*		
	4-NPDA	0.5			102 ± 25*	
With Activation	FOE 5043-Hydroxy	0	21 ± 4	10 ± 1	35 ± 4	115 ± 11
		1	16 ± 4	6 ± 1	40 ± 6	114 ± 16
		2	15 ± 7	13 ± 2	45 ± 8	108 ± 11
		4	19 ± 3	10 ± 4	47 ± 8	126 ± 17
		8	15 ± 2	9 ± 2#	44 ± 10	97 ± 7
		16	17 ± 4	6 ± 3#	43 ± 6	102 ± 13
		32	11 ± 2 <sup>B</sup> #	5 ± 1 <sup>B</sup> #	23 ± 9 <sup>B</sup> #	80 ± 17 <sup>B</sup> #
	2-AA	3	130 ± 10*#	50 ± 15*#	901 ± 212*#	1200 ± 221*#

NaN<sub>3</sub> = sodium azide; NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene

<sup>S</sup> = not used for assessment due to increased toxicity. Results were used only for assessment of bacteriototoxicity.

<sup>B</sup> = background lawn reduced, # = bacteriotoxic effect; \* = mutagenic effect

**Table 6.8.2/20-2: Summary of results of pre-incubation experiment**

Metabolic Activation	Test Group	Dose (µg/plate)	Revertant Colony Counts (Mean ±SD)			
			TA1535	TA1537	TA98	TA100
Without Activation	FOE 5043-Hydroxy	0	9 ± 3	10 ± 1	19 ± 5	103 ± 12
		1	12 ± 2	9 ± 2	30 ± 8	87 ± 13
		2	11 ± 4	6 ± 2	12 ± 3	82 ± 21
		4	8 ± 2	5 ± 2	12 ± 1	71 ± 8 <sup>B</sup>
		8	4 ± 1	0 ± 0 <sup>B</sup>	8 ± 4 <sup>B</sup>	47 ± 17 <sup>B</sup>
		16	2 ± 1	0 ± 0 <sup>B</sup>	3 ± 1 <sup>B</sup>	15 ± 6 <sup>B</sup>
		32	0 ± 0 <sup>B</sup> #	0 ± 0 <sup>B</sup>	0 ± 0 <sup>B</sup> #	0 ± 0 <sup>B</sup> #
	NaN <sub>3</sub>	10	388 ± 63*			
	NF	0.2				414 ± 21*
	4-NPDA	10		60 ± 10*		
	4-NPDA	0.5			64 ± 7*	
With Activation	FOE 5043-Hydroxy	0	15 ± 5	10 ± 2	41 ± 9	124 ± 8
		1	12 ± 4	10 ± 3	37 ± 11	124 ± 22
		2	13 ± 6	9 ± 1	23 ± 6	119 ± 7
		4	11 ± 2	7 ± 2	19 ± 1	115 ± 15
		8	13 ± 2	9 ± 4	26 ± 4	113 ± 13
		16	12 ± 4	11 ± 3	28 ± 2	123 ± 25
		32	6 ± 3 <sup>B</sup> #	7 ± 2 <sup>B</sup> #	10 ± 4 <sup>B</sup> #	64 ± 5 <sup>B</sup> #
	2-AA	3	149 ± 3*	188 ± 9*	492 ± 47*	729 ± 41*

NaN<sub>3</sub> = sodium azide; NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene

<sup>B</sup> = background lawn reduced, # = bacteriotoxic effect; \* = mutagenic effect



<b>Conclusion</b>	FOE 5043-Sulfon is considered to be non-mutagenic in this <i>Salmonella typhimurium</i> reverse mutation assay.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /21; [REDACTED] 1992c</b>
<b>Title:</b>	FOE 5043 Sulfon - Study for acute oral toxicity in rats
<b>Document No:</b>	<b>M-004578-01</b>
<b>Report No:</b>	21893
<b>Guidelines:</b>	OECD 401 (1987); US-EPA Pesticide assessment guidelines, Series 81-1 (1984) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Name:	FOE 5043-Sulfon
Description:	Colorless crystals
Batch / Lot No.:	TE 86005, 17001-3/91
Purity:	99.3%
Stability of test compound:	guaranteed for study duration; expiry date: 1992-10-01

#### 2. Vehicle:

2% (v/v) Cremophor® EL in deionized water

#### 3. Test animals

Species:	Rat
Strain:	Wistar, Bor:WISW (SPF-Cpb)
Age:	Young adults, approx. 7-8 (males) and 10 (females) weeks
Weight at dosing:	males: 165 g - 184 g; females: 168 g - 187 g
Source:	[REDACTED]
Acclimatisation period:	at least 7 days
Diet:	Altromin® 1324 maintenance diet for rats and mice (Altromin GmbH & Co KG, Germany), <i>ad libitum</i> , except during a 17 hour fasting period prior to dosing
Water:	Tap water, <i>ad libitum</i>
Housing:	5 per sex in Makrolon® Type 3 cages with low-dust wood granules type S 8/15 (Ssniff, Germany) as bedding material.

## B. Study design and methods

### 1. Animal assignment and treatment

Dose:	50-100-150-170-200-300-1000 mg/kg bw
Application route:	Oral, gavage
Application volume:	10 mL/kg bw
Fasting time:	before administration: $17 \pm 1$ hour
Group size:	5 rats/sex, except at 300 mg/kg: 10/sex
Post-treatment observation period:	14 days
Observations:	clinical signs, mortality, body weight, gross necropsy

## II. Results and discussion

### A. Mortality

Mortalities occurred at 150 mg/kg bw and above for females and at 200 mg/kg bw and above for males. The results are summarised in the following table.

**Table 6.8.2/21-1: Result summary**

Animal Nos.	Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats							
21 - 25	50	0	0	5	--	--	0
31 - 35	100	0	0	5	--	--	0
41 - 45	150	0	5	5	52 min – 2 d	--	0
51 – 55	170	0	5	5	2 h – 2 d	--	0
11 - 15	200	3	5	5	2.25 h – 1 d	3.75 h – 1 d	60
61 – 65; 71 - 75	300	1	10	10	2.5 h – 2 d	5.5 h	10
1 - 5	1000	5	5	5	9 min – 1 d	48 min – 1 d	100
LD <sub>50</sub> > 170 < 200 mg/kg bw							
Female rats							
26 - 30	50	0	0	5	--	--	0
36 - 40	100	0	2	5	2.75 h – 1 d	--	0
46 - 50	150	2	5	5	51 min – 2 d	5.75 h – 1 d	40
56 - 60	170	0	2	5	3.75 h – 1 d	--	0
16 - 20	200	4	5	5	2.25 h – 1 d	3.5 h – 1 d	80
66 – 70, 76 - 80	300	4	10	10	29 min – 1 d	2 h – 1 d	40
6 - 10	1000	5	5	5	13 min – 4.25	2.25 h – 4.25 h	100
LD <sub>50</sub> > 150 < 200 mg/kg bw							

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

### B. Clinical observations

The following signs were observed in males at 150 mg/kg bw and above and in females at 100 mg/kg bw and above: apathy, reduced motility, piloerection and labored breathing. In the females there was also pallor, this occurring in the males only in higher doses. In both sexes at the higher doses there were additionally signs of cyanosis, narrowed palpebral fissures and staggering gait. Atony, cramped posture, prostration, spastic gait, no reflexes and soft faeces were also observed in isolated cases in the males and females. The following individual signs were also observed: salivation; prostration; vocalization on touching. The signs were mainly up to moderate in degree, occurred in some cases shortly after administration and lasted in the males and females to max. day 2 of the study. A dose of 100 mg/kg b.w. was tolerated by the males and a dose of 50 mg/kg b.w. by the females without signs occurring.

### C. Body weight

There were no treatment-related effects on body weight gain noted.

### D. Necropsy

*Animals that died during the study:* The three males of the 200 mg/kg bw dose group that died had a reddened glandular stomach and (small) intestine, as well as injected mesenteric vessels. Two of these males had a clear liquid in stomach, strongly circulated vessel, slightly reddened epididymes, and injected vessels on testes. In addition, one male each had severely distended vessels on testes, liquid diet in stomach, reddened adrenals and a somewhat patchy spleen. The one male of the 300 mg/kg bw dose group showed a distended stomach and reddened glandular stomach. All five high-dose males that died had a severely reddened glandular stomach and (severely) reddened small intestines. Four rats had also a dark liver, liquid in the stomach, and liquid contents in the intestine. Mesenteric vessels of the intestine tract were severely injected in three rats, while distended lungs, dark adrenals, and dark spleen were observed in 2 rats each. In addition, reddened pelvis, dark pancreas, enlarged stomach with slimy yellowish content, injected vessels on testes, bladder filled with clear liquid was observed in one rat each.

At 150 mg/kg bw two females died during the study. One had pale, mottled kidneys and clear liquid stomach content, as well as a reddened small intestine tract. The thoracic organs of this animal were not assessable. The second female had a dark liver, clear liquid in stomach, reddened small intestine tract with slimy red contents and a slightly reddened renal pelvis. At 200 mg/kg bw four females had a reddened glandular stomach. Three rats had a clear liquid in the glandular stomach, a reddened small intestine and severely injected vessels. The small intestines of two females were filled with a clear liquid. In addition, mottled or pale liver, isolated ulcerous foci of glandular stomach, mottled spleen and liquid content in the glandular stomach were observed in one rat each.

The four females of the 400 mg/kg bw dose group that died had all a reddened glandular stomach and intestinal tract. In addition, mottled liver, slightly distended lungs, liver lobulation, mottled lung, and a slightly distended stomach was observed in one rat each.

All five high-dose females had a severely reddened glandular stomach and a severely or slightly reddened intestinal tract. Four rats had also a mottled liver and liquid stomach contents. Three females had a dark liver and liquid in the small intestine. In addition, slightly distended lungs, distended stomach liver lobulation, mottled lung and a mottled spleen were recorded in one rat each.

*Animals sacrificed at termination:* There were no gross lesions observed in males up to and including 200 mg/kg bw sacrificed at termination. At 300 mg/kg bw four males had a small crater-like protrusion of the proventriculus. At the edge the crater was white and there were no mucous membrane on the inner surface. In addition, one of these rats had isolated thicker regions on proventriculus.

In females sacrificed at termination there were also no gross pathological findings up to and including 200 mg/kg bw. One female at 300 mg/kg bw had a small crater-like protrusion of the proventriculus. At the edge the crater was white and there were no mucous membrane on the inner surface.

<b>Conclusion</b>	FOE 5043-Sulfon is considered to be toxic after acute oral administration. The estimated acute oral LD <sub>50</sub> for male and female rats is > 150 and < 200 mg/kg bw.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /22; ██████████ 1992a</b>
<b>Title:</b>	FOE 5043-Sulfone - Study of the acute inhalation toxicity to rats in accordance with OECD guideline no. 403
<b>Document No:</b>	<b>M-004576-01</b>
<b>Report No:</b>	21784
<b>Guidelines:</b>	OECD 403 (1981), EC guideline 84/449/EEC B.2, US FIFRA guideline § 81-3 (1984) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-Sulfon
Lot/Batch no:	Colourless crystals
Purity:	TE 86005, 17001-3/91
Stability of test compound:	99.4%
	guaranteed for study duration;

#### 2. Vehicle:

Dust exposure : none  
Aerosol exposure: Polyethylene glycol 400 (PEG 400) / acetone (1:1)

#### 3. Test animals

Species:	Wistar rat
Strain:	Bor : WISW (SPF-bred)
Age:	2 to 3 months
Weight at dosing:	males: 163 g – 187 g, females: 163 g – 192 g
Source:	████████████████████
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	During acclimatization and during the study period in groups of

five in Makrolon® Type III cages; bedding: type S8/15 low-dust wood shavings (Ssniff, Soest, Germany)

## B. Study design and methods

### 1. Animal assignment and treatment

Dose:	Aerosol: 0-8.2-52.6-89.8-146.3 mg/m <sup>3</sup> air (actual concentration) Dust: 0-35.3-122.7 mg/m <sup>3</sup> (actual concentration)
Application route:	Inhalation, nose / head only
Exposure:	4 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	2 weeks
Observations:	mortality, clinical signs, body weights, rectal temperature (after aerosol exposure only), reflex measurements, gross necropsy

### 2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

	Dust			Aerosol				
Target concentration (mg/m <sup>3</sup> )	0	--	--	0	50	500	1000	1500
Analytical concentration (mg/m <sup>3</sup> )	0	35.3	122.7	0	8.2	52.6	89.8	146.3
Temperature (mean, °C)	21.5	23.1	nr	21.4	21.6	21.6	21.5	21.7
Relative humidity (mean, %)	18.8	12.9	nr	45.1	18.2	18.6	15.6	19.7
MMAD (µm)		>9	>9	1.45	1.45	1.43	1.43	1.39
GSD		-	-	1.46	1.45	1.45	1.44	1.43
Aerosol mass < 3 µm (%)	--	0	0	98	98	98	98	99

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; - = not applicable.  
nr = not reported

## Results and discussion

### A. Mortality

#### *Dust exposure*

There were no mortalities observed in the air control dose group in both sexes, as well as in females up to 122.7 mg/m<sup>3</sup> dust. One male rat each died at 35.3 and 122.7 mg/m<sup>3</sup>.

#### *Aerosol exposure*

There were no mortalities observed in male and female rats in the vehicle control group and at concentrations up to and including 52.6 mg/m<sup>3</sup>.

Four males and one female each died at concentrations of 89 and 146.3 mg/m<sup>3</sup>.

**Table 6.8.2/21-2: Result summary**

Dose (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats – Dust exposure						
Air control	0	0	5	--	--	0
35.3	1	5	5	4h – 14d	2d	20
122.7##	2	5	5	4h – 7d	2d – 3d	20
Male rats – Aerosol exposure						
0#	0	0	5	--	--	0
8.2	0	0	5	--	--	0

Dose (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
52.6	0	5	5	4h – 9d	--	0
89.8	4	5	5	4h – 9d	1d – 3d	80
146.3	4	5	5	4h – 6d	1d – 3d	80
LC <sub>50</sub> approximately 69 mg/m <sup>3</sup>						
Female rats – Dust exposure						
Air control	0	0	5	--	--	0
35.3	0	5	5	4h – 14d	--	0
122.7##	0	5	5	4h – 14d	--	0
Female rats – Aerosol exposure						
0#	0	0	5	--	--	0
8.2	0	0	5	--	--	0
52.6	0	5	5	4h – 7d	--	0
89.8	1	5	5	4h – 11d	3d	20
146.3	1	5	5	4h – 8d	3d	20
LC <sub>50</sub> > 146.3 mg/m <sup>3</sup>						

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

# vehicle control

## maximum technically attainable concentration

## B. Clinical observations

### *Dust exposure*

There were no clinical signs of toxicity observed in male and female rats of the air control group. At the two tested concentrations of 35.3 and 122.7 mg/m<sup>3</sup> dust, all male and female rats exhibited clinical signs. These signs consisted of respiratory sounds, difficult breathing, dyspnea and bradypnea, reduced activity, atony, piloerection, serous nasal discharge, salivation, reddened and bloody rhinarium, corneal opacity, periorbital skin regions reddened, bloody and swollen, head and limbs severely swollen and reddened, cachexia, vitreous humor lesion, distended abdomen.

### *Aerosol exposure*

There were no treatment-related clinical findings in males and females at 0 and 8.2 mg/m<sup>3</sup>.

At higher concentrations all male and female rats exhibited clinical signs. The clinical signs at 52.6 mg/m<sup>3</sup> consisted of dyspnea and bradypnea, respiratory sounds, difficult breathing, rhinarium reddened and bloody, serous nasal discharge, reduced activity, atony, piloerection.

At 89.8 mg/m<sup>3</sup> the following signs were observed: dyspnea and bradypnea, difficult breathing and respiratory sounds, atony, reduced activity, serous nasal discharge, bloody and blood-incrusted rhinarium, periorbital incrustations of blood, piloerection.

The signs observed at the highest concentration of 146.3 mg/m<sup>3</sup> were Dyspnea and bradypnea, difficult breathing and respiratory sounds, atony, reduced activity, serous nasal discharge, bloody and blood-incrusted rhinarium, bloody and blood-incrusted eyelids, periorbital incrustations of blood, cyanosis, cachexia (severe body weight loss), piloerection and unpreened hair coat.

**C. Reflex measurements***Dust exposure*

The battery of reflex measurements conducted on day 1 revealed no changes of reflexes in any animal of the air control group. At 35.3 and 122.7 mg/m<sup>3</sup> dust reduced grip strength and reduced reaction to external stimuli were observed in a few animals.

*Aerosol exposure*

The battery of reflex measurements conducted on day 1 revealed no changes of reflexes in any animal of the vehicle control group and at concentrations up to and including 52.6 mg/m<sup>3</sup>. At 89.8 and 146.3 mg/m<sup>3</sup> dust reduced grip strength and reduced reaction to external stimuli were observed in a few animals.

**D. Body weight***Dust exposure*

There was a treatment-related and toxicologically significant effect on body weight gain (reduction) at 35.3 and 122.7 mg/m<sup>3</sup> dust.

*Aerosol exposure*

There was a treatment-related and toxicologically significant reduction on body weight gain at 52.6 mg/m<sup>3</sup> and above on day 3 after exposure. Afterwards body weights increased.

**E. Rectal temperature***Aerosol exposure*

There was a statistically significant, concentration-related hypothermia at concentrations of 8.2 mg/m<sup>3</sup> and above. The hypothermia is considered to be related to a severe “sensory irritation”.

**D. Necropsy***Dust exposure – animals that died during the study*

Observed findings were distended lungs, bloody nose, pale spleen, pale liver, lobular pattern of liver, GI tract with yellowish-mucoid content or empty and distended, pale kidneys, and reddened renal pelvis.

*Dust exposure – animals sacrificed at termination*

One high-dose male and one low-dose female had lungs with hepatoid foci. In addition, one female at 35.3 and two females at 122.7 mg/m<sup>3</sup> had corneal opacity.

*Aerosol exposure – animals that died during the study*

Observed findings were Lungs distended, and / or with hepatoid foci; lungs reddened, mucosa of the small intestine reddened, foci; thorax with serous fluid; spleen and kidneys pale; small intestine with bloody mucoid, bloody nose, glandular stomach reddened; and liver with lobular pattern;

*Aerosol exposure – animals sacrificed at termination*

There were no gross lesions observed in males and females of all dose groups.

<b>Conclusion</b>	FOE 5043-Sulfon, both as an aerosol (high respirability) and as a dust (practically no respirability) exhibited a high acute inhalation toxicity to rats. The determined acute LC <sub>50</sub> values of male and female rats were approximately 69 mg/m <sup>3</sup> and >146 mg/m <sup>3</sup> after aerosol exposure.
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<b>New studies; not evaluated</b>	This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.  <b>Supportive information for justification of the active substance specification</b>
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<b>Report:</b>	<b>KCA 5.8.2 /23; [REDACTED] 1992d</b>
<b>Title:</b>	FOE 5043-Sulfon - Study for skin and eye irritation/corrosion in rabbits
<b>Document No:</b>	<b>M-004522-01</b>
<b>Report No:</b>	21156
<b>Guidelines:</b>	OECD 404 (1981), EEC Directive 84/449/EEC B.4 (1984), US-EPA TSCA Test guidelines 798.4470 (1985), US-EPA Pesticide assessment guidelines §81-5 (1984), OECD 405 (1987); EEC Directive 84/449/EEC B.5 (1984), US-EPA TSCA Test guidelines 798.4450 (1985), US-EPA Pesticide assessment guidelines §81-4 (1984) Deviations: none
<b>GLP</b>	Yes

## A. Materials

### 1. Test material:

Name:	FOE 5043-Sulfon
Description:	Colourless crystals
Lot/Batch no:	TE 86005, 17001-3/91
Purity:	99.4%
Stability of test compound:	guaranteed for study duration;
<b>2. Vehicle:</b>	None for skin irritation and first eye irritation test; Cremophor EL 2% for the repeated eye irritation test

### 3. Test animals

Species:	Rabbit
Strain:	New Zealand White, HC:NZW
Sex:	Females
Age:	adult
Weight at dosing:	Females: 3.0 – 3.8 kg
Source:	[REDACTED]
Acclimatisation period:	At least 14 days
Diet:	Standard diet “Ssniff K4” (Ssniff Spezialdiaeten GmbH, Soest, Germany), 100 – 120 g per animal per day
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in stainless steel cages with flat rod bases or plastic cages with perforated bases



## B. Study design and methods

### 1. Animal assignment and treatment (skin irritation)

Dose:	First experiment: 0.5 g (moistened with water) second experiment: 1% formulation in 2% Cremophor EL/ animal
Application route:	Dermal (area: approx. 6 cm <sup>2</sup> )
Duration:	4 hours
Group size:	3 females
Observations:	Mortality, clinical signs, skin effects, body weight (at beginning of study)

### 2. Animal assignment and treatment (eye irritation)

Dose	First experiment: 0.1 mL/animal second experiment: 0.1 mL of a 1% formulation in 2% Cremophor EL/ animal
Application route:	Single instillation to the conjunctival sac of one eye (eyes were rinsed with saline 24 h after application)
Group size:	3 females
Observations:	Mortality, clinical signs, eye effects, body weight (at beginning of study)

## Results and discussion

### A. Findings skin irritation

There were no mortalities or systemic intolerance reactions.

Dermal application of the undiluted test substance caused irritant effects within the first 7 days.

The mean irritation scores for the individual animals were 0.0, 2.0 and 2.0 for erythema and 0.0, 0.3 and 0.0 for oedema.

After dermal application of a 1% formulation of the test substance slight erythema were observed in all rabbits.

The mean irritation scores for the individual animals were 1.0, 1.0 and 1.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

All skin reactions were resolved by day 14.

The skin irritation observations are summarized in the Table 6.8.2/23-1.

**Table 6.8.2/23-1: Summary of irritant effects (Score)**

Time after patch removal	Animal #1		Animal #2		Animal #3	
	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema
First experiment (undiluted test substance)						
60 min	0	0	0	0	1	0
24 h	0	0	2	0	2	0
48 h	0	0	2	1	2	0
72 h	0	0	2	0	2	0
<b>Mean 24-72 h</b>	<b>0.0</b>	<b>0.0</b>	<b>2.0</b>	<b>0.3</b>	<b>2.0</b>	<b>0.0</b>
7 days	0	0	2	0	2	0
14 days	0	0	0	0	0	0
Second experiment (1% formulation)						
60 min	1	0	0	0	1	0
24 h	1	0	1	0	1	0
48 h	1	0	1	0	1	0
72 h	1	0	1	0	1	0
<b>Mean 24-72 h</b>	<b>1.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>
7 days	0	0	2	0	1	0
14 days	0	0	0	0	0	0

**B. Findings eye irritation**

There were no mortalities or systemic intolerance reactions.

Exposure of the undiluted test substance caused strong irritating reactions to the eyes of all three rabbits. Therefore the animals were sacrificed 72 hours after the test substance administration, and the test was repeated with a 1% aqueous formulation of the test substance.

Exposure of the 1% formulation of the test substance caused also severe eye reactions.

The eye observations are summarized in the Table 6.8.2/23-2.

**Table 6.8.2/23-2: Summary of irritant effects (Score) after undiluted application**

Animal No.	Observation	1 h	24 h	48 h	72 h	Mean scores	Other eye effects	Response
1s	Corneal opacity	ne*	2	2	3	<b>2.3</b>	Conjunctiva: whitish colored, hemorrhage, conjunctivae and eyelids: partly black colored Conjunctivae and nictitating membrane: strong formation of vessels	++
	Iris	ne*	2	2	ne#	<b>x</b>		
	Redness conjunctivae	2	3	3	ne	<b>x</b>		
	Chemosis conjunctivae	3	3	3	3	<b>3.0</b>		
2s	Corneal opacity	ne*	2	2	3	<b>2.3</b>		++
	Iris	ne*	2	2	ne#	<b>x</b>		
	Redness conjunctivae	2	3	3	ne	<b>x</b>		
	Chemosis conjunctivae	4	3	3	3	<b>3.0</b>		
3s	Corneal opacity	ne*	2	2	3	<b>2.3</b>		++
	Iris	ne*	2	2	ne#	<b>x</b>		
	Redness conjunctivae	2	3	3	ne	<b>x</b>		
	Chemosis conjunctivae	4	3	3	3	<b>3.0</b>		

\*ne = no evaluation possible due to the chemosis of the conjunctivae

# ne = no evaluation possible due to strong corneal opacity

ne = no evaluation possible

x = calculation not possible

s = due to strong eye effects animals were sacrificed after 72 hours

**Table 6.8.2/23-3: Summary of irritant effects (Score) after application of 1 % formulation**

Animal No.	Observation	1 h	24 h	48 h	72 h	Mean scores	Reversibility	Other eye effects	Response
4	Corneal opacity	0	1	1	1	<b>1.0</b>	7 days	Conjunctivae: whitish colored Conjunctivae and nictitating membrane: strong formation of vessels	+
	Iris	0	0	0	0	<b>0.0</b>	na		–
	Redness conjunctivae	2	2	2	3	<b>2.3</b>	14 days		+
	Chemosis conjunctivae	3	3	3	3	<b>3.0</b>	21 days		+
5	Corneal opacity	ne*	1	1	1	<b>1.0</b>	14 days	Conjunctivae: whitish colored Conjunctivae and nictitating membrane: strong formation of vessels; vascularization	+
	Iris	ne*	1	1	1	<b>1.0</b>	7 days		+
	Redness conjunctivae	2	2	2	3	<b>2.3</b>	14 days		+
	Chemosis conjunctivae	3	3	3	3	<b>3.0</b>	21 days		+
6	Corneal opacity	1	0	0	0	<b>0.0</b>	1 day	Conjunctivae and nictitating membrane: strong formation of vessels;	–
	Iris	0	0	0	0	<b>0.0</b>	na		–
	Redness conjunctivae	2	2	1	1	<b>1.3</b>	21		–
	Chemosis conjunctivae	3	2	1	1	<b>1.3</b>	7 days		–

\*ne = no evaluation possible due to the chemosis of the conjunctivae

Response for mean scores	Corneal opacity	Iritis	Conjunctival		
			redness	oedema	
– = negative	<1	<1	<2	<2	(Regulation (EC) No 1272/2008 and GHS)
	<2	<1	<2.5	<2	(Directive 1999/45/EC as amended)
+ = irritant	≥1 - <3	≥1 - <2	≥2	≥2	(Regulation (EC) No 1272/2008 (GHS) category 2)
	≥2 - <3	≥1 - <2	≥2.5	≥2	(Directive 1999/45/EC as amended)
++ = irreversible effects	≥3	≥1.5			(Regulation (EC) No 1272/2008 and GHS category 1)
serious damage	≥3	≥2			(Directive 1999/45/EC as amended)
na not applicable					

<b>Conclusion</b>	The test item FOE 5043-Sulfon was irritating to the skin. Based on the study results the test substance FOE 5043-Sulfon is severely irritating to eyes of rabbits.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /24; [REDACTED] 1994</b>
<b>Title:</b>	FOE 5043-Sulfone - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman)
<b>Document No:</b>	<b>M-004673-01</b>
<b>Report No:</b>	23001
<b>Guidelines:</b>	OECD 406; Directive 84/449/EEC (1984) Deviations: none
<b>GLP</b>	Yes

## A. Materials

### 1. Test materials:

Name:	FOE 5043-Sulfon
Description:	White crystals
Lot/Batch no:	17007/92
Purity:	99.1%
Stability of test compound:	guaranteed for study duration; expiry date: 1995-04-13

### 2. Vehicle:

Propylene glycol (1,2-propanediol)

### 3. Test animals:

Species:	Guinea pig
Strain:	Hsd/Win:DH (SPF-bred)
Age:	4 to 8 weeks
Sex:	Males
Weight at dosing:	292 - 399 g
Source:	[REDACTED]
Acclimatization period:	At least 7 days
Diet:	Altromin® 3020 maintenance diet for guinea pigs (ALTROMIN GmbH, Lage, Germany), <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	In Makrolon® type 4 cages with low-dust wood shavings as bedding. During acclimatization 5 per cage, during experiment 2 or 3 animals per cage.

## B. Study design and methods

### 1. Animal assignment and treatment

Dose	
Intradermal induction:	5% (= 20 mg test substance/animal)
Topical induction:	6% (= 30 mg test substance/animal)
Challenge:	1% (= 5 mg test substance/animal) and 0.5% (=2.5 mg test substance/animal)
Application route:	Intradermal, dermal
Application volume:	intradermal induction: 0.1 mL/injection

	topical induction: 0.5 mL/patch
	challenge: approx. 0.5 mL/patch
Duration:	topical induction: 48 hours, challenge: 24 hours
Group size:	20 in test item groups; 10 in control group
Observations::	mortality, clinical signs, skin effects, body weight (at beginning and termination of study)

## Results and discussion

### A. Findings

There were no mortalities or systemic intolerance reactions noted in any animal during the study. Body weight development was not affected by treatment.

After the second induction the application sites of 16 animals of the test substance group were encrusted. The incrustation was not resolved in all animals at termination.

After challenge exposure with 1% test substance skin findings were observed in all guinea pigs of the test substance group. After challenge with 0.5% 19 animals exhibited skin findings. In the control group skin findings were observed in 2 and 1 animal after exposure to 1% and 0.5% of the test substance, respectively.

A summary of the skin reactions observed after challenge exposure are given in the Table 5.8.2/24-1.

**Table 5.8.2/24-1: Number of animals exhibiting skin effects\*Summary of irritant**

	Test item group (20 animals)			Control group (10 animals)		
	Test item patch		Control patch	Test item patch		Control patch
Hours	24	48	Total	24	48	Total
Challenge 1%	20/20	20/20	20/20	0/20	0/20	2/10
Challenge 0.5%	19/20	18/20	19/20	0/20	0/20	1/10

\* After patch removal.

The last two reliability checks performed in the laboratory with 2-mercaptobenzothiazole confirmed the sensitivity and reliability of the test method.

<b>Conclusion</b>	Based on the study results FOE 5043-Sulfon does possess a skin sensitizing potential.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /25; ██████████ 1993</b>
<b>Title:</b>	FOE 5043-Sulfone - Study to assess the sensory irritation potential to mice (RD50 determination)
<b>Document No:</b>	<b>M-004601-01</b>
<b>Report No:</b>	22729
<b>Guidelines:</b>	ASTM E981-84 (Exposure technique in accordance with OECD 403 and EC guideline 84/449/EEC B.2) Deviations: not specified
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-Sulfon
Lot/Batch no:	white crystals
Purity:	17004+5/91
Stability of test compound:	99.2-99.9%
	guaranteed for study duration; expiry date: 1993-02-03

#### 2. Vehicle:

None

#### 3. Test animals

Species:	Mouse
Strain:	OF1 (SPF-bred)
Age:	approximately 5 to 7 weeks
Weight at dosing:	Mean: 29 g
Source:	██████████
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	During acclimatization and during the study period in groups of four in Makrolon® Type II cages; bedding: type S8/15 low-dust wood shavings (Ssniff, Soest, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	Vapour: 0-4.3-9.8-13.3 mg/m <sup>3</sup> air (actual concentration)
Application route:	Inhalation, nose / head only
Exposure:	45 minutes
Group size:	4 males/group
Post-treatment observation period:	1 week

Observations:	mortality, clinical signs, body weights, lung function test, gross necropsy
Calculations:	RD <sub>50</sub> (minimum smoothed respiratory rate); decrease in respiratory rate

## 2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

Target concentration (mg/m <sup>3</sup> )	0	7.6	16.1	23.3
Analytical concentration (mg/m <sup>3</sup> )	Air control	4.3	9.8	13.3
Temperature (mean, °C)	22	42	50	54

## Results and discussion

### A. Mortality

One rat at 4.3 mg/m<sup>3</sup> died on study day 4.

**Table 6.8.2/25-1: Result summary**

Dose (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Vapour exposure						
0	0	0	4	--	--	0
4.3	1	1	4	1d – 4d	4d	25
9.8	0	0	4	--	--	0
13.3	0	0	4	--	--	0

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

# air control

### B. Clinical observations

Except for the one mouse that died, no clinical signs were observed.

The one mouse of the low dose group that died showed bradypnea and reduced motility from day 1 to day 4. However, it was not clear if these signs were treatment-related.

### C. Lung function test

The tests showed that the test substance vapor induces a concentration-related decrease in the respiratory rate. The decrease in frequency is attributed to a reflex bradypnea and is indicated by a pause between inspiration and expiration. The changes were found to be largely reversible during the recovery period.

Based on the decrease in the respiratory rate, the RD<sub>50</sub> was calculated to be **8.9 mg/m<sup>3</sup> air**.

**Table 6.8.2/25-2: Results for respiratory decrease**

Concentration (mg/m <sup>3</sup> air)	Respiratory Decrease (%)
4.3	25
9.8	47
13.3	69



**D. Body weight**

There was no treatment-related and toxicologically relevant effect on body weight gain.

**D. Necropsy***Animals that died during the study*

In the one low-dose animal that died on study day 4 the lungs were bright red.

*Animals sacrificed at termination*

There were no gross lesions observed in any rat of all dose groups.

<b>Conclusion</b>	<p>A severe sensory irritation potential was observed in mice exposed to FOE 5043-Sulfon vapor for approximately 45 minutes. The changes that were observed are characteristic of an upper respiratory tract irritant. The observed respiratory changes and their relatively rapid reversibility are regarded as characteristic of a sensory irritant vapor.</p> <p>Based on the most sensitive parameter (i.e. respiratory rate), 0.3 mg FOE 5043-Sulfon /m<sup>3</sup> is regarded as the non-irritant threshold concentration.</p>
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /26; [REDACTED] 1992b</b>
<b>Title:</b>	FOE 5043-Sulfone - Range-finding study of the subacute inhalation toxicity to rats (exposure: 5x6h)
<b>Document No:</b>	<b>M-004571-01</b>
<b>Report No:</b>	21390
<b>Guidelines:</b>	EC Guideline 84/449/EEC; OECD 403 and 412; Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-Sulfon
Lot/Batch no:	colourless crystals
Purity:	TE 86005, 17004+5/91
Stability of test compound:	99.2%
	guaranteed for study duration; expiry date:

#### 2. Vehicle:

None

#### 3. Test animals

Species:	Rat
Strain:	Wistar Bor:WISW (SPF-Cpb)
Age:	Approximately 2 to 3 months
Weight at dosing:	Mean males: 210 ± 9 g; mean females: 185 ± 7 g
Source:	[REDACTED]
Acclimatisation period:	at least 1 week
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	During acclimatization and during the study period in groups of five in Makrolon® Type III cages; bedding: type S8/15 low-dust wood shavings (Ssniff, Soest, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	Vapour: 0-0.5-3.5-16.3 mg/m <sup>3</sup> air (actual concentration)
Application route:	Inhalation, nose / head only
Exposure:	5 x 6 h/day
Group size:	10 rats/sex/group
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, body weights, rectal temperature,

reflex tests, organ weights, gross necropsy (interim after 3 days and terminal)

## 2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

Target concentration (mg/m <sup>3</sup> )	Air control	0.5	4.0	15.0
Analytical concentration (mg/m <sup>3</sup> )	Air control	0.5	3.5	16.3
Temperature (mean, °C)	21.36	21.62	21.68	21.80
Relative humidity (mean, %)	33.38	11.22	28.94	15.92
MMAD (µm)	Not reported	Not reported	Not reported	2.03
GSD				1.57
Aerosol mass < 3 µm (%)				81

## Results and discussion

### A. Mortality

In the high-dose group 4 males and 4 females died between days 1 and 4. The surviving rats of this dose group were terminated on day 7.

No mortalities were observed at lower concentrations.

**Table 6.8.2/26-1: Result summary**

Animal Nos.	Concentration (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats							
1-10	Air control	0	0	10	--	--	0
11-20	0.5	0	0	10	--	--	0
21-30	3.5	0	10	10	2 d – 6 d	--	0
31-40	16.3	4	10	10	0 d – 7 d**	1 d – 2 d	40
Female rats							
41-50	Air control	0	0	10	--	--	0
51-60	0.5	0	0	10	--	--	0
61-70	3.5	0	10	10	2 d – 6 d	--	0
71-80	16.3	4	10	10	0 d – 7 d**	2 d – 4 d	40

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

\*\* All rats were sacrificed on day 7 (1<sup>st</sup> day of study = day 0)

### B. Clinical observations

No clinical signs were observed in rats of the air control and low-dose group.

At 3.5 mg/m<sup>3</sup> bradypnea and dyspnea, piloerection and unpreened hair coat, slight respiratory sounds, and a mild serous nasal discharge was observed in a few rats.

At the highest concentration of 16.3 mg/m<sup>3</sup> the following clinical signs were observed: unpreened hair coat, piloerection, cyanosis, reduced activity, sternal recumbency (prostration), atony, high-stepping

gait, bradypnea and dyspnea, difficult breathing, respiratory sounds, serous to bloody nasal discharge, blood-incrusted rhinarium, emaciation.

### C. Reflex tests

No change in reflex behavior was observed in the air control group, as well as up to concentrations of 3.5 mg/m<sup>3</sup>. At 16.3 mg/m<sup>3</sup> reduced reaction to touch were observed in the rats.

**Table 6.8.2/26-2: Summary of effects observed during reflex tests**

Concentration (mg/m <sup>3</sup> )	Type of reflex (study day)			
	Startle reflex touch (day 4)	Tail-pinch response (day 4)	Startle reflex touch (day 7)	Tail-pinch response (day 7)
Males				
Air control	0/10	0/10	0/10	0/10
0.5	0/10	0/10	0/10	0/10
3.5	0/10	0/10	0/10	0/10
16.3	6/10	6/10	0/10	0/10
Females				
Air control	0/10	0/10	0/10	0/10
0.5	0/10	0/10	0/10	0/10
3.5	0/10	0/10	0/10	0/10
16.3	6/10	6/10	0/10	0/10

\* 1<sup>st</sup> number = number of rats with abnormal reflexes, 2<sup>nd</sup> number = total number of animals examined

### D. Rectal temperature

There were no treatment-related changes observed at concentrations up to and including 3.5 mg/m<sup>3</sup>. A statistically significant effect on rectal temperature was determined in the 15 mg/m<sup>3</sup> group (see Table below).

**Table 6.8.2/26-3: summary of rectal temperature measurements**

Concentration (mg/m <sup>3</sup> )	Males			Females		
	Rectal temperature on day			Rectal temperature on day		
	0	4	7	0	4	7
Air control	37.8	37.8	37.9	37.9	38.0	38.5
0.5	37.8	37.8	37.6	37.8	37.8	38.4
3.5	37.3	37.5	37.8	37.7	37.3	38.3
16.3	31.0++	35.0+	37.1+	30.8++	30.2+	37.5+

+ = significant different from control p < 0.05

++ = significant different from control p < 0.01

### E. Body weight

Treatment-related reduction in body weights were observed in both sexes at concentrations of 3.5 mg/m<sup>3</sup> and above (see Table below).

**Table 6.8.2/26-4: Summary of rectal temperature measurements**

Concentration (mg/m <sup>3</sup> )	0	4	7	14	21
	Males				
Air control	211	207	221	242	263
0.5	206	203	215	247	272
3.5	207	199+	212	243	268
16.3	210	152++	173++		
	Females				
Air control	186	183	185	194	199
0.5	187	185	189	198	200
3.5	190	183	192+	194	200
16.3	185	143++	160++		

+ = significant different from control p &lt; 0.05

++ = significant different from control p &lt; 0.01

**F. Organ weights**

Up to and including 3.5 mg/m<sup>3</sup>, there was no significant change in the organ to brain weight ratio. High-dose animals had increased relative lung and liver weights, males showed also increased relative brain weights. In addition, in rats of the 15 mg/m<sup>3</sup> group, the relative organ to brain weights of heart, kidney and spleen weights were reduced.

**Table 6.8.2/26-5: Summary of absolute organ weights on day 7**

Concentration (mg/m <sup>3</sup> )	Absolute organ weighs (mg)					
	Heart	Lung	Spleen	Liver	Kidneys	Brain
	Males					
Air control	806	1101	402	9461	1558	1663
0.5	714+	1135	451	8462	1505	1583
3.5	758	1102	429	8674	1506	1641
16.3	669+	1158	252++	8359	1273++	1629
	Females					
Air control	657	989	373	6902	1252	1560
0.5	728	1054	388	6982	1330	1664
3.5	708	1061	464	6834	1314	1678
16.3	650	1160+	260+	8326	1139	1578

+ = significant different from control p &lt; 0.05

++ = significant different from control p &lt; 0.01

**Table 6.8.2/26-6: Summary of relative organ weights on day 7**

Concentration (mg/m <sup>3</sup> )	Absolute organ weights (mg/100 g body weight)					
	Heart	Lung	Spleen	Liver	Kidneys	Brain
Males						
Air control	357	488	178	4181	689	736
0.5	335	533	212+	3975	707	743
3.5	357	519	202	4089	710	773
16.3	386	670++	145+	4823+	734	940++
Females						
Air control	352	531	201	3692	669	837
0.5	387	559	205	3701	705	883
3.5	368	552	241	3551	683	872
16.3	406	726++	162	5212++	712	989

+ = significant different from control p &lt; 0.05

++ = significant different from control p &lt; 0.01

**Table 6.8.2/26-7: Summary of relative organ to brain weights on day 7**

Concentration (mg/m <sup>3</sup> )	Relative organ to brain weights (mg/100 g brain weight)				
	Heart	Lung	Spleen	Liver	Kidneys
Males					
Air control	49	66	24	569	94
0.5	45	72	29	537	96
3.5	46	67	26	529	92
16.3	41+	71	16++	513	78++
Females					
Air control	42	64	24	445	81
0.5	44	63	23	421	80
3.5	42	63	28	408	78
16.3	41	74	17+	529	72

+ = significant different from control p &lt; 0.05

++ = significant different from control p &lt; 0.01

**G. Necropsy***Animals that died during the study:*

The three males of the 16.3 mg/m<sup>3</sup> group that died had distended, reddish lungs, a pale spleen. Two males had also a reddish duodenum mucosa and hepatized lungs. The duodenum content of one male was also reddish, mucus.

The four high-dose females that died during the study had all a pale spleen, a reddish duodenum mucosa and bloody mucus content in the duodenum. Three had also a distended, reddish lung, while one had only a reddish lung. The lungs of two females showed also hepatization. A pale liver and a red fore stomach were observed in one female.

*Animals sacrificed at interim sacrifice (day 7):*

There were no gross pathological findings observed in males and females at concentrations up to and including 3.5 mg/m<sup>3</sup>.

Surviving males and females of the high-dose group were all sacrificed on day 7. In high-dose males at interim sacrifice the following findings were observed: small spleen (3 rats), distended lungs (3 rats), reddish or pale lung (1 rat each). Three high-dose females had a distended and pale lung. Small spleens were observed in two high-dose females.

*Animals sacrificed at termination (day 21):*

There were no gross lesions observed in males and females up to and including 3.5 mg/m<sup>3</sup> sacrificed at termination.

<b>Conclusion</b>	Based on the study results the NOEC for FOE 5043-Sulfon was determined to be 0.5 mg/m <sup>3</sup> . Significant clinical findings were observed starting at 3.5 mg/m <sup>3</sup> . The most prominent changes (irritation of the respiratory tract mucosae and hypothermia caused by irritation) were observed in rats exposed to 16.3 mg/m <sup>3</sup> . This concentration was within the lethal range. The cause of death is considered to be causally related to lung damage resulting from irritation.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /27; ██████████ 1994</b>
<b>Title:</b>	FOE 5043-Sulfone - Study of the subacute inhalation toxicity to rats in according with OECD guideline no. 412
<b>Document No:</b>	<b>M-004779-01</b>
<b>Report No:</b>	22918
<b>Guidelines:</b>	OECD 412 (1981); EC guideline 84/449/EEC B.8, US-EPA FIFRA § 82-4 (1984) Deviations: Relative humidity was lower as recommended by OECD 412. This had no detectable negative effect on the outcome of the study. Particle size distribution not reported
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-Sulfon
Lot/Batch no:	colourless crystals
Purity:	17004+5/91
Stability of test compound:	99.2%
	guaranteed for study duration; expiry date: 1993-01-02

#### 2. Vehicle:

None

#### 3. Test animals

Species:	Rat
Strain:	Wistar Bor:WISW (SPF-Cpb)
Age:	Approximately 2 to 3 months
Weight at dosing:	Mean males: 190 g; mean females: 170 g
Source:	██████████
Acclimatisation period:	Approximately 2 weeks
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	During acclimatization and during the study period in groups of five in Makrolon® Type III cages; bedding: type S8/15 low-dust wood shavings (Ssniff, Soest, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	Vapour: 0-0.47-2.04-7.63 mg/m³ air (actual concentration)
Application route:	Inhalation, nose / head only
Exposure:	6 h/day 5 times / week; 4 consecutive weeks



Group size:	10 rats/sex/group
Observations:	mortality, clinical signs, body weights, rectal temperature, reflex tests, ophthalmology, clinical chemistry, hematology, urinalysis, organ weights, gross necropsy, histopathology

## 2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

Target concentration (mg/m <sup>3</sup> )	Air control	0.5	2	8
Analytical concentration (mg/m <sup>3</sup> )	Air control	0.47	2.04	7.63
Temperature (mean, °C)	23.2	22.5	23.1	22.8
Relative humidity (mean, %)	15.1	17.5	16.4	17.8
MMAD (µm)	Not reported	Not reported	Not reported	Not reported
GSD				
Aerosol mass < 3 µm (%)				

## Results and discussion

### A. Mortality

No mortalities were observed in any dose group.

**Table 6.8.2/27-1: Result summary**

Animal Nos.	Concentration (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats							
1-10	Air control	0	0	10	--	--	0
11-20	0.5	0	0	10	--	--	0
21-30	2	0	10	10	2 d – E	--	0
31-40	8	0	10	10	1 d – E	--	0
Female rats							
41-50	Air control	0	0	10	--	--	0
51-60	0.5	0	0	10	--	--	0
61-70	2	0	10	10	18 d – E	--	0
71-80	8	0	10	10	1 d – E	--	0

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

E signs until end of study

### B. Clinical observations

No clinical signs were observed in rats of the air control and low-concentration group.

At 2 mg/m<sup>3</sup> high-stepping gait (sporadic), reduced mobility (sporadic), bradypnea, labored breathing, sporadic rales, piloerection, unpreened fur, serous nasal discharge, sneezing, and sporadic atony.

At the highest concentration of 8 mg/m<sup>3</sup> the following clinical signs were observed: high-stepping gait, reduced motility, bradypnea, labored breathing, rales, gasping, piloerection, serous nasal discharge, unpreened fur, sneezing, atony, cachexia, distended abdomen.

The severity of the signs was most pronounced in the animals at 8 mg/m<sup>3</sup>. Females tended to be more sensitive than the male rats. Reflex bradypnea induced by sensory irritation is regarded as the most

sensitive clinical parameter. As regards this end point, distinct convalescence was observed on the exposure-free weekends.

### C. Reflex tests

Up to and including 8 mg/m<sup>3</sup>, the reflex tests did not reveal any abnormal findings that would indicate specific neurological changes. Individual animals of the 8 mg/m<sup>3</sup> group exhibited, to some degree, a reduced "righting response" and a reduced reactivity to noises. The quantitative determination of the grip strength (all paws) revealed that grip strength tended to be weakened temporarily in the female rats of the 8 mg/m<sup>3</sup> group.

**Table 6.8.2/27-2: Summary of effects observed during reflex tests**

Concentration (mg/m <sup>3</sup> )	Reduced righting response (day 3)	Type of reflex (study day) Startle reflex / sound: no reaction (day 10)	Reduced righting response (day 21)
Males			
Air control	0/5	0/5	0/5
0.5	0/5	0/5	0/5
2	0/5	0/5	1/5
8	1/5	0/5	0/5
Females			
Air control	0/5	0/5	0/5
0.5	0/5	0/5	0/5
2	0/5	0/5	1/5
8	3/5	2/5	0/5

\* 1<sup>st</sup> number = number of rats with abnormal reflexes, 2<sup>nd</sup> number = total number of animals examined

### D. Rectal temperature

When compared to control, there was a slight hypothermia noted at 2 and 8 mg/m<sup>3</sup>.

**Table 6.8.2/27-3: summary of rectal temperature measurements**

Concentration (mg/m <sup>3</sup> )	Rectal temperature (°C) on day					
	0	4	9	16	23	30
Males						
Air control	38.2	38.7	38.3	38.4	38.5	38.0
0.5	38.0	38.6	38.3	38.4	38.6	38.1
2	37.6	37.7+	37.1+	37.9	38.4	37.8
8	36.4	37.5+	36.4++	38.0	37.3++	37.1++
Females						
Air control	38.4	39.0	38.7	39.0	38.9	38.3
0.5	38.5	38.6	38.6	38.9	38.8	38.0
2	38.3	38.3	37.9+	38.4	38.8	38.1
8	36.9+	38.0	36.9+	38.2+	37.7	37.1

+ = significant different from control  $p \leq 0.05$  (ANOVA)

++ = significant different from control  $p \leq 0.01$  (ANOVA)

### E. Body weight

Treatment-related reduction in body weights were observed in both sexes at concentrations of 2 mg/m<sup>3</sup> and above (see Table below). As can be seen in the table below, the animals clearly gained weight during the exposure-free weekends.

**Table 6.8.2/27-4: Summary of body weight measurements**

Concentration (mg/m <sup>3</sup> )	Mean body weight (g)								
	0	4	7	11	14	18	21	25	28
<b>Males</b>									
Air control	190	181	195	192	205	209	221	225	240
0.5	190	181	194	191	204	207	218	219	235
2	194	183	198	193	207	205	220	214	234
8	191	168++	186+	170++	188++	183++	205	188++	209++
<b>Females</b>									
Air control	171	167	171	167	172	174	174	180	186
0.5	170	165	168	167	170	172	172	175	183
2	167	161	167	163	167	165+	169	169+	177
8	174	155+	170	157+	169	161++	174	167+	182

+ = significant different from control  $p \leq 0.05$  (U-test)

++ = significant different from control  $p \leq 0.01$  (U-test)

### F. Haematology

There was a treatment-related increase in coagulation time observed at 2 mg/m<sup>3</sup> and in females at 8 mg/m<sup>3</sup>. There were some other statistical significant changes, but since there were not concentration-related and were also within the range of historical controls, these changes were considered not toxicological relevant.

There were also treatment-related effects on the leukocyte differential count in females at concentrations of 2 mg/m<sup>3</sup> and above (i.e. a relative increase in the segmented leukocytes and monocytes but no effect on the absolute leukocyte count)

**Table 6.8.2/27-5: summary of haematology and leucocyte differential count**

Concentration (mg/m <sup>3</sup> )	LEU (10 <sup>9</sup> /L)	MCHC (g/L ERY)	THRO (10 <sup>9</sup> /L)	HQUICK (sec)	LYM (%)	SEGM (%)	MONO (%)
<b>Males</b>							
Air control	4.5	318	895	36.6	88.6	9.6	1.7
0.5	4.6	320	786++	37.2	85.4	12.6	2.0
2	4.4	319	809+	37.9	90.0	7.8	1.8
8	4.1	313	863	37.7	82.1	14.6	3.2
<b>Historical control data - males</b>							
HCD: $\pm 2S$	3.0-9.8	288-323	801-1547	25.1-39.0	78-97	1-18	Up to 7
HCD: $\pm 3S$	1.3-11.5	279-332	614-1733	21.7-42.5	73-100	Up to 22	Up to 10
<b>Females</b>							
Air control	3.2	320	768	33.0	90.4	8.6	0.5
0.5	3.8	327+	771	33.6	90.6	8.8	0.4
2	4.0	323	782	34.7	90.6	7.9	1.4+
8	3.0	313++	830	35.8++	82.9++	13.5+	3.3++
<b>Historical control data - females</b>							
HCD: $\pm 2S$	2.5-8.6	287-318	883-1475	24.2-33.2	81-98	1-16	Up to 5
HCD: $\pm 3S$	1.0-10.0	279-326	736-1623	22.0-35.5	77-100	Up to 19	Up to 6

+ = significant different from control  $p < 0.05$  (U-test)

++ = significant different from control  $p < 0.01$  (U-test)

HCD = historical controls

## G. Clinical chemistry

### Blood clinical chemistry

Primarily in the females of the 8 mg/m<sup>3</sup> group, the plasma-cholesterol and plasma-bilirubin concentrations were slightly reduced, and the plasma ASAT and ALAT tended to be higher. The plasma-chloride concentration was marginally reduced in male and female rats at 8 mg/m<sup>3</sup>. No toxicologically relevant changes were observed in female rats of the 2 mg/m<sup>3</sup> group with the exception of a reduction in the plasma-cholesterol concentration which was not concentration-related.

There were some other statistical significant changes, but since there were not concentration-related and were also within the range of historical controls, these changes were considered not toxicological relevant.

In addition, there were marginal but not concentration-related changes in urea and creatinine values. However, these findings were not toxicologically relevant, since these parameters essentially dependent on feed and water consumption and muscular activity.

Slightly reduced blood, urea and creatinine values, in contrast to increased values, are therefore pathognostically not relevant, especially in inhalation toxicity studies.

**Table 6.8.2/27-6: Summary of clinical chemistry**

Concentration (mg/m <sup>3</sup> )	ASAT (U/L)	ALAT (U/L)	Total BILI (mmol/L)	Cholesterol (mmol/L)	Chloride (mmol/L)
Males					
Air control	47.6	48.7	1.4	1.93	98
0.5	51.3	42.5+	1.4	1.69+	99
2	50.0	44.0	1.3	1.56+	98
8	57.2	45.6	1.4	1.71	96++
HCD: ± 2S	25.4-73.5	35.7-76.0	1.4-3.1	1.40-2.53	96-102
HCD: ± 3S	17.1-84.8	25.6-84.0	1.0-3.5	1.12-2.81	94-104
Females					
Air control	48.9	39.1	1.3	1.66	100
0.5	41.0+	37.6	1.4	1.56	99
2	44.6	37.6	1.3	1.24++	100
8	56.2	46.8	1.0++	1.31++	95+
HCD: ± 2S	25.4-76.5	31.0-65.8	1.2-3.1	1.26-2.54	98-104
HCD: ± 3S	16.4-88.5	22.3-74.5	0.7-3.6	0.94-2.86	96-106

+ Statistically significant different from control p ≤ 0.05

++ Statistically significant different from control p ≤ 0.01

### Protein electrophoresis

In both sexes at 8 mg/m<sup>3</sup> there was a treatment-related shift in relative albumin/globulin, without evidence of toxicologically relevant, concentration-related effects on the total protein concentration or on the relative protein composition. No effects were observed at lower concentrations.

### Examinations in liver tissue

The hepatic O-demethylase activity was significantly reduced in the male rats of the 2 and 8 mg/m<sup>3</sup> groups, and the hepatic N-demethylase activity was increased in the female rats. The hepatic cytochrome P-450 activity was significantly reduced in the males of the 8 mg/m<sup>3</sup> group.

**Table 6.8.2/27-7: Summary of examinations in liver tissue**

Concentration (mg/m <sup>3</sup> )	Triglycerides [μmol/g]	O-demethylase [mU/g]	N-demethylase [mU/g]	P450 [nmol/g]
Air control	4.38	10.9	144.2	41.5
0.5	4.76	10.6	131.0	43.9
2	4.68	8.9++	146.6	39.4
8	4.46	8.8++	115.5+	33.6++
Air control	4.32	7.7	65.5	34.9
0.5	4.08	7.9	71.9	33.1
2	4.39	7.0	78.3++	30.8
8	4.26	8.2	85.7++	32.5

+ Statistically significant different from control  $p \leq 0.05$  (U-test)

++ Statistically significant different from control  $p \leq 0.01$  (U-test)

No historical control data for liver tissue examinations available

### H. Urinalysis

There were no treatment-related effects observed at any concentration.

### I. Ophthalmology

There were no treatment-related effects observed at any concentration.

### K. Organ weights

There was a treatment-related reduction of absolute and relative thymus weight, as well as relative thymus-to-brain weight observed at 8 mg/m<sup>3</sup>. In males there was also a treatment-related reduction in spleen weights observed at that concentration.

In the female rats of this group, the heart weights were marginally increased and the lung weights statistically significantly increased. First indications of an increased thymus involution were found already in the 2 mg/m<sup>3</sup> group.

However, most of the organ weight changes summarised in the tables below are considered to be due to the body weight changes.

**Table 6.8.2/27-8: Summary of absolute organ weights**

Concentration (mg/m <sup>3</sup> )	Terminal BW (g)	Absolute organ weighs (mg)						
		Liver	Brain	Kidneys	Lung	Heart	Thymus	Thyroid
	Males							
Air control	240	8982	1885	1587	1209	814	290	8
0.5	235	8412	1815	1459	1205	779	273	8
2.0	234	7993+	1808	1499	1168	782	211+	8
8.0	209++	7309+	1749	1387+	1068++	731+	177++	9
		<b>Spleen</b>	<b>Adrenals</b>	<b>Testes</b>				
Air control	240	478	49	2811				
0.5	235	489	50	3043				
2.0	234	470	47	2946				
8.0	209++	388	55	2773				
	Females							
Air control	186	6933	1788	1189	994	669	241	8
0.5	183	6515	1763	1171	977	642	231	8
2.0	177	6167	1737	1184	949	626	198	8
8.0	182	6430	1742	1245	1075	718	166+	7
		<b>Spleen</b>	<b>Adrenals</b>	<b>Ovaries</b>				
Air control	186	447	65	136				
0.5	183	432	65	120				
2.0	177	389	66	128				
8.0	182	414	70	129				

+ = significant different from control p &lt; 0.05 (ANOVA)

+ = significant different from control p &lt; 0.01 (ANOVA)

**Table 6.8.2/27-9: Summary of relative organ weights**

Concentration (mg/m <sup>3</sup> )	Relative organ weighs (mg/100 g bodyweight)						
	Liver	Brain	Kidneys	Lung	Heart	Thymus	Thyroid
	Males						
Air control	3688	781	657	501	337	119	3
0.5	3547	767	616	508	329	115	3
2.0	3489	791	656	510	342	92+	3
8.0	3637	886	697	541	369	87+	5
	<b>Spleen</b>	<b>Adrenals</b>	<b>Testes</b>				
Air control	337	20	1163				
0.5	329	21	1285				
2.0	342	21	1292				
8.0	369	28+	1388++				
Concentration (mg/m <sup>3</sup> )	Relative organ weighs (mg/100 g bodyweight)						
	Liver	Brain	Kidneys	Lung	Heart	Thymus	Thyroid
	Females						
Air control	3703	956	635	530	357	128	4
0.5	3558	963	640	533	351	126	4
2.0	3532	998	679	546	361	113	5
8.0	3706	1010	721++	622++	416++	95+	4
	<b>Spleen</b>	<b>Adrenals</b>	<b>Ovaries</b>				
Air control	239	35	72				
0.5	236	36	65				
2.0	224	38	73				
8.0	239	40	73				

+ = significant different from control p &lt; 0.05 (ANOVA)

++ = significant different from control p &lt; 0.01 (ANOVA)

**Table 6.8.2/27-10: Summary of relative organ to brain weights**

Concentration (mg/m <sup>3</sup> )	Relative organ weighs (mg/100 g bodyweight)					
	Liver	Kidneys	Lung	Heart	Thymus	Thyroid
	Males					
Air control	475	85	64	43	15	0.4
0.5	465	81	67	43	15	0.4
2.0	444	83	65	43	12+	0.4
8.0	418	79	61	42	10++	0.5
	<b>Spleen</b>	<b>Adrenals</b>	<b>Testes</b>			
Air control	25	3	149			
0.5	27	3	168			
2.0	26	3	163			
8.0	22	3	159			
Concentration (mg/m <sup>3</sup> )	Relative organ weighs (mg/100 g bodyweight)					
	Liver	Kidneys	Lung	Heart	Thymus	Thyroid
	Females					
Air control	388	66	56	37	13	0.5
0.5	371	67	56	37	13	0.4
2.0	354	68	55	36	11	0.5
8.0	369	72	62+	41	10+	0.4
	<b>Spleen</b>	<b>Adrenals</b>	<b>Ovaries</b>			
Air control	25	4	4			
0.5	25	4	4			
2.0	22	4	4			
8.0	24	4	4			

+ = significant different from control  $p < 0.05$  (ANOVA)

+ = significant different from control  $p < 0.01$  (ANOVA)

### L. Necropsy

There were no treatment-related organ damage observed in males and females up to and including 8 mg/m<sup>3</sup> sacrificed at termination.

### M. Histopathology

Irritation-induced morphological changes in the turbinates, nasopharynx and larynx were determined at 0.5 mg/m<sup>3</sup>.

At 2 mg/m<sup>3</sup> and above there was marked inflammatory infiltration in the upper respiratory tract caused by irritation observed. A concentration-related hyperplasia of the goblet cells in the nasal septum and an epithelial hyperplasia (larynx) with, and without keratinization were also found.

In addition, in the 8 mg/m<sup>3</sup> group there were necrotic, degenerative/atrophic changes in the olfactory epithelium combined with extensive round cell infiltration in the entire nasopharynx. In males at 8 mg/m<sup>3</sup> and females at 2 and 8 mg/m<sup>3</sup> degenerations in the olfactory epithelium were observed. The sinus catarrh of the mandibular lymph nodes which was found frequently is considered to be causally related to the inflammatory changes in the upper respiratory tract.

There were no treatment-related findings observed in other organs.

<b>Conclusion</b>	Based on the most sensitive end point (inflammatory changes in the upper respiratory tract, sensory irritation), 0.5 mg/m <sup>3</sup> were not tolerated without specific effects, a NO(A)EC for FOE 5043-Sulfon could not be determined. The LO(A)EC was determined to be 0.5 mg/m <sup>3</sup> .
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**FOE 5043-acetate**

<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /28; ██████████ 1994</b>
Title:	FOE 5043 Acetate - Study for acute oral toxicity in rats
Document No:	<b>M-004640-01</b>
Report No:	23279
Guidelines:	OECD 401 (1987), US-EPA Pesticide Assessment Guidelines , Series 81-1 (1984), Directive 67/548/EEC amended by Directive 92/69/EEC B.1 Deviations: none
GLP	Yes

**Materials and methods****A. Materials****1. Test material:**

Name: FOE 5043-Acetate  
Description: beige crystals  
Batch / Lot No.: 17025/93  
Purity: 96.90%  
Stability of test compound: guaranteed for study duration; expiry date: 1994-07-11

**2. Vehicle:**

2% (v/v) Cremophor® EL in deionized water

**3. Test animals**

Species: Rat  
Strain: Wistar, Hsd/Win:Wu (SPF-bred)  
Age: Young adults, approx. 7 -8 (males) and 10-11 (females) weeks  
Weight at dosing: males: 165 g - 191 g; females: 176 g - 189 g  
Source: ██████████  
Acclimatisation period: at least 7 days  
Diet: Altromin® 1324 maintenance diet for rats and mice (Altromin GmbH & Co KG, Germany), *ad libitum*, except during a 17 hour fasting period prior to dosing  
Water: Tap water, *ad libitum*  
Housing: During acclimatization 5 per sex in Makrolon® Type 3 cages. During the experimental period from day 2 onwards individually in Makrolon® Type 2A cages. Low-dust granules type S 8/15 were used as bedding material.

**B. Study design and methods****1. Animal assignment and treatment**

Dose: 50-200-1000 mg/kg bw  
Application route: Oral, gavage

Application volume:	10 mL/kg bw
Fasting time:	before administration: $17 \pm 1$ hour
Group size:	5 rats/sex
Post-treatment observation period:	14 days
Observations:	clinical signs, mortality, body weight, gross necropsy

## Results and discussion

### A. Mortality

Mortalities occurred at 1000 mg/kg bw in males and females. The results are summarised in the following table.

**Table 6.8.2/28-1: Result summary**

Animal Nos.	Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats							
1 - 5	50	0	0	5	--	--	0
21 - 25	200	0	5	5	5 min – 3 h	--	0
11 - 15	1000	1	5	5	5 min – 4 d	40 min	20
LD <sub>50</sub> > 1000 mg/kg bw							
Female rats							
16 – 20	50	0	0	5	--	--	0
26 - 30	200	0	5	5	5 min – 3 h	--	0
6 - 10	1000	3	5	5	2 min – 2 d	1 h – 2 h	60
LD <sub>50</sub> > 200 - < 1000 mg/kg bw							

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

### B. Clinical observations

The following signs were observed at 200 mg/kg bw and above: decreased or increased motility, poor reflexes, unspecific behavioral disturbances, decreased reactivity, uncoordinated and spastic gait, spasmodic state, labored breathing, increased salivation, orbital margins red incrustated.

The signs observed occurred within minutes after administration. They were mostly reversible on study days 1 or 2 and lasted latest until day 4 of the study.

### C. Body weight

There were no treatment-related effects on body weight gain noted.

### D. Necropsy

*Animals that died during the study:* The one male of the high-dose group that died had slightly collapsed lungs and a pale discolored spleen.

All three high-dose females that died during the study had slightly collapsed lungs. Two showed also slight, dark red discoloration of the liver, and a slight pale discolored spleen. The third rat had a moderately spotted and discolored liver.

*Animals sacrificed at termination:*

One male of the low-dose group had markedly enlarged testes. One female of the mid-dose group had moderate spotted, discolored lungs.

No other gross lesions were observed in rats of all three dose groups sacrificed at termination.

<b>Conclusion</b>	FOE 5043-Acetate is considered to be moderately toxic after acute oral administration. The determined acute oral LD <sub>50</sub> values of male and female rats were >1000 mg/kg bw and >200 < 000 mg/kg bw, respectively.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /29; ██████████ 1996</b>
<b>Title:</b>	FOE 5043 Acetat (intermediate product of FOE 5043) - Study for acute inhalation toxicity in rats according to OECD no. 403
<b>Document No:</b>	<b>M-004734-01</b>
<b>Report No:</b>	25414
<b>Guidelines:</b>	OECD 403 (1983); EC Guideline 92/69/EEC B.2 (1992), US-EPA health effects guideline Acute exposure - inhalation toxicity (1982), US-EPA Hazard evaluation division: Standard evaluation procedure, inhalation toxicity testing(1988), JMAFF 59 NohSan no. 4200 (1985) Deviations: none
<b>GLP</b>	Yes

## Materials and methods

### A. Materials

#### 1. Test material:

Description:	FOE 5043-Acetate
Lot/Batch no:	Beige, crystalline, solid
Purity:	17025/93
Stability of test compound:	96.9%
	guaranteed for study duration; expiry date: 1994-07-11

#### 2. Vehicle:

Polyethylene glycol 400 (PEG 400) / ethanol solution (1/1, v/v)

#### 3. Test animals

Species:	Wistar rat
Strain:	Hsd Win:WU
Age:	2 to 3 months
Weight at dosing:	males: 196 g – 209 g, females: 200 g – 209 g
Source:	████████████████████
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula diet (Altromin ® 1324; Altromin GmbH, Germany), <i>ad libitum</i>
Water:	tap water, <i>ad libitum</i>
Housing:	Singly in conventional Makrolon® Type II cages; bedding: type S8/15 low-dust wood granulate (Ssniff, Soest, Germany)

### B. Study design and methods

#### 1. Animal assignment and treatment

Dose:	0 – 2350 mg/m <sup>3</sup> air (actual concentration)
Application route:	inhalation
Exposure:	4 hours
Group size:	5 rats/sex/group

Post-treatment observation period: 2 weeks

Observations: mortality, clinical signs, body weights, body temperature, reflex measurements, gross necropsy

## 2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

	Group 1	Group 2
Target concentration (mg/m <sup>3</sup> )	Control (vehicle)	100000
Analytical concentration (mg/m <sup>3</sup> )	0	2350
Test substance concentration in vehicle (% w/v)	--	30
Temperature (mean, °C)	24	24
Relative humidity (mean, %)	32	32
MMAD (µm)	1.6	1.5
GSD	2.0	2.0
Aerosol mass < 3 µm (%)	83	84

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; - = not applicable.

### A. Mortality

There were no mortalities observed during the study.

**Table 6.8.2/29-1: Result summary**

Dose (mg/m <sup>3</sup> )	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Male rats						
0	0	0	5	--	--	0
2350	0	0	5	--	--	0
LC <sub>50</sub> > 2350 mg/m <sup>3</sup>						
Female rats						
0	0	0	5	--	--	0
2350	0	0	5	--	--	0
LC <sub>50</sub> > 2350 mg/m <sup>3</sup>						

\* 1<sup>st</sup> number = number of dead animals, 2<sup>nd</sup> number = number of animals with toxic signs

3<sup>rd</sup> number = number of animals used

### B. Clinical observations

There were no clinical signs of toxicity observed in any animal.

### C. Reflex measurements

The battery of reflex measurements conducted on day 1 revealed no changes of reflexes in any animal.

### D. Body weight

There were no treatment-related effects on body weight and body weight gain noted.

### E. Rectal temperature

The female rats of group 2 showed a marginal decrease of the rectal temperatures when compared to the control animals. No differences were observed in male rats.

**D. Necropsy**

There were no treatment-related gross-pathological findings observed in any animal.

<b>Conclusion</b>	FOE 5043-Acetat is considered to be non-toxic after acute inhalation exposure. The determined acute LC <sub>50</sub> values of male and female rats were >2350 mg/m <sup>3</sup> , the maximum technically attainable concentration.
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<b>New studies; not evaluated</b>	<p>This study was available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414.</p> <p><b>Supportive information for justification of the active substance specification</b></p>
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<b>Report:</b>	<b>KCA 5.8.2 /30; [REDACTED] F.; 1994</b>
<b>Title:</b>	FOE 5043 Acetat - Study for skin and eye irritation/corrosion in rabbits
<b>Document No:</b>	<b>M-004662-01</b>
<b>Report No:</b>	23062
<b>Guidelines:</b>	<p>OECD 404 (1992), EEC Directive 84/449/EEC B.4 (1984), US-EPA TSCA Test guidelines 798.4470 (1985), US-EPA Pesticide assessment guidelines §81-5 (1984), OECD 405 (1987); EEC Directive 84/449/EEC B.5 (1984), US-EPA TSCA Test guidelines 798.4450 (1985), US-EPA Pesticide assessment guidelines §81-4 (1984)</p> <p>Deviations: description and scoring of corneal effects could be more accurate; additional examinations of aqueous humour were not conducted. These slight deviations do not affect the validity of the study.</p>
<b>GLP</b>	Yes

## A. Materials

### 1. Test material:

Name:	FOE 5043-Acetate
Description:	Beige coloured crystalline
Lot/Batch no:	17025/93
Purity:	96.9%
Stability of test compound:	guaranteed for study duration;

### 2. Vehicle:

None

### 3. Test animals

Species:	Rabbit
Strain:	New Zealand White, HC:NZW
Sex:	Females
Age:	adult
Weight at dosing:	Females: 3.2 – 4.0 kg
Source:	[REDACTED]
Acclimatisation period:	At least 14 days
Diet:	Standard diet “Ssniff K4” (Ssniff Spezialdiaeten GmbH, Soest, Germany), 100 – 120 g per animal per day
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in stainless steel cages with flat rod bases or plastic cages with perforated bases

## B. Study design and methods

### 1. Animal assignment and treatment (skin irritation)

Dose:	0.5 g (moistened with water)
Application route:	Dermal (area: approx. 6 cm <sup>2</sup> )
Duration:	4 hours
Group size:	3 females
Observations:	Mortality, clinical signs, skin effects, body weight (at beginning of study)

### 2. Animal assignment and treatment (eye irritation)

Dose	0.1 mL/animal
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Application route:	Single instillation to the conjunctival sac of one eye (eyes were rinsed with saline 24 h after application)
Group size:	3 females
Observations:	Mortality, clinical signs, eye effects, body weight (at beginning of study)

## II. Results and discussion

### A. Findings skin irritation

There were no mortalities or systemic intolerance reactions.

Dermal application of the undiluted test substance caused only very slight erythema in one animal 1 hour after patch removal. No other skin reactions were observed in any animal at any time point.

The mean irritation scores for the individual animals were 0.0, 0.0 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

The skin irritation observations are summarized in the Table 6.8.2/30-1.

**Table 6.8.2/30-1: Summary of irritant effects (Score)**

Time after patch removal	Animal #1		Animal #2		Animal #3	
	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema	Erythema and eschar formation	Oedema
First experiment (undiluted test substance)						
60 min	0	0	0	0	1	0
24 h	0	0	0	0	0	0
48 h	0	0	0	1	0	0
72 h	0	0	0	0	0	0
<b>Mean 24-72 h</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.0</b>	<b>0.0</b>
7 days	0	0	0	0	0	0

### B. Findings eye irritation

There were no mortalities or systemic intolerance reactions. Exposure of the undiluted test substance caused only slight conjunctival redness and chemosis 1 hour after application. No other eye reactions were observed.

The eye observations are summarized in the Table 6.8.2/30-2.



**Table 6.8.2/30-2: Summary of irritant effects (Score) after undiluted application**

Animal No.	Observation	1 h	24 h	48 h	72 h	Mean scores	Response	Reversibility
1s	Corneal opacity	0	0	0	0	<b>0.0</b>	–	na
	Iris	0	0	0	0	<b>0.0</b>	–	na
	Redness conjunctivae	1	0	0	0	<b>0.0</b>	–	na
	Chemosis conjunctivae	1	0	0	0	<b>0.0</b>	–	na
2s	Corneal opacity	0	0	0	0	<b>0.0</b>	–	na
	Iris	0	0	0	0	<b>0.0</b>	–	na
	Redness conjunctivae	1	0	0	0	<b>0.0</b>	–	na
	Chemosis conjunctivae	1	0	0	0	<b>0.0</b>	–	na
3s	Corneal opacity	0	0	0	0	<b>0.0</b>	–	na
	Iris	0	0	0	0	<b>0.0</b>	–	na
	Redness conjunctivae	1	0	0	0	<b>0.0</b>	–	na
	Chemosis conjunctivae	1	0	0	0	<b>0.0</b>	–	na

Response for mean scores	Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema	
– = negative	<1	<1	<2	<2	(Regulation (EC) No 1272/2008 and GHS)
	<2	<1	<2.5	<2	(Directive 1999/45/EC as amended)
+ = irritant	≥1 - <3	≥1 - <2	≥2	≥2	(Regulation (EC) No 1272/2008 (GHS) category 2)
	≥2 - <3	≥1 - <2	≥2.5	≥2	(Directive 1999/45/EC as amended)
++ = irreversible effects	≥3	≥1.5			(Regulation (EC) No 1272/2008 and GHS category 1)
serious damage	≥3	≥2			(Directive 1999/45/EC as amended)
na not applicable					

<b>Conclusion</b>	The test item FOE 5043-Sulfon was not irritating to the skin. Based on the study results the test substance FOE 5043-Acetate is not irritating to eyes of rabbits.
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### B.6.8.3 Endocrine disrupting properties

It should be noted that to date, no clear criteria are available to define endocrine disrupting properties.

The flufenacet toxicology database has been updated over the past years with a number of OECD and US EPA guideline studies. Flufenacet has no effects on reproductive indices nor fertility nor reproductive tissues and organs as shown in the multi-generation study. Flufenacet is not a developmental toxicant. Mechanistic data already submitted for the initial evaluation of flufenacet indicated that effects on thyroid hormone levels and minimal changes in thyroid gland histopathology are secondary to increased T4 clearance by the liver.

Detailed analysis of all these apical toxicological studies under inclusion of scientific and regulatory hazard principles in discussion at present no evidence of endocrine disrupting properties are seen and flufenacet does not fall under the interim definition for endocrine disruption. Therefore, based on a complete toxicological data set, there is no evidence of endocrine disrupting properties of flufenacet.

#### B.6.8.3.1 Mechanistic Study of Thyroid Hormone Effects in Rats

<b>Previous evaluation</b>	<b>In DAR for original approval (1997);</b>  Technical grade FOE 5043: Evidence for an extrathyroidal mechanism to explain alterations in circulating thyroid hormone concentration following exposure of the male rat to the experimental Acctanilid studies were presented and evaluated during the EU process for Annex I listing.
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<b>Report:</b>	<b>██████████; 1995e</b>
<b>Title:</b>	Technical grade FOE 5043: Evidence for an extrathyroidal mechanism to explain alterations in circulating thyroid hormone concentration following exposure of the male rat to the experimental Acctanilid
<b>Document No:</b>	NA
<b>Report No:</b>	Bayer Corporation, unpublished report no. 7685 (addendum: 7685) of July 21. 1995 (addendum: Oct. 09, 1995)
<b>Guidelines:</b>	For this type of study no specific method is applicable
<b>GLP</b>	Yes

## I. Materials and methods

### A. Materials

#### 1. Test material:

FOE 5043
Synonym(s): flufenacet
Supplier: Bayer AG, Leverkusen, Germany
Stability of test compound: stable for the duration of the study

#### 2. Test animals

Species: rat
Strain: CDF[F-344]/BR
Sex: Male
Weight prior first dosing: Males: approx. 180 – 269 g

Source:	████████████████████
Acclimatisation period:	1 week (with the exception of the bile duct-cannulated rats which were subject to a 3-day acclimation period)
Diet:	Purina Mills Rodent Lab Chow 5001-4 in "etts" form, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Housing:	Individually in either suspended stainless steel wire-mesh cages or in polycarbonate shoebox-type enclosures (bile ductcannulated rats) containing Alpha-dri bedding.
Environmental conditions:	<ul style="list-style-type: none"> <li>• Temperature: 18-26°C</li> <li>• Humditiy: 40-70%</li> <li>• Air changes: not reported</li> <li>• Photoperiod: 12 hr light/dark cycle</li> <li>•</li> </ul>
Preparation of rats	Male rats were surgically thyroidectomized and provided thyroid hormone replacement therapy via implanted osmotic minipumps capable of maintaining a T4/triiodothyronine (T3) serum concentration for ~4 weeks at a level comparable to that of euthyroid controls. Seven days after minipump implantation, thyroidectomized + T4/T3 (TX + T4/T3) and non-thyroidectomized intact rats (NTX) were fed diets containing 0, 25, 1000, or 3000 ppm FOE 5043 for up to 3 weeks. In addition, a direct assessment of the functional integrity of the FOE 5043-exposed thyroid gland as measured by its capacity to take up iodide ion was examined experimentally.

## B. Study design and methods

### 1. Animal assignment and treatment:

Route of administration:	Oral (diet)
Exposure:	3 to 13 weeks
Group size:	30/dose or 6/dose
Dose levels:	0, 25, 400, 1000, 1500, 1600, 2000, 2500, 3000, or 4000 ppm. 0-400-1600-3000 ppm for 3, 6 or 13 weeks 0-21.5-84.3-144.9 mg/kg bw/d 0-25-1000-3000 ppm for 3 weeks 0-1.7-70.5-224.2 mg/kg bw/day

### 2. Dose preparation and analysis:

Dose preparation:	<p>An acetone/corn oil mixture was used as a vehicle to dissolve the test substance prior to mixing with the dietary carrier.</p> <p>The control diet (including the acetone/corn oil mixture) was prepared the same as the treated diet, excluding only the test chemical.</p>
Analysis:	<p>The homogeneity, stability, and concentration of FOE 5043 in its dietary matrix were analytically verified by a comparative analysis of the concentration of 9 samples taken from 3 distinct sections (3 samples/section) of the mixing bowl. The stability of the AI of FOE 5043, when mixed in the diet and stored at room and freezer (approx. - 23°C) temperatures, was confirmed for 14 and 28 days, respectively. Additionally, the concentration of AI in the various test diets was periodically confirmed during the course of each experiment as well.</p>
<b>3. Observations:</b>	<p>mortality, clinical signs, body weight, food consumption, specific test (see below).</p>
<b>Initial toxicity screening:</b>	<p>10 rats/dose/time point received diets containing FOE 5043 at 0, 400, 1600 or 3000 ppm for 3, 6 and 13 weeks. Additional groups of 10 rats stayed on control diet for 4 weeks after the exposure of FOE 5043 and served as recovery animals. After 3, 6, 13 and 17 weeks respective animals were sacrificed and liver and thyroid were weighed and tissues were sampled for histopathological examination. Before sacrifice blood samples were taken for analyses of T3, T4 and TSH in serum.</p>
<b>Estimation of the metabolic response and bioavailability of FOE 5043:</b>	<p>To obtain a broad characterization of the metabolic response and bioavailability of FOE 5043 in the rat, 30 animals per dosage group were treated with 400, 1000, 1500, 2000, 2500, or 4000 ppm of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 as a dietary admixture for approximately 5 weeks. During exposure to the chemical, blood was periodically drawn from the orbital sinus of the rats. Two hundred microliter aliquots of the sample were then oxidized to CO<sub>2</sub> (Tri-Carb, Hewlett-Packard Model 306), which was trapped in a Carbosorb solution. The radioactive content present in each sample as CO<sub>2</sub> determined by LSC.</p>

**Comparative response to FOX 5043 exposure: thyroidectomized (TX) animals made euthyroid with hormonal replacement (TX + T4/T3) vs intact nonthyroidectomized animals (NTX)** Two groupings of animals, NTX and TX + T4/T3, were each exposed to doses (4-6 animals per dose group) of 0, 25, 1000, or 3000 ppm of FOE 5043 in their diet for 3 weeks. Rats were surgically TX approximately 7 days prior to initiation of dosing (Day 0).

To confirm the integrity of the thyroidectomy procedure itself, a third group of 6 animals was TX and maintained, without hormonal replacement, on control feed for the duration of the experiment. For those animals receiving hormonal replacement, osmotic mini-pumps containing a mixture of T3 and T4, were implanted subcutaneously and concomitantly at the time of thyroidectomy. Mini-pumps provided an estimated delivery rate of 4.0 µg T4/kg bw/day and 20 µg T3/kg bw/day.

**Serum thyroid hormone determinations:** At 3, 6, 13 and 17 weeks blood was consistently drawn for analysis between the hours of 0800 and 0930. Depending upon the requirements of the experiment, the sample was obtained either from the retroorbital sinus or the inferior vena cava. Once clotted, serum was isolated by centrifugation and stored at -20°C pending assay. With the exception of thyrotropin (TSH), all circulating thyroid hormone concentrations were measured by using commercially available kits, characterized for use with rat serum. The specific hormones measured, the intra-assay coefficient of variation, and the minimum detectable concentration, respectively, were detected for total T4, free T4, total T3, and free T3, and TSH.

#### **Thyroidal iodide uptake**

Following exposure to FOE 5043, iodide uptake was determined at various times by measuring the ratio of free and unreacted <sup>125</sup>I found in the thyroid to that found in the serum.

Animals (20/dosage group; 4 groups of 5 control and 5 treated animals were placed on study 1 day apart) received either 0 or 1000 ppm of FOE 5043 as a dietary admixture for 3 weeks. On Day 21 5 treated and 5 control animals received an ip injection of approximately 25 mCi/kg bw (2.5 mL/kg bw) of carrier-free [<sup>125</sup>I]Na. Prior to injection, [<sup>125</sup>I]Na (specific activity approximately 17.4 µCi/µg). At 2, 3, 4, or 5 hours after administration of the tracer, animals were asphyxiated in a CO<sub>2</sub> chamber and killed by exsanguination. The thyroids were excised and weighed, and then the radioactive content in the serum and thyroid was determined with a gamma counter. Background counts were subtracted and data were calculated and expressed as a percentage of total <sup>125</sup>I administered.

**Postmortem procedures:**

Animals were killed either by CO<sub>2</sub> asphyxiation or by exsanguination via the inferior vena cava while under CO<sub>2</sub> anesthesia. Organ weights of thyroid and liver were determined. For histopathologic examination, liver, thyroid, pituitary, and hypothalamic tissue were taken and preserved in 10% buffered formalin. Representative sections of the tissues collected were processed, embedded in paraffin, sectioned, mounted, and stained with hematoxylin and eosin for examination under the light microscope. Portions of some liver and thyroid tissue were also preserved in universal fixative to allow for examination under the electron microscope. In some cases hypothalamic and pituitary tissue were immunohistochemically stained to permit a more detailed morphologic assessment of thyrotropin releasing and thyrotropin stimulating centers within each gland, respectively.

**Serum thyroid hormone determinations:**

Depending upon the requirements of the experiment, blood samples were obtained either from the retroorbital sinus or the inferior vena cava. Once clotted, serum was isolated by centrifugation and stored at -20°C pending assay. With the exception of thyrotropin (TSH), all circulating thyroid hormone concentrations were measured by using commercially available kits, characterized for use with rat serum. The specific hormones measured, the intra-assay coefficient of variation, and the minimum detectable concentration, respectively, were detected for total T4, free T4, total T3, and free T3, and TSH.

**Thyroidal iodide uptake:**

Following exposure to FOE 5043, iodide uptake was determined at various times by measuring the ratio of free and unreacted  $^{125}\text{I}$  found in the thyroid to that found in the serum.

Animals (20/dosage group; 4 groups of 5 control and 5 treated animals) received either 0 or 1000 ppm of FOE 5043 as a dietary admixture for 3 weeks. On Day 21 (Day 0, initiation of dosing for each group of 10) and between the hours of 0800 and 0930, 5 treated and 5 control animals received an ip injection of approximately 25  $\mu\text{Ci/kg bw}$  (2.5 ml/kg bw) of carrier-free  $^{125}\text{I}]\text{Na}$ . Prior to injection,  $^{125}\text{I}]\text{Na}$  (spec act approximately 17.4  $\mu\text{Ci}/\mu\text{g}$ ) was diluted with 0.9% NaCl to give a solution of approximately 10  $\mu\text{Ci/ml}$ . At 2, 3, 4, or 5 hr after administration of the tracer, animals were asphyxiated in a  $\text{CO}_2$  chamber and killed by exsanguination. The thyroids were excised and weighed, and then the radioactive content in the serum and thyroid was determined with a gamma counter. Background counts were subtracted and data were calculated and expressed as a percentage of total  $^{125}\text{I}$  administered.

**Determination of hepatic UDP-GT and 5'-deiodinase activities:**

Liver tissue was perfused with ice-cold saline, frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  pending assay. The activity of uridine diphosphate glucuronosyltransferase (UDP-GT activity) was determined by measuring the disappearance of p-nitrophenol and expressed as nanomoles p-nitrophenyl glucuronide formed per minute per unit of liver or liver protein. The estimation of 5'-deiodinase (5'DI) activity was based on the procedure described by Jones et al. (1988). Briefly, liver tissue was homogenized, centrifuged, and an aliquot of the supernatant incubated at  $37^\circ\text{C}$  for 10 min in the presence of phosphate buffer, dithiothreitol, and T4. The level of hepatic deiodinase activity measured was expressed as picograms T3 formed per minute per unit of liver or liver protein. The protein content of the hepatic microsomal preparations was estimated by the method of Bradford (1976).

**Perchlorate discharge test:**

Animals (6/dose group) received either 0 or 1000 ppm FOE 5043 as a dietary admixture for 21 days. On Day 21 all animals received an i.p. injection of approximately 33  $\mu\text{Ci/kg bw}$  (0.4 ml/kg bw) of carrier-free [ $^{125}\text{I}$ ]Na. Six hours after injection of the tracer,  $\text{KClO}_4$  was administered (10 mg/kg bw; ip); approximately 2.5 min after injection of the  $\text{ClO}_4^-$ , the animal was asphyxiated in a  $\text{CO}_2$  chamber and sacrificed by exsanguination. Thyroids were excised and weighed, and the radioactive content in the blood and thyroid determined with a gamma counter. Background counts were subtracted and data calculated and expressed as a percentage of total  $^{125}\text{I}$  administered.

Positive control group was treated with 200 mg/kg bw/day PTU for 4 days p.o.. approximately 24 hours after the final dose of PTU animals received one i.p. injection of 10 mg/kg bw of  $\text{ClO}_4^-$ .

**TRH challenge test:**

To assess the functional integrity of the pituitary gland 6 rats/dose received either 0 or 1000 ppm FOE 5043 as a dietary admixture for 21 days. On Day 21 blood was drawn via the retroorbital sinus for determination of circulating levels of thyrotropin. Samples were collected at  $10 \pm 5$  minutes before and then again at  $15 \pm 2$  and  $60 \pm 5$  minutes after a tail vein injection of approximately 5  $\mu\text{g TRH/kg bw}$ .

**[ $^{125}\text{I}$ ]T<sub>4</sub> clearance test:**

5 rats/dose received either 0 or 1000 ppm of FOE 5043 as a dietary admixture for 21 days. On Day 21 all animals received an iv injection of approximately 8  $\mu\text{Ci/kg bw}$  [ $^{125}\text{I}$ ]T<sub>4</sub> into a tail vein. Blood was drawn via the retroorbital sinus at 4, 8, 24, 48, 72, and 96 hrs after administration of the tracer. Blood samples were centrifuged, and the radioactive content of an aliquot ( $\leq 0.1$  ml) of serum was determined with a gamma counter.

Background counts were subtracted and data for each time point calculated and expressed as a percentage of the total [ $^{125}\text{I}$ ]T<sub>4</sub> administered per ml of serum.

The clearance of [ $^{125}\text{I}$ ]T<sub>4</sub> was estimated for each rat using the total area under the plasma concentration vs time curve (AUC), as calculated by the trapezoidal rule and applying the equation: clearance (Cl) = iv dose/AUC.



**Biliary excretion of [<sup>125</sup>I]T<sub>4</sub>:**

Bile duct-cannulated animals were obtained from Hilltop Lab Animals, Inc. Animals (6/dose group) received either 0 or 1000 ppm of FOE 5043 as a dietary admixture for 14 days. On Day 14 all animals were administered an iv injection of approximately 9 µCi/kg bw of [<sup>125</sup>I]T<sub>4</sub> into a lateral tail vein. Animals were anesthetized with a 3:3:1 mixture of ketamine, xylazine, and acepromazine, respectively, prior to administration of the tracer. During the bile collection process, the rats were placed in a Bollman restraining cage. In addition to bile, liver and blood samples were collected and their radioactive content determined.

**Statistical Analysis:**

For statistical comparisons of continuous data involving 2 groups, a two-tailed Student's t test (unpaired) was used. Continuous data that were examined statistically and involved a comparison of 3 or more groups were initially evaluated for equality or homogeneity of variance using Bartlett's test (Snedecor and Chochran, 1967). Further statistical analysis of the data included a one-way analysis of variance (Snedecor and Chochran, 1967) followed by Dunnett's test (Dunnett, 1955; Dunnett, 1964). For the Bartlett test, a probability (p) value < to 0.001 was considered significant; for all other statistical tests, differences with p values < 0.05 were considered statistically significant.

**4. Statistical Analysis:**

Data were analyzed for statistically significant differences either by Student's t test (unpaired) or a one-way analysis of variance (Snedecor and Chochran, 1967) followed by Dunnett's test (Dunnett, 1955, 1964). Differences with p values ≤ 0.05 were considered statistically significant. All statistical evaluations were performed using software from either INSTEM Computer Systems, SAS Institute Inc., or Jandel Scientific (Corte Madera, CA).

**II. Results and discussion****A. Initial toxicity screening**

Test substance intake

**Table with test substance intake (mg a.s/kg bw/day)**

	13-week exposure			
Dose (ppm)	0	400	1600	3000
Mean test substance intake (mg/kg body weight)	0	21.5±1.3	84.3±3.4	144.9±13.7

Body weight

<b>Dose level</b>	<b>0 ppm</b>	<b>400 ppm</b>	<b>1600 ppm</b>	<b>3000 ppm</b>
Bodyweight day 0	186.6	184.4	188.8	190.3
Bodyweight day 7	200.7	203.3	206.7	203.3
Bodyweight day 14	215.5	217.3	220.1	217.3
Bodyweight day 21	227.5	232.8	234.3	229.9
Bodyweight day 28	239.9	246.2	244.1	230.1
Bodyweight day 35	253.6	258.9	257.6	244.8
Bodyweight day 42	264	270.5	267.3	253.3
Bodyweight day 49	274.5	279	277.4	261.8
Bodyweight day 56	281	286.8	282.8	267.3
Bodyweight day 63	288.6	294.3	291.4	273.9
Bodyweight day 70	296.1	301.9	297	279.8
Bodyweight day 75/76/77	304.7	304.6	309.3	291.7
Bodyweight day 84	293.3	295.4	294.5	272.8*
Bodyweight gain days 0-7	14.1	18.9	17.9	13
Bodyweight gain days 7-14	14.8	14	13.4	14
Bodyweight gain days 14-21	12	15.5	14.2	12.6
Bodyweight gain days 0-21	40.9	48.4	46	39.6

## Food consumption

<b>Dose level</b>	<b>0 ppm</b>	<b>400 ppm</b>	<b>1600 ppm</b>	<b>3000 ppm</b>
Food consumption Day 7	15.6	15.91	16.04	16.14
Food consumption Day 14	15.99	15.99	15.89	15.91
Food consumption Day 21	15.37	15.58	15.78	15.86
Food consumption Day 28	15.68	16.32	15.52	16.62
Food consumption Day 35	15.4	15.83	15.85	15.89
Food consumption Day 42	15.39	15.72	15.96	15.72
Food consumption Day 49	16.03	16.42	15.74	15.53

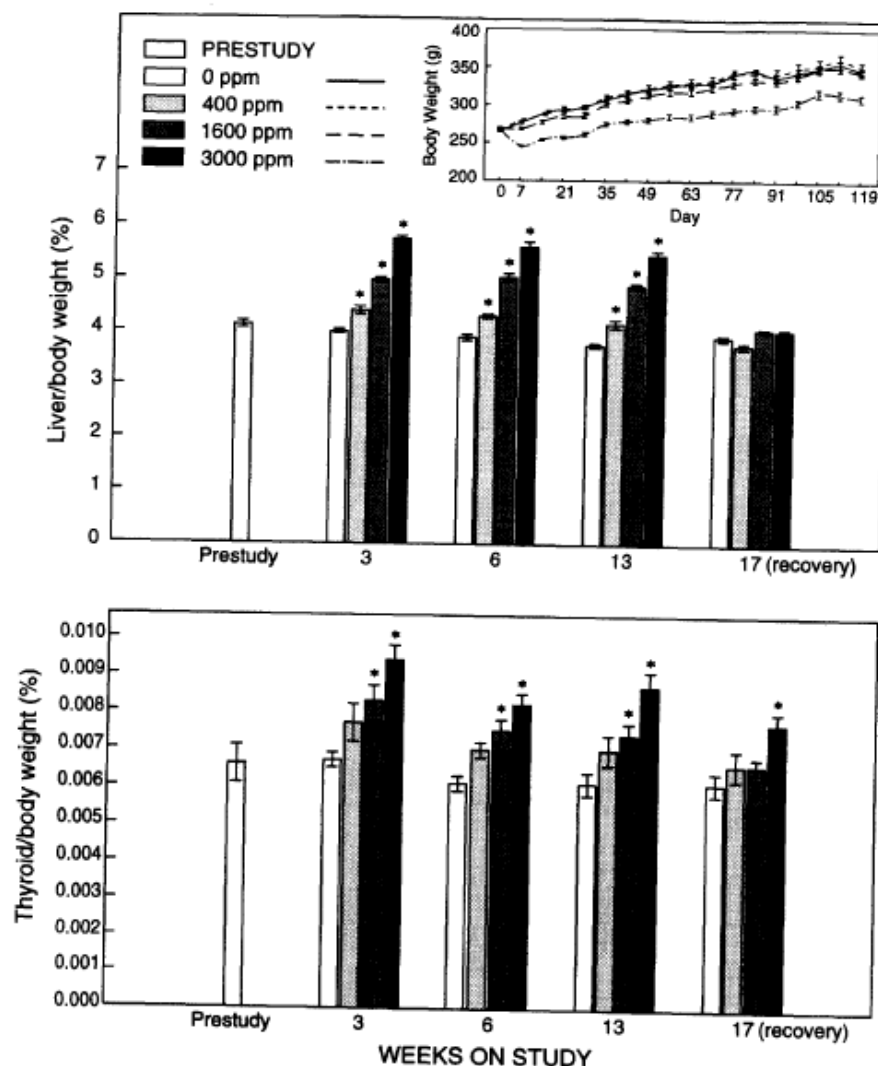
Food consumption Day 56	15.77	15.99	15.95	16.33
Food consumption Day 63	15.49	15.82	15.88	15.94
Food consumption Day 70	16.19	16.58	16.15	15.98
Food consumption Day 77	15.76	15.77	15.5	15.23
Food consumption Day 84	13.82	14.16	14.48	14.65

#### Organ weights

Following 3, 6, or 13 weeks of treatment with FOE 5043, dose-dependent increases in liver weight and liver-to-body weight ratios were measured relative to controls in animals administered 0, 400, 1600, or 3000 ppm FOE 5043. Elevations in thyroid-to-body weight ratios were also observed in the 1600- and 3000-ppm groups. However, a decline in final body weights in comparison to control animals was noted in mid- and high-dose group.

#### **Organ weights after 13 weeks exposure**

<b>Dose level</b>	<b>0 ppm</b>	<b>400 ppm</b>	<b>1600 ppm</b>	<b>3000 ppm</b>
BW at terminal kill	307.4	304	305	284.3*
Liver weight (rel.)	4.04	4.125	4.355*	5.112*
Thyroid weight (rel.)	0.0063	0.0063	0.0067	0.00740*



Each bar represents the  $\bar{x} \pm \text{SE}$  for groups of 10 animals. An asterisk (\*) indicates statistical significance relative to control by one-way analysis of variance and Dunnett's tests;  $p \leq 0.05$ .

#### Histopathology of thyroid and liver

Histologically, a multifocal hypertrophy of the follicular epithelium of the thyroid gland was observed at Weeks 3, 6, and 13 in 3000-ppm animals.

Histopathological examination of the liver revealed an increase in the incidence of diffuse centrilobular hypertrophy at Weeks 3, 6, and 13 in 400-, 1600-, and 3000-ppm animals. Electron microscopic evaluation of 3000-ppm 13-week livers showed no nuclear changes suggestive of cellular injury (e.g., destruction of nuclear pores, aggregation of protein, clumping and peripheralization of chromatin). Cytoplasmic organelle changes consisted of an increased proliferation of the smooth endoplasmic reticulum, which correlated with increased liver weights and hepatocytomegaly noted previously. Though cytoplasmic changes were noted in the liver, electron microscopic evaluation of 3000-ppm 13-week thyroids showed no indication of either nuclear or cytoplasmic ultrastructural variations.

The histological appearance of both the pituitary and the hypothalamus, following both conventional and immunohistochemical microscopic analysis, was unremarkable at all time intervals and levels of exposure.

Dose (ppm)	Liver												Thyroid											
	Hepatic Cell Hypertrophy												Follicular Cell Hypertrophy											
	+ <sup>b</sup>				++				+++				+				++				+++			
	3	6	13	17	3	6	13	17	3	6	13	17	3	6	13	17	3	6	13	17	3	6	13	17
0	0 <sup>c</sup>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	3	2	2	-	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-
1600	2	1	0	-	3	9	8	-	0	0	2	-	0	0	0	-	0	0	0	-	0	0	0	-
3000	0	0	0	0	10	10	2	0	0	0	8	0	4	5	2	0	0	0	0	0	0	0	0	0

<sup>a</sup>Animals were treated for 3, 6, or 13 weeks with 0, 400, 1600, or 3000 ppm FOE 5043 as a dietary admix. Ten (10) animals were killed for histopathological determinations at each time point, including control and high-dose recovery animals that remained on study for 4 additional weeks (Weeks 13-17) receiving only control feed.

<sup>b</sup>Severity grades are designated as follows: +, minimal; ++, slight; and +++, moderate.

<sup>c</sup>Number of animals with a particular finding from each group of 10 killed at 3, 6, 13, or 17 weeks.

#### Thyroid hormone levels

A dose-related, comparable, and consistent decline in serum T4 concentration was measured in 400-, 1600-, and 3000-ppm animals after 3, 6, and 13 weeks of treatment. Compound-related declines and increases in serum T3 and TSH levels, respectively, were measured at Weeks 3 and 6 but both parameters had returned to or were approaching control concentrations by Week 13. Following removal of the chemical from the feed at 13 weeks, all effects described above had been reversed by Week 17.

In summary, (1) the consistency of the pattern of change of FOE 5043-induced declines in T4 blood levels observed in 400-3000-ppm animals with respect to magnitude, time, and dose; (2) the 2-3 week time-to-steady state, established for 400-4000-ppm animals under non-saturated conditions, suggested by the FOE 5043 bioavailability/absorption profile depicted in Appendix A, Fig. 6, and (3) the exposure regimen used in the lifetime bioassay with FOE 5043 in the rat (25, 400, and 800 ppm) formed the basis for the decision to use 3-week exposures at a low dose of 25 and a top dose of either 1000 or 3000 ppm FOE 5043 admixed in the diet to conduct the mechanistic investigations described next.

#### FOE 5043 Intake

For the 3-week exposure period, the mean daily intake of the FOE 5043 (mg/kg bw/day  $\pm$  SE), calculated from feed consumption, body weight, and diet analysis data, for animals administered the chemical at nominal dietary concentrations of 25, 1000, or 3000 ppm was  $1.7 \pm 0.1$ ,  $70.5 \pm 4.1$ , and  $224.2 \pm 35.0$ , respectively. FOE 5043 was not detected in control feed.

	13-week exposure			
Dose (ppm)	0	25	1000	3000
Mean test substance intake (mg/kg body weight)	0	1.7 $\pm$ 0.1	70.5 $\pm$ 4.1	224.2 $\pm$ 35.0

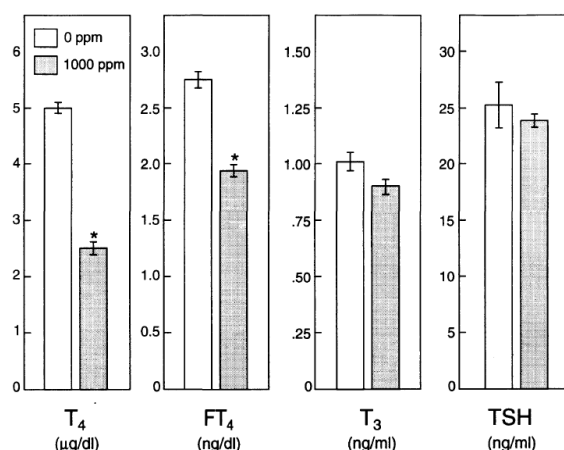
### Effect of FOE 5043 on Body Weight and Food Consumption

Over 21 days of continuous and uninterrupted dietary exposure to FOE 5043, declines in body weight and increases in food consumption were noted at a concentration of 3000 ppm. However, neither body weight nor food consumption were affected at exposure levels up to an including 1000 ppm in the feed.

### Effect of FOE 5043 on Serum Thyroid Hormone Concentrations

Statistically significant declines relative to controls of 50 and 30%, respectively, in circulating levels of total and free T4 were measured in 21-day 1000-ppm FOE 5043-treated animals. However, no effect on serum levels of either T3 or TSH over the same exposure interval was indicated. Most notably, the lack of change in TSH is curious as it suggests the possibility of an FOE 5043-induced interference with the regulation of thyroid hormone as the result of a compromised pituitary gland that is unable to respond appropriately to a depressed serum T4 concentration.

Circulating Thyroid Hormones after 21-d exposure to FOE 5043		
Dose levels (ppm)	0	1000
T3 [ng/mL]	1.01	0.9
T4 total [µg/dL]	5	2.51*
T4 free [ng/dL]	2.75	1.94*
TSH [ng/mL]	25.28	23.87



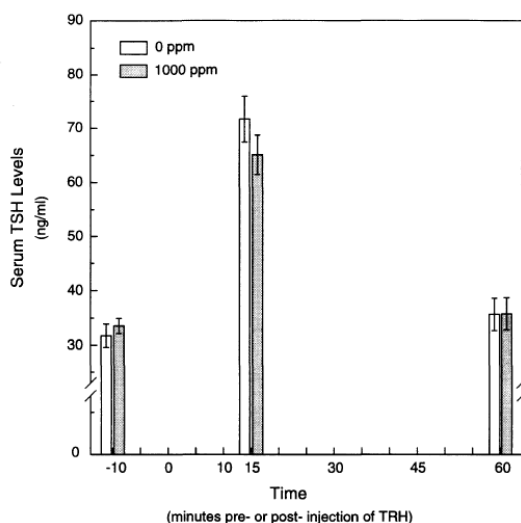
Effect of FOE 5043 on circulating concentrations of total and free thyroxine (T<sub>4</sub>), triiodothyronine (T<sub>3</sub>), and thyrotropin (TSH) in the male rat. Animals were treated for 21 days with 0 or 1000 ppm FOE 5043 as a dietary admixture to the feed. Each bar represents the mean  $\pm$  SE for groups of 4-5 animals.

\* = statistically significant different from controls by Student's t-test (unpaired);  $p \leq 0.05$

### Effect of FOE 5043 on the Response of the Pituitary Gland to Thyrotropin Releasing Hormone

In the face of a marked decline in serum T<sub>4</sub> concentration (as noted above), a functionally-competent pituitary gland would be expected to respond, as a result of the apparent release of negative feedback inhibition accompanying a fall in serum T<sub>4</sub> levels, with an increased TSH secretion from the pituitary, presumably to drive the thyroid to restore circulating T<sub>4</sub> levels to normal. In this procedure the functional status of the FOE 5043-exposed pituitary gland was assessed in terms of its response

(release of TSH into the circulation) to an exogenous challenge with the hypothalamic and TSH-regulating tripeptide thyrotropin releasing hormone (TRH). Circulating TSH levels in the serum approximately 10 minutes before and approximately 15 and 60 minutes after administration of TRH are shown below.



Despite the apparent lack of a compensating elevation in serum TSH levels in the face of a marked decline in FOE 5043-induced circulating T4 concentrations, the data from this experiment provide no indication of pituitary impairment as a result of treatment with FOE 5043. As illustrated above, the response of the pituitary gland of the control and treated animals at the two time points evaluated following the TRH challenge was unequivocally comparable. In addition, histopathological examination conducted previously on the pituitary as well as the hypothalamus of rats administered up to 3000 ppm FOE 5043 in the diet for 13 weeks provided no morphological evidence of a chemically-mediated interference with the homeostasis of these glands.

#### **Effect of FOE 5043 and PTU on Perchlorate-Induced Discharge of Radioiodide from the Thyroid Gland**

The effect on control, FOE 5043-, and PTU-treated animals to a pulse dose of  $\text{ClO}_4^-$  is shown in the Table below. As can be seen, no significant difference in the response to perchlorate was observed between control and FOE 5043-treated animals, suggesting that in this experiment exposure to FOE 5043 did not interfere with the capacity of the thyroid gland to take up and organify iodide ion during the process of hormonogenesis. In contrast, the response to  $\text{ClO}_4^-$  of the animals treated with the positive control PTU, a thioamide known to inhibit the production of T4 at both the organification (inhibition of peroxidase activity) and the coupling level, was characterized, relative to both FOE 5043 and control animals, by a significantly lower thyroid/blood ratio of exogenously administered radiolabeled iodine.

The Effect on the Process of Thyroidal Organification as Measured by the Perchlorate Discharge Test, Following Exposure of the Male Rat to either FOE 5043 or Propylthiouracil<sup>a</sup>

Treatment	Thyroid (% <sup>125</sup> I dose/g) <sup>b</sup>	Blood (% <sup>125</sup> I dose/ml)	Thyroid/ Blood Ratio
Control (0 ppm)	1741 ± 58 <sup>c</sup>	0.39 ± 0.06	4906 ± 562
FOE 5043 (1000 ppm)	1352 ± 168	0.31 ± 0.03	4366 ± 339
Propylthiouracil	16.9 ± 4.2 <sup>d</sup>	0.35 ± 0.05	47.6 ± 6.6 <sup>d</sup>

<sup>a</sup> Rats were treated for 21 days with 0 or 1000 ppm FOE 5043 as a dietary admix. On Day 21 (Day 0, initiation of dosing) all animals received an ip injection of [<sup>125</sup>I]Na. Six (6) hours after administration of the tracer, all animals were dosed with potassium perchlorate (10 mg/kg body wt; ip); approximately 2.5 min after injection of the perchlorate, the animal was sacrificed, and the radioactive content of the thyroid and the blood was determined in a gamma counter. To serve as a positive control, a group of 5 rats were administered propylthiouracil, a thioamide capable of inhibiting the synthesis of thyroxine, at a dosage of 200 mg/kg body wt/day (po) for 4 days prior to administration of the perchlorate on Day 4.

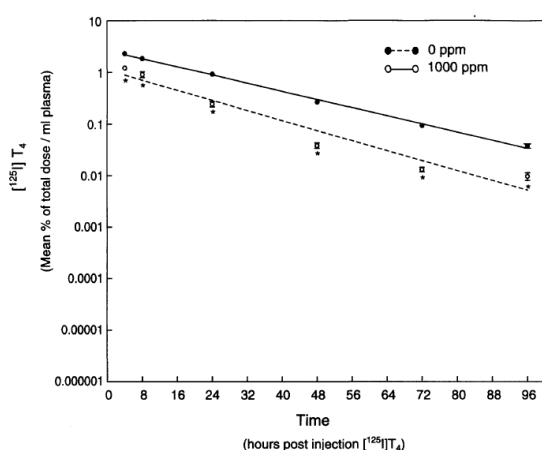
<sup>b</sup> Data are expressed in terms of the percentage of the total dose of [<sup>125</sup>I]Na administered per unit weight of thyroid tissue or per unit volume of blood.

<sup>c</sup> Each value represents the mean ± standard error for 5-6 rats per dose group.

<sup>d</sup> Indicates statistically significant difference relative to control by Student's t-test (unpaired);  $p \leq 0.05$ .

### Effect of FOE 5043 on Clearance of [<sup>125</sup>I]Thyroxine from the Circulation

Mean plasma [<sup>125</sup>I]T<sub>4</sub> disappearance curves for control and FOE 5043-treated animals are shown in the graph below. The graph illustrates that treatment with FOE 5043 significantly increased the capacity of the rat to clear an exogenously administered dose of [<sup>125</sup>I]T<sub>4</sub> from the plasma. By 4 hrs after administration of the T<sub>4</sub> tracer, blood levels of radiolabeled T<sub>4</sub> (expressed as the mean percentage of administered dose per milliliter of plasma) in the FOE 5043-exposed animals were markedly lower than controls given a comparable dose. Quantitatively, this difference was expressed in terms of an overall mean plasma clearance ( $\bar{x} \pm \text{SE}$ ) rate for the entire sampling period of  $1.98 \pm 0.09$  ml/hr for the controls compared to a statistically significantly and approximately 3-fold higher rate of  $5.96 \pm 0.71$  ml/hr for the FOE 5043-exposed animals.



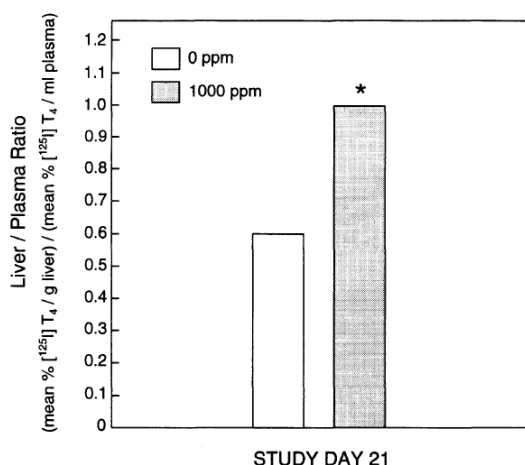


Effect of FOE 5043 on the clearance of [ $^{125}$ I]T<sub>4</sub> from the circulation of the male rat as shown by mean [ $^{125}$ I]T<sub>4</sub> disappearance curves plotted semi-logarithmically. Animals were treated for 21 days with 0 or 1000 ppm of FOE 5043 as a dietary admixture to the feed. On Day 21 (Day 0, initiation of dosing), rats received an iv injection of [ $^{125}$ I]T<sub>4</sub>. At various times after administration of the tracer, blood was drawn from the orbital sinus, and the radioactive content of the plasma determined using a gamma counter. Radioactivity was expressed in terms of the mean percentage of the IOC total dose of [ $^{125}$ I]T<sub>4</sub> administered per ml of plasma. Each data point represents the  $\bar{x} \pm \text{SE}$  for groups of 6 animals. An asterisk (\*) indicates statistical significance relative to control by Student's t test (unpaired);  $p \leq 0.05$ .

[ $^{125}$ I]T <sub>4</sub> clearance hrs after iv injection		
% [ $^{125}$ I]T <sub>4</sub> dose/ml 4 hrs	2.31	1.23*
% [ $^{125}$ I]T <sub>4</sub> dose/ml 8 hrs	1.86	0.91*
% [ $^{125}$ I]T <sub>4</sub> dose/ml 24 hrs	0.92	0.24*
% [ $^{125}$ I]T <sub>4</sub> dose/ml 48 hrs	0.27	0.04*
% [ $^{125}$ I]T <sub>4</sub> dose/ml 72 hrs	0.09	0.01*
% [ $^{125}$ I]T <sub>4</sub> dose/ml 96 hrs	0.03	0.01*

### Effect of FOE 5043 on the Hepatocellular Binding of [ $^{125}$ I]Thyroxine

The distribution of radioactivity between the liver and plasma (expressed as the percentage of administered dose per unit tissue) of control and FOE 5043-treated animals at 4 hr after administration of a radiolabeled dose of T<sub>4</sub> is shown below. A statistically significant 66% increase in the liver/plasma ratio of the administered dose in treated animals relative to controls was measured.



Effect of FOE 5043 on the hepatic uptake of [ $^{125}$ I]T<sub>4</sub> from the circulation of the male rat. Animals were treated for 21 days with 0 or 1000 ppm of FOE 5043 as a dietary admixture to the feed. On Day 21 (Day 0, initiation of dosing) rats received an iv injection of [ $^{125}$ I]T<sub>4</sub>. Approximately 4 hrs after administration of the tracer, animals were sacrificed by exsanguination and the radioactive content of the liver and plasma determined in a gamma counter. Radioactivity was expressed in terms of the mean percentage of the total dose of [ $^{125}$ I]T<sub>4</sub> administered per g of liver and per ml of plasma. Each bar represents the  $\bar{x} \pm \text{SE}$  for groups of 6 animals. An asterisk (\*) indicates statistical significance relative to control by Student's t test (unpaired);  $p \leq 0.05$ .

### Effect of FOE 5043 on Hepatic UDP-GT and 5'DI Activities

The activities of the membrane-bound enzymes UDP-GT and 5'DI in the liver following 3 weeks of exposure to FOE 5043 are shown in Tables 2 and 3, pages 33 and 34, respectively. Both enzymes have fundamental roles in the regulation and disposition of thyroid hormones, which are metabolized primarily in the liver (Hill et al., 1989). Insofar as chemical induction of one or both metabolizing enzymes could account for or contribute to the reduction in circulating T4 concentration, which was observed in the FOE 5043-exposed animals, the activity of both enzymes was assessed in the liver. Statistically significant and dose-related increases in UDP-GT activity were measured in the 1000- and 3000-ppm groups following 3 weeks of dietary dosing. These changes would be consistent with the postulate that FOE 5043 is indirectly mediating reductions in serum T4 levels through stimulation of the liver's capacity for glucuronidation. However, FOE 5043 appeared to have the opposite effect on 5'DI as the activity of the enzyme appeared to trend downward with dose.

Effect of FOE 5043 on Hepatic Uridine Diphosphate Glucuronosyltransferase Activity in the Male Rat<sup>a</sup>

Dose (ppm)	Activity (nmol/min) <sup>b</sup>	
	mg protein <sup>-1</sup>	g liver <sup>-1</sup>
0	1.5 ± 0.1 <sup>c</sup>	188.7 ± 20.1
25	2.3 ± 0.4	300.6 ± 59.7
1000	5.5 ± 0.6 <sup>d</sup>	827.0 ± 88.9 <sup>d</sup>
3000	10.1 ± 0.8 <sup>d</sup>	1401.2 ± 53.8 <sup>d</sup>

<sup>a</sup> Rats were treated for 21 days with 0, 25, 1000, or 3000 ppm FOE 5043 as a dietary admix. On Day 21 (Day 0, initiation of dosing) animals were sacrificed and the livers removed for estimation of uridine diphosphate glucuronosyltransferase activity.

<sup>b</sup> Data are expressed as nanomoles p-nitrophenyl glucuronide formed per minute per milligram of microsomal protein or per gram liver.

<sup>c</sup> Each value represents the mean ± standard error for 5 rats per dose group.

<sup>d</sup> Indicates statistically significant difference relative to control by oneway analysis of variance and Dunnett's tests;

p ≤ 0.05.

Effect of FOE 5043 on Hepatic 5'-Deiodinase Activity in the Male Rat<sup>a</sup>

Dose (ppm)	Activity (picograms/min) <sup>b</sup>	
	mg protein <sup>-1</sup>	g liver <sup>-1</sup>
0	6.8 ± 0.8 <sup>c</sup>	862.6 ± 84.6
25	6.1 ± 0.3	837.3 ± 50.8
1000	4.3 ± 0.4 <sup>d</sup>	649.3 ± 40.5
3000	3.9 ± 0.3 <sup>d</sup>	580.0 ± 24.8 <sup>d</sup>

<sup>a</sup> Rats were treated for 21 days with 0, 25, 1000, or 3000 ppm FOE 5043 as a dietary admix. On Day 21 (Day 0, initiation of dosing) animals were sacrificed and the livers removed for estimation of 5'-deiodinase activity.

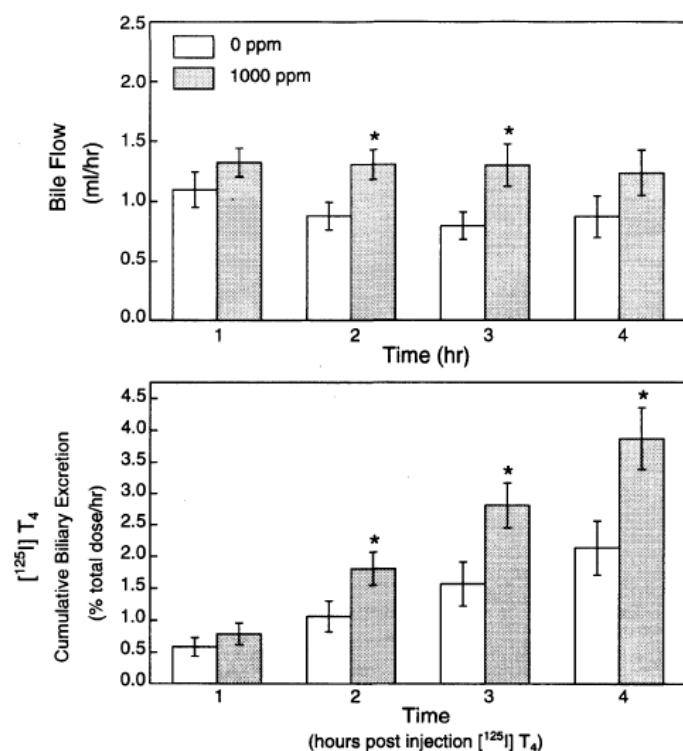
<sup>b</sup> Data are expressed as picograms T3 formed per minute per milligram of microsomal protein or per g liver.

<sup>c</sup> Each value represents the mean ± standard error for 5 rats per dose group.

<sup>d</sup> Indicates statistically significant difference relative to control by oneway analysis of variance and Dunnett's tests;  
 $p \leq 0.05$ .

### Effect of FOE 5043 on Bile Flow and the Biliary Excretion of [<sup>125</sup>I]Thyroxine

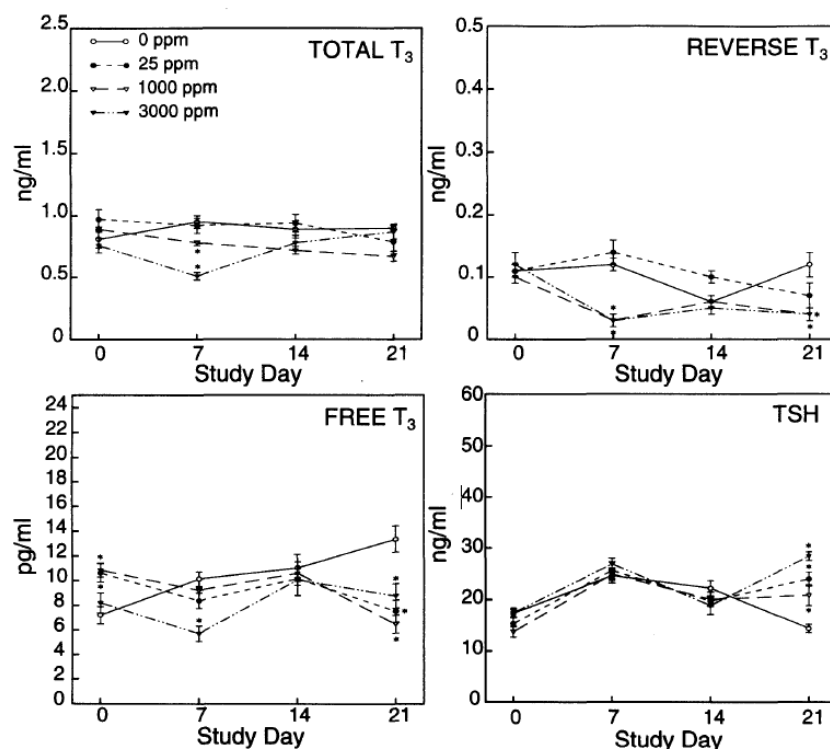
As is typically seen with microsomal enzyme inducers with the ability to reduce circulating levels of thyroid hormone, both bile flow and the biliary excretion of exogenously supplied [<sup>125</sup>I]T<sub>4</sub> (expressed as the percentage of the administered dose excreted per hour) were significantly increased in FOE 5043-treated animals (see below). For the 4 hrs following administration of the T<sub>4</sub> tracer, the cumulative biliary excretion of radioactivity was increased, most likely as a function of the modest increase in bile flow, by 45% with respect to control.



Effect of FPOE 5043 on bile flow and biliary excretion of [<sup>125</sup>I]T<sub>4</sub> in male rats. Bile duct cannulated rats were treated for 14 days with 0 or 1000 ppm FOE 5043 in diet. On day 14 (Day 0 = initiation of dosing), all rats received an iv injection of [<sup>125</sup>I]T<sub>4</sub> and bile was collected at 1 hour-intervals. The cumulative biliary excretion of the T<sub>4</sub>-tracer, expressed in terms of percentage of the total dose of [<sup>125</sup>I]T<sub>4</sub> administered, was determined over a 4-hour period. Each bar represents the  $\bar{x} \pm \text{SE}$  for groups of 6 animals. An asterisk (\*) indicates statistical significance relative to control by Student's t test (unpaired);  $p \leq 0.05$ .

### Comparative response to FOX 5043 exposure: thyroidectomized (TX) animals made euthyroid with hormonal replacement (TX + T<sub>4</sub>/T<sub>3</sub>) vs intact non-thyroidectomized animals (NTX)

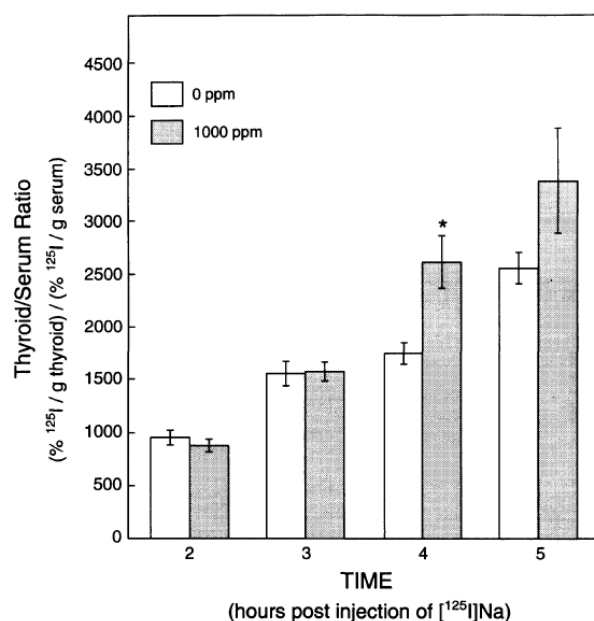
Total and free serum T<sub>4</sub> concentrations for non-thyroidectomized intact rats and thyroidectomized rats receiving thyroid hormone replacement (TX + T<sub>4</sub>/T<sub>3</sub>) are shown in the following graph.



Effect of FOE 5043 on serum concentrations of total and free triiodothyronine (T<sub>3</sub>), reverse T<sub>3</sub>, and thyrotropin (TSH) in the intact nonthyroidectomized male rat. Each data point represents the  $\bar{x} \pm \text{SE}$  for groups of 5 animals. An asterisk (\*) indicates statistical significance relative to control by one-way analysis of variance and Dunnett's tests;  $p \leq 0.05$ .

Both the NTX and the TX + T<sub>4</sub>/T<sub>3</sub> animal groupings consisted of 4 dose groups (4-6 animals/group) which received FOE 5043 in their feed at concentrations of 0, 25, 1000, or 3000 ppm for 3 weeks. Approximately 7 days after minipump implantation and before exposure to FOE 5043 (Day 0), total T<sub>4</sub> serum levels in all 4 dose groups comprising the TX + T<sub>4</sub>/T<sub>3</sub> animals were determined to be roughly equivalent. It should be noted, however, that all values were higher than normal euthyroid (~6.0 µg/dl) control animals, possibly reflecting a greater than expected rate of delivery of the replacement hormones. The experimental data indicate that comparable declines in circulating levels of both total and free T<sub>4</sub> with respect to dose, time, and magnitude were observed in both the NTX and the TX + T<sub>4</sub>/T<sub>3</sub> animals.

As the pump represents the sole source of thyroid hormone in the TX animals, these findings suggest the changes in serum T<sub>4</sub> concentration observed in FOE 5043-exposed rats are mediated extrathyroidally through a separate and distinct mechanism not involving direct chemical interference with either the synthesis or secretory functions of the thyroid gland itself. In addition to T<sub>4</sub>, the effects of FOE 5043 on TSH, as well as total, free, and reverse T<sub>3</sub> serum concentrations for intact NTX animals, were measured and are shown in below.



Effect of FOE 5043 on thyroidal uptake of iodide ion in the male rat.

Radioactivity was expressed in terms of the percentage of the total dose of <sup>125</sup>I administered per unit tissue weight.

Each bar represents the  $\bar{x} \pm \text{SE}$  for groups of 5 animals. An asterisk (\*) indicates statistical significance relative to control by Student's t test (unpaired);  $p \leq 0.05$ .

Unlike FOE 5043-induced alterations in T<sub>4</sub>, which had clearly emerged in 1000- and 3000-ppm animals by 7 days and then persisted out to Day 21 of the study, a clear and consistent pattern of change with respect to dose and/or time was not apparent for any of the associated thyroid parameters that were measured. Although alterations in serum T<sub>3</sub> parameters and TSH were noted sporadically in 1000- and 3000-ppm animals, no consistent trend was evident for either endpoint. Considering the erratic nature of the serum T<sub>3</sub> response coupled with the lack of or delayed nature of the TSH response, despite the apparent release of negative feedback suppression provided by a reduced serum T<sub>4</sub> concentration which was seen by 7 days into the study, there would appear to be some additional form(s) of interference occurring here, possibly secondary to the non-thyroidally mediated action of FOE 5043 on circulating levels of T<sub>4</sub>.

With respect to the question of a specific target site in which to attribute the changes in blood levels of T<sub>4</sub>, the comparative changes in liver and thyroid weights measured in the NTX and TX + T<sub>4</sub>/T<sub>3</sub> animals (see Table below) correspond well and are consistent (as is the thyroidal uptake data presented next) with the hypothesis that the thyroidal effects accompanying exposure to FOE 5043 are not due to a direct effect on the synthesizing/secreting functions of the thyroid gland but are secondary to a chemically induced stimulation of the metabolizing capacity of the liver.

Effect of FOE 5043 on Liver and/or Thyroid Comparative  
Organ Weights in Intact Male Rats and Thyroidectomized Male Rats Receiving  
Thyroid Hormone Replacement by Osmotic Minipump<sup>a</sup>

Dose (ppm)	Terminal body weight (g)	Liver weight (g)	Liver/body weight ratio (%)	Thyroid weight (g)	Thyroid/body weight ratio (%; $\times 10^{-2}$ )
<b>NON-THYROIDECTOMIZED</b>					
0	253.7 $\pm$ 14.4 <sup>b</sup>	10.824 $\pm$ 1.019	4.228 $\pm$ 0.202	0.016 $\pm$ 0.001	0.62 $\pm$ 0.02
25	262.6 $\pm$ 4.6	11.203 $\pm$ 0.343	4.263 $\pm$ 0.080	0.017 $\pm$ 0.001	0.66 $\pm$ 0.02
1000	253.1 $\pm$ 4.4	12.796 $\pm$ 0.733	5.043 $\pm$ 0.203 <sup>c</sup>	0.018 $\pm$ 0.001	0.71 $\pm$ 0.02
3000	239.6 $\pm$ 5.8	15.136 $\pm$ 0.419 <sup>c</sup>	6.316 $\pm$ 0.035 <sup>c</sup>	0.019 $\pm$ 0.001	0.80 $\pm$ 0.04 <sup>c</sup>
<b>THYROIDECTOMIZED + T<sub>4</sub>/T<sub>3</sub></b>					
0	257.8 $\pm$ 3.8	10.361 $\pm$ 0.136	4.021 $\pm$ 0.054	---	---
25	268.5 $\pm$ 2.8	11.020 $\pm$ 0.202	4.103 $\pm$ 0.039	---	---
1000	265.7 $\pm$ 5.1	13.469 $\pm$ 0.391 <sup>c</sup>	5.067 $\pm$ 0.094 <sup>c</sup>	---	---
3000	237.0 $\pm$ 6.7	14.398 $\pm$ 0.574 <sup>c</sup>	6.079 $\pm$ 0.213 <sup>c</sup>	---	---

<sup>a</sup>Intact non-thyroidectomized rats (NTX) and thyroidectomized rats (TX) receiving a continuous infusion of thyroid hormone (TX + T<sub>4</sub>/T<sub>3</sub>) were treated for 21 days with 0, 25, 1000, or 3000 ppm FOE 5043 as a dietary admix.

<sup>b</sup>Data represent the mean  $\pm$  standard error for 4-6 rats per dose group.

<sup>c</sup>Indicates statistically significant difference relative to control by one-way analysis of variance and Dunnett's tests;  $p \leq 0.05$ .

### Material and methods:

FOE 5043, batch number: 17001/90, purity: - 93.7% (screening tests); Fl. 036 of July 04, 1991, purity: ~ 97.2% (main test), formulated in acclon/corn oil (to dissolve FOE 5043 prior to mixing with the diet). administration: orally by diet to rats (CDF [F-344]/BR) for 3 weeks or 13 weeks dosage (a.i.): 0 - 400 - 1600 - 3000 ppm (13 weeks), equivalent to: 0, 21.5, 84.3, 144.9 mg/kg bw/day; 0 - 25 - 1000 ppm (3 weeks), equivalent to: 0, 1.6, 70.6 mg/kg bw/day

### Findings:

Dose-related and equivalent declines in total and free serum T<sub>4</sub> levels were measured at weeks 1, 2 and 3 in both TX-T<sub>4</sub>/T<sub>3</sub> and NTX animals. The functional status of the thyroid gland as measured by the capacity of the organ to take up radiolabeled iodide remained unchanged in NTX 1000-ppm FOE 5043-exposed animals. Alterations in serum concentrations of thyrotropin as well as total, free, and reverse T<sub>3</sub> were also noted in both TX + T<sub>4</sub>/T<sub>3</sub>, and NTX animals; however, a compound-related trend was difficult to discern. Dose-related increases in absolute and relative liver weights were measured in both TX + T<sub>4</sub>/T<sub>3</sub> and NTX animals.

<b>Conclusion</b>	As a sole source of thyroid hormone in the TX + T <sub>4</sub> /T <sub>3</sub> animals was that provided by the pump, coupled with the indications of a functionally unimpaired FOE 5043-exposed thyroid gland provided by the iodide uptake test, these data suggest that FOE 5043-induced alterations in serum thyroid hormone levels, most notably T <sub>4</sub> are being mediated indirectly. Specifically, a chemically-induced increase in the hepatic T <sub>4</sub> metabolism, implied by the gross and histopathologic changes in the liver, rather than through a mechanism of direct chemical interference with the synthesizing/secretory functions of the thyroid gland is strongly suggested.
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### Summary of mechanistic study in rats

In a mechanistic study, male rats were provided thyroid hormone replacement therapy via osmotic minipumps and then fed diets of FOE 5043. The data suggested that FOE 5043-induced alterations in serum thyroid hormone levels, most notably serum thyroxine (T<sub>4</sub>), are being mediated indirectly.

Specifically, a chemically-induced increase in hepatic  $T_4$  metabolism, implied by the gross and histopathologic changes in the liver, rather than through a mechanism of direct chemical interference with the synthesis/secreting functions of the thyroid gland is strongly suggested

**B.6.9 Medical data****B.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies**

<b>Report:</b>	<b>KCA 5.9.1 /1; Steffens, W.; 2014;</b>
<b>Title:</b>	Occupational Medical Experiences with Flufenacet
<b>Document No:</b>	<b>M-475871-01</b>
<b>Report No:</b>	na
<b>Guidelines:</b>	Not applicable Deviations: not applicable
<b>GLP</b>	<b>No (Not applicable)</b>

**Materials and methods****A. In-company experience:**

Name:	FOE 5043
Physical state:	Off-white powder and flakes
Production/Processing plant:	Kansas City, Missouri, USA
Number of employees handling the product:	24
Production period:	Since 1997 and ongoing
Amount produced:	500 to 1500 tons/year in one annual campaign
Personal safety measures:	Work clothing, safety shoes, helmet, nitrile chemical protection gloves, goggles, half or full mask with OV/P 100 cartridge

**B. Occupational Medical Experiences**

No. of workers exposed:	24 including HazMat team members
Commenced on:	1997
Examination intervals:	Annually
Medical examinations:	History and full physical examination for HazMat members
Laboratory testing:	FBC, liver enzymes, creatinine, urea, uric acid, Na, K, Ca, Fe, hepatitis B antigen, proteins, urine status for HazMat Team members
Technical examinations:	Audiogram and lung function testing for all workers, stress ECG for HazMat team members every 5 years, ECG annually
Other technical details:	Not applicable

<b>Conclusion</b>	<p><u>In-company experience:</u> There were no unusual occurrences or complaints recorded.</p> <p><u>Medical assessment:</u> Occupational medical surveillance of employees from the Flufenacet plant performed annually since 1997 as described above, not directly related to exposures, did not reveal any unwanted effects in the workers. During the production period since 1997 no accidents with Flufenacet occurred in the workers.</p>
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	<u>No further consultations of the Medical Service due to work or contact with Flufenacet were required.</u>
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### **B.6.9.2 Data collected on humans**

No cases of human poisoning have been reported up to now.

### **B.6.9.3 Direct observations**

Up to now there are no direct observations available.

### **B.6.9.4 Epidemiological studies**

Up to now there are no epidemiological studies available.

### **B.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests**

#### **Signs and Symptoms of Poisoning**

No human poisoning cases have been published; in animal experiment neurotoxicity has been observed, though only after repeated application of high doses.

In humans the formation of methemoglobin and resulting cyanosis can be expected in severe cases.

**Methemoglobinemia** is the oxidation of Fe<sup>++</sup> in hemoglobin to Fe<sup>+++</sup>, which cannot bind nor transport oxygen. Thus methemoglobinemia causes a hypoxemia and consecutively hypoxia in tissues and organs.

Methemoglobin can very easily and quickly be measured with many hemoglobin analysers.

- 10% of methemoglobin will cause bluish-grey cyanosis, best seen on lips, fingertips, and earlobes, but spreading to all of the skin with increasing concentrations.
- 20% and more of methemoglobin will cause signs and symptoms as headache, nausea, vertigo, drowsiness, somnolence, shortness of breath, tachycardia.
- 60-80% of methemoglobin may be fatal.

**Note:** Due to the discoloration of the skin oxygen saturation cannot be measured with fingertip sensors.

**Note:** Due to a competition for metabolic enzymes alcohol greatly increases the formation of methemoglobin.

Therefore any consumption of alcohol is strictly forbidden for 48 hours after the incident.

### **B.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment**

#### **First Aid:**

- Remove patient from exposure/terminate exposure.
- Thorough skin decontamination with copious amounts water and soap, if available with polyethylenglykol 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 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!

**Treatment:**

- Gastric lavage should be considered in cases of significant ingestions within the first (2) hour(s).
- The application of activated charcoal and sodium sulphate (or other cathartic) can be considered in significant ingestions.

As there is no antidote, treatment has to be symptomatic and supportive.

However:

- In case of proven methemoglobinemia:  
The human organism is able to reduce methemoglobin to hemoglobin without further intervention. However, this will take days and thus is not feasible in significant intoxications. Therapy will aim at increasing oxygen transport and reversing the hemoglobin oxidation/reducing Fe<sup>+++</sup> to Fe<sup>++</sup>.

Methemoglobin should be measured before and during therapy (most hemoglobin analysers can measure methemoglobin).

- If *methemoglobin level is less than 20%*, administer 100% oxygen; additionally 1g of ascorbic acid (vitamin C) may be given orally or intravenously. The reducing effect of vitamin C is weak, but in these cases sufficient.
- If *methemoglobin level is greater than 20%* treat with 100% oxygen and administer a reducing agent: Methylene Blue or Toluidine Blue. These will be effective within 10-20 minutes. Additionally high doses (> 1g) of ascorbic acid/vitamin C intravenously can be considered.

- **Methylene Blue:**

- 1% solution (10 mg/mL) intravenously at 0.1-0.2 mL/kg body weight (1-2 mg/kg bw) during ca. 5 minutes.
- A 60 kg person would thus receive 6 to 12 mL Methylene Blue 1% intravenously.
- If required this dose may be repeated after 30 minutes.
- The maximum daily dose is 7 mg/kg bw.

- **Toluidine Blue:**

- 3% solution (30 mg/ml) intravenously at 0.07 to 0.13 mL/kg bw (2-4 mg/kg bw).
- A 60 kg person would thus receive about 4 to 8 mL Toluidine Blue 3% intravenously.
- If required this dose may be repeated after 30 minutes.

**Note:** Both Methylene Blue and Toluidine Blue can cause methemoglobinemia themselves in case of overdose.

A known deficiency of G-6-PDH is a contraindication against both drugs.

Paravenous injection has to be avoided as it can cause severe tissue necrosis.

### **B.6.9.7 Expected effects of poisoning**

After strong intoxication cyanosis due to methemoglobinemia is expected based on animal data.

## Overall summary and conclusion

The following overall summary is taken from the Monograph amended by the new information of this supplemental dossier. New information is written in bold letters. The code “FOE 5043” of the active substance has been replaced by its common name “flufenacet” where appropriate.

The biokinetic and metabolism study on rats showed a high degree of absorption of radioactivity followed by fast elimination from the body. After oral administration of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 more than 87% of the recovered radioactivity was excreted via urine and faeces within 72 hours in all dose groups tested. The plasma curve analysis after dosing of [fluorophenyl-UL-<sup>14</sup>C]- and [thiadiazole-2-<sup>14</sup>C]-labelled FOE 5043 revealed that only the fluorophenyl part of the molecule underwent enterohepatic circulation. Absorption commenced immediately after administration. The concentration in the different organs and tissues were relatively low and showed only slight differences with respect to dose and sex.

The identification rate ranged from 60 to 75% of the recovered radioactivity in the experiments with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 and was 92% on average in the experiments with [thiadiazole-2-<sup>14</sup>C]FOE 5043. After application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 all metabolites identified contained only the fluorophenyl moiety of the active ingredient, because the thiadiazole ring was cleaved off prior to further metabolism. This was confirmed by the results obtained after application of [thiadiazole-2-<sup>14</sup>C]FOE 5043. The major metabolites were the glucuronic acid of thiadone (M24), the oxalylacetic acid conjugate of thiadone (M26) and free thiadone (M09).

Glutathione conjugation appeared to be the major, and possibly the exclusive, metabolic pathway for [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in rats. Although the glutathione itself was not detected, the presence of a variety of glutathione-derived metabolites provided sufficient evidence for the glutathione pathway. Almost all metabolites identified were glutathione related compounds. The major metabolite in all dose groups was the N-acetylcysteine conjugate of fluorophenylacetanilide (M10).

For a better understanding of the biokinetic behaviour and metabolism of some FOE 5043 plant metabolites, the bioavailability of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043-oxalate as well as [thiadiazole-2-<sup>14</sup>C]-N-glucoside was investigated after oral administration to rats. Both compounds were excreted unchanged with urine and faeces. Due to the extremely low residues in tissues and carcass, there should be no detectable residues in animal tissues neither from the acetamide moiety nor from the thiadiazole moiety of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues.

An additional metabolism study with [thiadiazole-5-<sup>14</sup>C]flufenacet revealed an almost complete excretion of the radiolabel 48 hours after oral administration at a dose level of 1 mg/kg bw. The renal route was the predominant excretion route. Chromatographic profiling of the radioactive residues in the urine yielded a less polar metabolite at a portion of 6.5% of the dose. It was identified as thiadone. An additional very polar metabolite was identified as trifluoroacetate. It amounted to approx. 10% of the oral dose. This metabolite was also identified in the plasma. It can therefore be concluded that the trifluoroacetate metabolite is covered in toxicity studies of the parent substance flufenacet in the rat.

**Flufenacet** was found to have a low to moderate order of acute toxicity when administered orally in mice and rats. Non-specific clinical signs of toxicity were observed on the day of dosing and included

ataxia, labored breathing, decreased activity and, lacrimal, nasal, and perianal staining. All deaths occurred on days 0-5. The principal clinical signs in surviving animals resolved within a few days after dosing.

A low order of acute toxicity was demonstrated in acute dermal and inhalation toxicity studies. Clinical signs, but no mortalities, were seen at the limit dose, 2000 mg/kg, in the dermal toxicity study. Four-hour inhalation exposure to a liquid aerosol containing **flufenacet** at a concentration of 3,740 mg/m<sup>3</sup> produced clinical symptoms, but no mortalities. Thus, by the routes of exposure relevant to workers, **flufenacet** has a low order of acute toxicity.

Eye and skin irritation studies also demonstrated favorable characteristics. **Flufenacet** is not irritating to skin and essentially non-irritating to eyes. The results of the dermal sensitization study revealed equivocal evidence of allergenic potential. **Both** maximization tests **were** positive; the more practice relevant Buehler test was negative **as well as the Local Lymph Node assay on mice. Furthermore, flufenacet does not show a phototoxic potential.**

The summary table on acute toxicity studies presented in the monograph (Table 5.10.1a) has been reformatted and updated with the results of the new studies conducted with flufenacet of this supplemental dossier, please refer to Table 5- 2.

**Table 5- 2: Summary of acute toxicity studies\***

Route/Study	Species	Sex	Results	Reference
Oral <sup>1)</sup>	Rat	M F	LD <sub>50</sub> : 1617 mg/kg bw 589 mg/kg bw	██████████, 1993 M-004865-02-1
Oral <sup>2)</sup>	Rat	M	LD <sub>50</sub> : 683 mg/kg bw	██████████, 1992 M-004864-01-1
Oral	Mouse	M F	LD <sub>50</sub> : 1331 mg/kg bw 1756 mg/kg bw	██████████, 1991 M-004850-01-1
Dermal	Rat	M F	LD <sub>50</sub> : >2000 mg/kg bw >2000 mg/kg bw	██████████, 1992 M-004843-01-1
Inhalation (aerosol, 4h)	Rat	M F	LC <sub>50</sub> : >3740 mg/m <sup>3</sup> >3740 mg/m <sup>3</sup>	██████████, 1990 M-004844-01-1
Skin irritation	Rabbit	M	Not irritating	██████████, 1992 M-004846-01-1
Eye irritation	Rabbit	M	Not irritating	██████████, 1992 M-004847-01-1
Skin sensitisation Buehler method	Guinea pig	M	Not sensitizing	██████████, 1992 M-004845-01-1
Skin sensitisation M&K method	Guinea pig	M	Sensitizing	██████████, 1994 M-004637-01-1
<b>Skin sensitisation M&amp;K method</b>	<b>Guinea pig</b>	<b>F</b>	<b>Sensitizing</b>	<b>██████████, 1995 M-004677-01-1</b>
<b>Skin sensitization Local lymph node assay</b>	<b>Mouse</b>	<b>F</b>	<b>Not sensitizing</b>	<b>██████████, 2004 M-090513-01-1</b>
<b>In vitro 3T3 NRU phototoxicity test</b>	<b>BALB/c 3T3 cells</b>		<b>Not phototoxic</b>	<b>Heppenheimer, 2013 M-464615-01-1</b>

\* New studies, i.e. studies previously not submitted, are written in bold  
M = male, F= female; <sup>1)</sup> animals were fasted (overnight); <sup>2)</sup> animals were non-fasted

The subacute dermal toxicity study on rats demonstrated that **flufenacet** was moderately toxic after repeated dermal administration. The liver was the primary target organ with secondary effects on thyroid hormone levels. Increased liver weights with correlative histopathological findings of

centrilobular hepatocytomegaly, and decreased thyroxin (T4) and free thyroxin levels were observed in the subacute dermal toxicity study.

Mechanistic studies on thyroid effects suggested that the changes in serum hormone levels of T4 are being mediated indirectly through an increase in the biotransformation and excretion of thyroid hormone in the liver. Thus, the functional status of the thyroid and pituitary gland are not affected by treatment with flufenacet.

**The liver was also the primary target organ after subacute (5x 6hours and 20x 6hours) inhalation exposure with secondary effects on the thyroid hormone levels. Increased liver weights with correlating clinical- and histopathological findings were observed. The inhalation toxicity studies revealed also alterations in the nasal cavity and larynx, in kidney-, hematologic/spleen-, and thyroid-related endpoints.**

The two-generation study with **flufenacet** revealed no evidence of reproductive toxicity. Dose levels including levels overtly toxic to parental animals had no effect on gonadal function, estrous cycles, mating behavior, conception, parturition, lactation, weaning, and the off-spring's ability to achieve adulthood and successfully reproduce. The study unequivocally demonstrated that **flufenacet** is not a reproductive toxin.

Teratology/embryotoxicity studies using rats and rabbits revealed no evidence of teratogenicity or embryotoxicity. At maternally toxic dose levels, reduced fetal bodyweights, and increased incidences of delayed ossification and skeletal variation were observed. Thus, **flufenacet** is not teratogenic or embryotoxic and it does not cause primary fetotoxicity.

Mutagenicity studies with **flufenacet** were consistently negative. Point mutation assays in bacteria and mammalian cells revealed no evidence of mutagenic potential. In vitro and in vivo cytogenetic studies revealed no evidence of clastogenicity, and an unscheduled DNA synthesis assay using primary rat hepatocytes revealed no evidence of genotoxic activity. Thus, **flufenacet** is not mutagenic, clastogenic or genotoxic.

**The summary of results on genotoxicity presented in the monograph in summary “Table 5.10.1b” has been reformatted and updated with the results of the new studies conducted with flufenacet of this supplemental dossier, please refer to Table 5- 3.**

**Table 5- 3: Summary of genotoxicity testing\***

Study	Test system	Results		Reference
		activation	non-activation	
<i>In-vitro</i>				
Bacterial reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	negative	negative	Herbold, 1995 <a href="#">M-004696-01-1</a>
Bacterial reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	negative	negative	Sokolowski, 2010 <a href="#">M-395211-01-1</a>
Mammalian cell gene mutation test (HGPRT)	Chinese hamster lung fibroblasts V79	negative	negative	Brendler-Schwaab, 1994 <a href="#">M-004634-01-1</a>
Mammalian chromosome aberration test	Chinese hamster ovary cells CHO	negative	negative	Gahlmann, 1995 <a href="#">M-004692-01-1</a>
Unscheduled DNA synthesis (UDS) assay	Primary rat hepatocytes	negative	negative	Brendler-Schwaab, 1992 <a href="#">M-004577-01-1</a>
<i>In-vivo</i>				
Micronucleus test	Mouse bone marrow	negative		<div></div> , 1993 <a href="#">M-004588-01-1</a>

\* New studies, i.e. studies that were not previously submitted, are written in bold

Subchronic and chronic feeding studies revealed similar findings in mice, rats, and dogs. The primary toxicological effects observed in all three species after long-term exposure involved structural and/or functional alterations in liver-, kidney-, hematologic/spleen-, and thyroid-related endpoints. Eye effects were also observed and included cataracts in mice and rats, scleral mineralization in rats, and vacuolization of the ciliary body epithelium and cystic vacuolization of the peripheral optic retina in dogs. As discussed below, an increased incidence of axonal swelling was observed in the brain and spinal cord of rats and dogs exposed to high levels of **flufenacet** which saturate metabolic pathways.

Oncogenicity studies in mice and rats revealed no evidence of oncogenic potential. No treatment-related increased incidences of benign or malignant neoplastic changes were observed in any tissue at any dose level in either species. **Flufenacet** is not oncogenic or carcinogenic.

The neurotoxic potential of **flufenacet** has been thoroughly investigated and well characterized in studies using mice, rats and dogs. The neuropathological changes as assessed by both light and electron microscopy examinations appear to be metabolic lesions. In animals chronically exposed to high dose levels of **flufenacet**, similar lesions were observed in several high-oxygen demand tissues, the eye, brain and kidney. The data, taken collectively, demonstrate that these pathologic changes are due to limitations in glutathione interdependent pathways and antioxidant stress. Toxicokinetic data from the chronic dog study demonstrated saturation of metabolic pathways at the mid and high dose levels where these changes were observed. The pathological changes observed in the brain and spinal cord of **flufenacet**-treated animals primarily consisted of an increased incidence or exacerbation of a morphological change (i.e., axonal swelling) occurring spontaneously in untreated animals. Thus, prolonged exposure to high dose levels of **flufenacet** which saturate metabolic pathways causes a slight increase in the incidence of a normal morphologic change.

**A developmental neurotoxicity study was conducted based on thyroid-related findings and therefore, the potential for affecting development of the nervous system. In this study flufenacet did not cause any neurotoxic effect in parental and offspring animals. Treatment-related findings consisted of reduced food consumption and a reduction in maternal body weights during gestation and in males at mid and high-dose. Body weights were also reduced in mid- and**

high-dose F1-offspring and secondary to the lower body weights the F1 offspring exhibited a delay in development (eye opening, preputial separation).

Comparative thyroid sensitivity assays with flufenacet in neonatal and adult (pregnant and lactating) female rats did not give any indication for neonatal susceptibility to thyroid-related neurodevelopmental effects. Dietary exposure during pregnancy revealed no adverse effects in dams and foetuses at any dose level.

Dietary exposure during pregnancy and lactation until post natal day 21 induced only a slight decrease in maternal body weight gain resulting in lower body weight and decreases in T4 and T3 with thyroid follicular cell hypertrophy in two dams. In post natal day 21 pups, high-dose flufenacet revealed reduced body weight gain resulting in lower body weight and slightly lower T3 values in male and female pups.

Flufenacet administration once daily by gavage in pre-weaning rats (PND 10-20) of 1.7 mg/kg bw/day had no effect on the thyroid or any other endpoint measured.

Toxicological studies conducted with FOE 5043-hydroxy, FOE 5043-(TDA)-sulfone and FOE-acetate are considered supportive to justify the limits of specified impurities.

During the previous EU review, the toxicological properties of the plant and/or soil metabolites (FOE-oxalate (M01), FOE-sulfonic acid (M02), FOE-thioglycolate sulfoxide (M04), and thiadone (M09)) were investigated in acute oral toxicity to rats and/or mutagenicity and/or their bioavailability in rats.

The genotoxic properties of several metabolites (FOE-oxalate (M01), FOE-sulfonic acid (M02), FOE-methylsulfone (M07), FOE 5043-trifluoroethanesulfonic acid (M44) and trifluoroacetate (TFA) (M45)) were further investigated in the recommended *in vitro* and if necessary *in vivo* genotoxicity assays. Overall, all metabolites are considered to be non-genotoxic.

In addition, TFA (M45) is of low acute toxicity with a LD50 above 2000 mg/kg bw without any evidence of acute effects based on clinical signs and necropsy findings. After repeated administration the liver was the target organ, with effects that were adaptive and reversible. Moreover, the 14-day mechanistic study showed that liver effects are related to peroxisome proliferation, a mode of action not relevant for humans. Furthermore, the developmental toxicity study in rats showed neither maternal nor developmental effects which are considered to be adverse up to the highest dose tested.

A toxicological assessment of several metabolites based on commonality assessments, structure similarity considerations, evaluation of genotoxicity and further toxicological studies as well as exposure calculations revealed that all plant metabolites are considered to be toxicologically adequately investigated and uncritical for human health.

The summary table “Table 5.10.1b” presented in the monograph has been reformatted and updated in order to provide an overview of the NO(A)ELs and main findings at the LO(A)EL in toxicity studies conducted with flufenacet relevant for setting of reference values, please refer to Table 5- 4.

## Reference values

During the previous evaluation for Annex I listing of flufenacet, reference values were based on a comprehensive toxicological database. Over the past years the toxicological database of flufenacet has

been updated with a number of OECD and US EPA guideline studies. During the previous evaluation the study endpoints were established as no-observed effect levels (NOELs) whereas, for the more recently conducted toxicological studies no-observed adverse effect levels (NOAELs) are established.

**Table 5- 4: Summary of NOAELs and main findings at LOAEL in toxicity studies relevant for setting reference values**

Study	Sex	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Main findings seen at LO(A)EL	Reference
Rat 21-day dermal	M F	1000 1000	-- --	No adverse effects noted. T4 ↓, liver findings considered adaptive response to treatment.	██████████, 1995 <a href="#">M-004981-01-1</a>
<b>Rat 1-week (5x6h) inhalation</b>	M, F	<b>~14 48 mg/m<sup>3</sup></b>	<b>~66 225 mg/m<sup>3</sup></b>	<b>T4 ↓ Liver: rel. weight ↑</b>	██████████, 2008 <a href="#">M-300005-01-1</a>
<b>Rat 4-week (20x6h) inhalation</b>	M, F	<b>~7 19 mg/m<sup>3</sup></b>	<b>~81 220 mg/m<sup>3</sup></b>	<b>HB ↓, HCT ↓, RETI ↑, HEINZ ↑, AP ↓, TG ↓, Liver: enzymes ↑, rel. weight ↑, spleen: weight ↑, histopathological changes in nasal cavity and larynx, spleen, testes, thyroid, liver</b>	██████████, 2008 <a href="#">M-302961-01-1</a>
Rat acute neuro- toxicity, oral	M F	75 50	200 75	Unspecific clinical signs (uncoordinated gait, decreased activity) NOEL neurotoxicity 450/150 mg/kg bw (males/females highest doses tested with survivors).	██████████, 1995 (amended 1998) <a href="#">M-004986-02-1</a>
Rat 90-day neurotoxicity feeding	M F	7.3 8.4	38 43	Microscopic lesions in brain and spinal cord (increased incidence of swollen axons in the cerebellum-medulla oblongata) NOEL neurobehavioral effects: 38/43 mg/kg bw/d	██████████., 1995 <a href="#">M-005014-01-2</a>
Rat 90-day feeding	M F	-- <sup>a)</sup> 7.2	6.0 29	HB ↓, T4 ↓, GLUC ↓, Liver: weight ↑, hepatocellular swelling, cell degeneration or necrosis; spleen: brown granular pigment accumulation within red pulp; kidney: mild renal proximal tubule injury	██████████, 1995 <a href="#">M-004999-01-1</a>
Rat 2-year feeding	M F	1.2 1.5	19 24	BWG↓, structural and/or functional alterations in liver-, kidney-, haematopoietic-, thyroid-related endpoints.	██████████, 1995, <a href="#">M-005062-02-1</a>
Rat oral (gavage) developmental	Dam Fetal	25 25	125 125	Maternal: BW ↓, food consumption ↓ Fetal: BW ↓, delayed ossification and/or skeletal variation ↑ in some skeletal elements	██████████, 1995 <a href="#">M-004976-02-1</a>
Rabbit oral (gavage) developmental	Dam Fetal	25 25	25 125	Maternal: soft stool, BW gain ↓ during treatment, histopathological changes of the liver Fetal: skeletal variation ↑	██████████, 1995 <a href="#">M-004979-01-1</a>
Rat 2-generation feeding	M F	<b>37.4 8.2</b>	37 41	BW ↓ in P females during pre-mating No reproductive effects observed at any dose level.	██████████, 1995 <a href="#">M-004984-03-1</a>
<b>Rat developmental neurotoxicity feeding</b>	<b>Dam Pup</b>	<b>1.7/3.0 (DG 6-21/DL 1-12)</b>	<b>8.3/15</b>	<b>Dam: BW ↓, food intake ↓ (gestation) Pup: BW/BWgain ↓, rel. food intake ↑, delayed development (eye opening, preputial separation)</b>	██████████, 2000 <a href="#">M-026105-01-1</a>
<b>Rat, mechanistic study thyroid feeding</b>	<b>Dam Fetal</b>	<b>35 35 (DG 6-20)</b>	<b>-- --</b>	No adverse effects observed at any dose level.	██████████, 2012 <a href="#">M-435619-01-1</a>
<b>Rat, mechanistic study thyroid feeding</b>	<b>Dam Pup</b>	<b>13 13 (DG 6 - DL 4/DL 21)</b>	<b>65 65</b>	<b>Dam: BW gain ↓, T4/T3 ↓, Liver: rel. weight ↑, thyroid: follicular cell hypertrophy in 2 of 13 dams Pup: BW ↓</b>	B██████████, 2012 <a href="#">M-435313-01-1</a>



Study	Sex	NOAEL mg/kg bw/day	LOAEL	Main findings seen at LO(A)EL	Reference
Dog 90-day feeding	M F	1.7 1.7	7.2 6.9	ALAT ↓, LDH ↑, albumin ↓, globulin ↑, T4 ↓, GLUC ↓, Spleen: pigment, kidney: rel. weight ↑	██████████, 1995 <a href="#">M-004977-02-1</a>
Dog 1-year feeding	M F	1.3 1.1	28 27	Hb ↓, Hct ↓, MCV ↓, MCH ↓, MCHC ↓, CHOL ↑, GLUC ↓, T4/T3 ↓, ALAT ↓, AP ↑, albumin ↓, Liver, heart, kidney: abs. + rel. weight ↑	██████████, 1995, 1997 <a href="#">M-005001-02-2</a>
Mouse 90-day feeding	M F	18 25	64 91	T4 ↓ Liver: rel. weight ↑	██████████ 1995 <a href="#">M-004985-01-1</a>
Mouse 20-month feeding	M F	7.4 9.4	30 77	MethHb ↑ Ocular cataracts ↑	██████████, 1995 <a href="#">M-005060-02-1</a>

<sup>a)</sup> The subchronic NOEL for males was established on the basis of the toxicity profile which emerged through the first year of the 2-year rat study.

M = male, F = female, ↑ = increase, ↓ = decrease, DG = Day of gestation, DL = day of lactation

BW = body weight,

### Acceptable Daily Intake (ADI) derivation

At Annex I inclusion for flufenacet an ADI of 0.005 mg/kg bw/day was set based on an increased incidence of a spontaneous background lesion in the kidneys observed at the LOAEL of 1.2 mg/kg bw/day of the 2-year rat study by using a safety factor of 250 (Review Report for flufenacet 7469/VI/98- Final, 3 July 2013). This finding was not considered as adverse.

Flufenacet is not a reproductive or developmental toxicant and it is not mutagenic or carcinogenic. It does induce neurotoxicity, but only after prolonged, repeated exposures to high dose levels exceeding animal's capacity to rapidly metabolize and eliminate it. Clear threshold exists for all toxicological effects observed in studies with flufenacet. The more recently conducted studies in rats did not reveal lower NOAELs or more sensitive endpoints. **Therefore, the rationale for the establishment of the ADI has not changed.**

### Acceptable Operator Exposure Level (AOEL) derivation

At Annex I inclusion for flufenacet an AOEL of 0.017 was set based on the NOEL of 1.7 mg/kg bw/day of the 90-day and 1-year toxicity studies in dogs by using a safety factor of 100 (Review Report for flufenacet 7469/VI/98- Final, 3 July 2013).

However, according to the monograph the AOEL was derived from the NOEL of 1.7/1.67 mg/kg bw/day established after 1 year exposure to flufenacet in the chronic rat study and derived from the 90-day dog study, respectively. The NOELs were based on minimal lower hemoglobin and thyroxine (T4) concentrations in rats and changes in clinical chemistry and higher relative kidney weight in dogs at the respective LOELs of 6.0 or 6.9 mg/kg bw/day. These findings observed at the LOELs were considered adaptive changes due to primary effects on the liver and resulting in secondary effects.

Due to the almost complete absorption of flufenacet from the gastrointestinal tract a correction for oral bioavailability is not needed.

**Since no lower NOELs were determined in the more recently conducted studies, the systemic AOEL of 0.017 mg/kg bw/day is still considered to be a valid value for the protection of**

**operators with regard to the exposure to flufenacet.**

### **Acute Reference Dose (ARfD) derivation**

At Annex I inclusion for flufenacet an ARfD of 0.017 mg/kg bw was set based on the NOEL of 1.7 mg/kg bw/day of the 90-day and 1-year toxicity studies in dog by using a safety factor of 100 (Review Report for flufenacet 7469/VI/98- Final, 3 July 2013). However, no rational can be found for the selection of these study end points in the monograph or in the review report. Obviously the same rational as for the AOEL derivation was used for setting an ARfD.

**B.6.10. References relied on****LITERATURE SEARCH DATA**

For flufenacet and its metabolites, a total of 3489 references were identified and evaluated for potential relevance. No publication has been identified which would indicate that a side-effect on human health, the environment and non-target species may exist, which would require to adapt any of the risk assessments in the flufenacet supplementary (renewal) dossier.

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection on claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1 /03	██████████ ██████████ ██████████ ██████████	2010	The metabolism of FOE 5043 in rats - Amendment no. 1 to final report ████████████████████ ████████████████████ ████████████████████, Report No.: 106665-1, Edition Number: M-384235-01-1 Date: 2010-06-25 GLP/GEP: yes, unpublished ...also filed: KCA 5.1.1 /02	Y	Y	Evaluation of pharmacokinetic data; new EU data requirement	Bayer CropScience
KCA 5.1 /04	██████████	2012	[Thiadiazole-5-14C]flufenacet: Supportive experiment for the identification of metabolites in the urine of the rat - Final report ████████████████████ No.: EnSa-12-0439, Edition Number: M-441499-01-1 Date: 2012-11-07 GLP/GEP: yes, unpublished ...also filed: KCA 5.1.1 /03	Y	Y	Investigation of occurrence in rat of major plant metabolite identified with new label	Bayer CropScience
KCA 5.1.1 /01	██████████ ██████████ ██████████	1995a	The metabolism of FOE 5043 in rats ████████████████████ ████████████████████ ████████████████████, Report No.: MR106665, Edition Number: M-002247-01-1 Date: 1995-02-17 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection on claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /02	██████████ ██████████ ██████████ ██████████	2010	The metabolism of FOE 5043 in rats - Amendment no. 1 to final report ████████████████████ ████████████████████ ████████████████████, Report No.: 106665-1, Edition Number: M-384235-01-1 Date: 2010-06-25 GLP/GEP: yes, unpublished <b>...also filed: KCA 5.1 /03</b>	Y	Y	Evaluation of pharmacokinetic data; new EU data requirement	Bayer CropScience
KCA 5.1.1 /03	██████████	2012	[Thiadiazole-5-14C]flufenacet: Supportive experiment for the identification of metabolites in the urine of the rat - Final report ████████████████████, Report No.: EnSa-12-0439, Edition Number: M-441499-01-1 Date: 2012-11-07 GLP/GEP: yes, unpublished <b>...also filed: KCA 5.1 /04</b>	Y	Y	Investigation of occurrence in rat of major plant metabolite identified with new label	Bayer CropScience
KCA 5.1.1 /04	██████████	2014	[Thiadiazole-5-14C]flufenacet: Metabolic stability and profiling in liver microsomes from rats and humans for inter-species comparison ████████████████████ ████████████████████ ████████████████████, Report No.: EnSa-13-0826, Edition Number: M-475336-01-1 Date: 2014-01-22 GLP/GEP: yes, unpublished	Y	Y	New EU-registration requirement	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection on claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.1 /01	██████████ ██████████ ██████████ ██████████	1991	Experimental acute oral toxicity study with technical grade FOE 5043 in mice - Supplemental submission to EPA MRID no. 43850010 - Bayer Corporation Agriculture Division report no. 101914 ████████████████████ ████████████████████ ████████████████████ Report No.: BC6013, Edition Number: M-004850-01-1 EPA MRID No.: 43850010 Date: 1991-09-25 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.1 /02	██████████	1992	Acute oral toxicity study with FOE 5043 in nonfasted male rats - Supplemental submission to EPA MRID no. 43850008 - Bayer Corporation Agriculture Division report no. 102671 ████████████████████ ████████████████████ ████████████████████ Report No.: BC6686, Edition Number: M-004864-01-1 EPA MRID No.: 43850008 Date: 1992-04-23 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.1 /03	██████████ ██████████ ██████████ ██████████	1993	Acute oral toxicity study with technical grade FOE 5043 in rats ████████████████████ ████████████████████ ████████████████████ Report No.: BC6916, Edition Number: M-004865-02-1 Date: 1993-01-20 ...Amended: 1996-06-11 GLP/GEP: yes, unpublished	Y			Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection on claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.2 /01	██████ ██████ ██████ ██████	1992	Acute dermal toxicity study with technical grade FOE 5043 in rats - Supplemental submission to EPA MRID no. 43441106 - Bayer Corporation Agriculture Division report no. 100656 ████████████████████ ████████████████████ ████████████████████ No.: BC5477, Edition Number: M-004843-01-1 EPA MRID No.: 43441106 Date: 1992-03-31 GLP/GEP: yes, unpublished	Y			Bayer CropScience
KCA 5.2.3 /01	████████ ██	1990	Acute four-hour inhalation toxicity study with technical grade FOE 5043 in rats ████████████████████ ████████████████████ ██████ ████████████████████, Report No.: BC5362, Edition Number: M-004844-01-1 Date: 1990-10-26 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.4 /01	██████ ██████ ██████ ██████	1992a	Primary dermal irritation study with technical grade FOE 5043 in rabbits ████████████████████ ████████████████████ ██████ ████████████████████, Report No.: BC6350, Edition Number: M-004846-01-1 Date: 1992-01-23 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.5 /01	████████ ██ ████████ ██	1992b	Primary eye irritation study with technical grade FOE 5043 in rabbits ████████████████████ ████████████████████ ████████████████████, Report No.: BC6349, Edition Number: M-004847-01-1 Date: 1992-01-27 GLP/GEP: yes, unpublished	Y			Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection on claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.6 /01	[REDACTED]	1994	FOE 5043 - Study for the skin sensitization effect in guinea pigs (Maximization test of Magnusson and Kligman) [REDACTED] [REDACTED] [REDACTED], Report No.: 23560, Edition Number: M-004637-01-1 Date: 1994-12-16 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.6 /02	[REDACTED] [REDACTED] [REDACTED] [REDACTED]	1992c	Dermal sensitization study with technical grade FOE 5043 in guinea pigs [REDACTED] [REDACTED] [REDACTED], Report No.: BC3991, Edition Number: M-004845-01-1 Date: 1992-03-31 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.2.6 /03	[REDACTED]	1995	FOE 5043 - Study for the skin sensitization effect in guinea pigs (Maximization test Magnusson and Kligman) [REDACTED] [REDACTED] [REDACTED], Report No.: 23924, Edition Number: M-004677-01-1 Date: 1995-04-11 GLP/GEP: yes, unpublished	Y	Y	Confirmation of end point/study result	Bayer CropScience
KCA 5.2.6 /04	[REDACTED]	2004	FOE 5043 - Local lymph node assay in mice (LLNA/IMDS) [REDACTED] [REDACTED] [REDACTED], Report No.: AT01491, Edition Number: M-090513-01-1 Date: 2004-09-21 GLP/GEP: yes, unpublished	Y	Y	Confirmation of end point/study result by a new study type	Bayer CropScience

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KCA 5.2.7 /01	Heppenheim er, A.	2013	Flufenacet TC: Cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1561200, Edition Number: M-464615-03-1 Date: 2013-09-12 ... <b>Amended: 2013-12-18</b> GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience
KCA 5.3.2 /01	██████████ ██████████ ██████████	1995a	FOE 5043: 13-week subchronic feeding study in beagle dogs ████████████████████ ████████████████████ ████████████████████, Report No.: BC7563, Edition Number: M-004977-02-1 Date: 1995-03-28 ... <b>Amended: 1997-07-18</b> GLP/GEP: yes, unpublished	Y			Bayer CropScience
KCA 5.3.2 /02	██████████ ██████████ ██████████ ██████████	1995a	Technical grade FOE 5043: A 13-week range-finding toxicity study in the mouse ████████████████████ ██████████ ████████████████████, Report No.: BC7720, Edition Number: M-004985-01-1 Date: 1995-07-17 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.3.2 /03	██████████ ██████████ ██████████ ██████████	1995b	Technical grade FOE 5043: A subchronic toxicity testing in the rat ████████████████████ ██████████ ████████████████████, Report No.: BC7733, Edition Number: M-004999-01-1 Date: 1995-07-19 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience



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KCA 5.3.2 /04	██████████ ██████████ ██████████	1995b	Technical grade FOE 5043 : A chronic toxicity feeding study in the Beagle dog ██ ██████████ ██ Report No.: BC7779, Edition Number: M-005001-02-2 Date: 1995-09-20 ...Amended: 1997-07-18 GLP/GEP: yes, unpublished	Y			Bayer CropScience
KCA 5.3.2 /05	Bongartz, R.	2012	Expert statement (non GLP) - Flufenacet (FOE 5043): Explanation of the chromatographic behaviour of FOE-thiadone in the extract of brain from dogs of the chronic feeding study Bayer CropScience, Report No.: EnSa-12/0266, Edition Number: M-430840-02-1 Date: 2012-05-08 ...Amended: 2012-07-04 GLP/GEP: no, unpublished	N	Y	Supplementary study information	Bayer CropScience
KCA 5.3.3 /01	██████████ ██████████ ██████████ ██████████	1995	Repeated dose 21-day dermal toxicity study with technical grade FOE 5043 in rats ██ ██████████ ██ Report No.: BC7682, Edition Number: M-004981-01-1 Date: 1995-09-29 GLP/GEP: yes, unpublished	Y			Bayer CropScience

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KCA 5.3.3 /02	████████	2008a	Flufenacet (FOE 5043) - 1-week inhalation pilot study in Wistar rats (exposure 6h/day, 5 days/week) ████████ ████████ ████████, Report No.: AT04505, Edition Number: M-300005-01-1 Date: 2008-04-10 GLP/GEP: no, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience
KCA 5.3.3 /03	████████	2008b	Flufenacet (FOE 5043) - 4-week subacute inhalation study in Wistar rats (exposure 6h/day, 5 days/week on four consecutive weeks) ████████ ████████ ████████, Report No.: AT04589, Edition Number: M-302961-01-2 EPA MRID No.: 47473101 Date: 2008-05-28 GLP/GEP: yes, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience
KCA 5.4.1 /01	Brendler-Schwaab, S.	1994	FOE 5043 - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 23538, Edition Number: M-004634-01-1 Date: 1994-12-09 GLP/GEP: yes, unpublished	N	Y	Guide line requirement	Bayer CropScience

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KCA 5.4.1 /02	Brendler-Schwaab, S.	1992	FOE 5043 - Test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 21885, Edition Number: M-004577-01-1 Date: 1992-12-03 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 5.4.1 /03	Herbold, B.	1995	FOE 5043 - Salmonella/microsome test plate incorporation and preincubation method Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 23948, Edition Number: M-004696-01-1 Date: 1995-04-24 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 5.4.1 /04	Gahlmann, R.	1995	FOE 5043 - In vitro mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 24340, Edition Number: M-004692-01-1 Date: 1995-10-04 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 5.4.1 /05	Sokolowski, A.	2010	Salmonella typhimurium reverse mutation assay with flufenacet techn. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1370100, Edition Number: M-395211-01-1 Date: 2010-11-18 GLP/GEP: yes, unpublished	N	Y	Requested by non-EU authorities ; SANCO/105 97/20 03 -rev. 10.1	Bayer CropScience

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KCA 5.4.2 /01	██████████ ████	1993	FOE 5043 - Micronucleus test on the mouse ████████████████████ ██████████ ████████████████████, Report No.: 22384, Edition Number: M-004588-01-1 Date: 1993-07-14 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.5 /01	██████████ ████ ██████████ ████	1995c	Technical grade of FOE 5043: An oncogenicity toxicity testing study in the mouse ████████████████████ ██████████ ████████████████████, Report No.: BC7795, Edition Number: M-005060-02-1 Date: 1995-10-03 ...Amended: 1996-06-14 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.5 /02	██████████ ████ ██████████ ████	1995d	Technical Grade FOE 5043: A combined chronic toxicity/oncogenicity testing study in the rat ████████████████████ ██████████ ████████████████████, Report No.: BC7798, Edition Number: M-005062-02-1 Date: 1995-10-03 ...Amended: 1997-06-30 GLP/GEP: yes, unpublished				Bayer CropScience
KCA 5.6.1 /01	██████████ ██████ ██████████ ████	1995	A two-generation dietary reproduction study in rats using technical grade FOE 5043 ████████████████████ ██████████ ████████████████████, Report No.: BC7695, Edition Number: M-004984-03-1 Date: 1995-06-19 ...Amended: 1997-07-15 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience

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KCA 5.6.2 /01	[REDACTED]	1995	A developmental toxicity study with orally administered FOE 5043 technical in the rat [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] Report No.: BC7471, Edition Number: M-004976-02-1 Date: 1995-01-10 ...Amended: 1997-07-21 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.6.2 /02	[REDACTED]	1995	A developmental toxicity study with orally administered FOE 5043 technical in the rabbit [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] Report No.: BC7661, Edition Number: M-004979-01-1 Date: 1995-05-22 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.7.1 /01	[REDACTED]	1995	An acute oral neurotoxicity screening study with technical grade FOE 5043 in Fischer 344 rats [REDACTED] [REDACTED] [REDACTED] [REDACTED] Report No.: BC7709, Edition Number: M-004986-02-1 Date: 1995-07-10 ...Amended: 1998-03-18 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience

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KCA 5.7.1 /02	██████████ ██████████ ██████████ ██████████ ██████████	1995	A subchronic dietary neurotoxicity screening study with technical grade Thiafluamide (FOE 5043) in Fischer 344 rats ██ ██████████ ██ Report No.: BC7796, Edition Number: M-005014-01-2 Date: 1995-10-09 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.7.1 /03	██████████ ██████████	2000	Developmental neurotoxicity study of technical grade Flufenacet administered orally via diet to CrI:CD BR VAF/Plus presumed pregnant rats ██ ██ ██ Bayer CropScience, Report No.: BC9333, Edition Number: M-026105-01-1 Date: 2000-09-12 GLP/GEP: yes, unpublished <b>...also filed: KCA 5.8.2 /05</b>	Y	Y	Requested by non-EU authorities	Bayer CropScience
KCA 5.8.1 /01	██████████ ██████████	1998	FOE 5043 Sulfonsaeure (plant metabolite of FOE 5043) - Study for acute oral toxicity in rats ██ ██████████ ██ Report No.: 27317, Edition Number: M-004749-01-1 Date: 1998-03-19 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience

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KCA 5.8.1 /02	Herbold, B. A.	2000a	FOE 5043-sulfonic-acid - Salmonella/microsome test - Plate incorporation and preincubation method Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 29473, Edition Number: M-019064-01-1 Date: 2000-01-19 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 5.8.1 /03	Herbold, B.	2000b	FOE 5043-Thioglycolate Sulfoxide - Salmonella/microsome test - plate incorporation and preincubation method Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 29871, Edition Number: M-032500-01-1 Date: 2000-05-12 GLP/GEP: yes, unpublished	N			Bayer CropScience
KCA 5.8.1 /04	██████ ██████ ██████ ██	2000	FOE 5043 Sulfonic acid - Plasmakinetik and excretion in urine in a rat study with single oral versus intravenous administration ████████████████████ ██████████████████ ██████████ Report No.: 30052, Edition Number: M-042251-01-1 Date: 2000-07-25 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience

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KCA 5.8.1 /05	Heimann, K. G.; Klamroth, E.	2000	Assessment of the toxicological significance of metabolite M4 (thioglycolate sulfoxide) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: MO-01-016035, Edition Number: M-069957-01-1 Date: 2000-02-03 GLP/GEP: no, unpublished	N	Y		Bayer CropScience
KCA 5.8.1 /06	██████████ ██████ ██████████ ██████	1993	Acute oral toxicity study with FOE 6457 (Thiadone, an FOE 5043 metabolite) in rats ████████████████████ ████████████████████ ████████████████████, Report No.: BC6979, Edition Number: M-004951-01-1 Date: 1993-04-27 GLP/GEP: yes, unpublished	Y	Y		Bayer CropScience
KCA 5.8.1 /07	Herbold, B.	2009	FOE 5043-Oxalate (Project: FOE 5043 (Flufenacet/AE F133402)) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05640, Edition Number: M-358953-01-1 Date: 2009-11-11 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience



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KCA 5.8.1 /08	Wollny, H. E.	2002	FOE 5043-Oxalate - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1277301, Edition Number: M-361724-01-1 Date: 2002-12-10 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /09	Nern, M.	2009	FOE 5043-oxalate (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05598, Edition Number: M-358043-01-1 Date: 2009-10-22 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /10	Wollny, H. E.	2009	FOE 5043-Sulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1277302, Edition Number: M-361158-01-1 Date: 2009-12-10 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience

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KCA 5.8.1 /11	Nern, M.	2010a	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - <i>In vitro</i> chromosome aberration test with Chinese hamster V79 cells Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05870, Edition Number: M-366380-01-1 Date: 2010-03-15 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /12	██████	2010b	FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse ████████████████████ ████████████████████ ████████████████████, Report No.: AT05913, Edition Number: M-368627-01-1 Date: 2010-05-04 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /13	██████	2010c	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - Unscheduled DNA synthesis test with male rat liver cells in vivo ████████████████████ ████████████████████ ████████████████████ Report No.: AT06167, Edition Number: M-397810-01-1 Date: 2010-12-07 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package	Bayer CropScience

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KCA 5.8.1 /14	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with FOE 5043-methylsulfone Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1454201, Edition Number: M-422370-01-1 Date: 2012-01-17 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /15	Wollny, H. E.	2012	FOE 5043-methylsulfone - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1454202, Edition Number: M-430571-01-1 Date: 2012-05-08 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /16	Bohnenberger, S.	2012	In vitro chromosome aberration test in Chinese hamster V79 cells with FOE 5043-methylsulfone Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1454203, Edition Number: M-437250-01-1 Date: 2012-08-23 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience

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KCA 5.8.1 /17	Sokolowski, A.	2011	Salmonella typhimurium reverse mutation assay with FOE 5043-Thiadone Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1423000, Edition Number: M-413989-01-1 Date: 2011-09-13 GLP/GEP: yes, unpublished	N	Y	Requested by non-EU authorities	Bayer CropScience
KCA 5.8.1 /18	Sokolowski, A.	2012	Salmonella typhimurium reverse mutation assay with FOE 5043-trifluoroethanesulfonic acid Na-salt Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1486601, Edition Number: M-434728-01-1 Date: 2012-07-13 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /19	Wollny, H. E.	2013	FOE 5043-trifluoroethanesulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1486603, Edition Number: M-446033-01-1 Date: 2013-02-01 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience

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KCA 5.8.1 /20	Bohnenberger, S.	2013	In vitro chromosome aberration test in Chinese hamster V79 cells with FOE 5043-trifluoroethanesulfonic acid Na-salt Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany Bayer CropScience, Report No.: 1486602, Edition Number: M-447404-01-1 Date: 2013-02-20 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package	Bayer CropScience
KCA 5.8.1 /21	Johnson, M.	2005	Trifluoroacetate (TFA): reverse mutation in five histidine-requiring strains of Salmonella typhimurium Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report No.: 2014/82, Edition Number: M-256628-01-1 Date: 2005-08-24 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience
KCA 5.8.1 /22	Ballantyne, M.	2005	Trifluoroacetate (TFA) - Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report No.: 2014/84-D6173, Edition Number: M-260699-01-1 Date: 2005-10-17 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience

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KCA 5.8.1 /23	Clare, G.	2005	Trifluoroacetate (TFA) - Induction of chromosome aberrations in cultured human peripheral blood lymphocytes Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom Bayer CropScience, Report No.: 2014/83-D6172, Edition Number: M-260807-01-1 Date: 2005-10-12 GLP/GEP: yes, unpublished	N	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience
KCA 5.8.1 /24	██████████ ██████████	2013	Sodium Trifluoroacetate - Acute oral toxicity study in rats ████████████████████ ████████████████████ ████████████████████, Report No.: 12/333-001P, Edition Number: M-444479-01-1 Date: 2013-01-14 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience
KCA 5.8.1 /25	██████████ ██████████ ██████████	2001	Trifluoroacetate - Exploratory 14-day toxicity study in the rat by dietary administration ████████████████████ ████████████████████ ████████████████████ No.: C016316, Report includes Trial Nos.: SA01136 Edition Number: M-202165-01-1 Date: 2001-09-14 GLP/GEP: no, unpublished	Y	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolites	Bayer CropScience
KCA 5.8.1 /26	██████████ ██████████ ██████████	2005	Sodium trifluoroacetate (TFA) - 28-day toxicity study in the rat by dietary administration ████████████████████ ████████████████████ ████████████████████ Report No.: SA05054, Edition Number: M-259106-01-1 Date: 2005-10-11 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience

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KCA 5.8.1 /27	[REDACTED]	2007	Sodium trifluoroacetate (TFA) 90-day toxicity study in the rat by dietary administration [REDACTED] [REDACTED] [REDACTED] Report No.: SA06080, Edition Number: M-283994-01-1 Date: 2007-02-16 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Bayer CropScience
KCA 5.8.1 /28	[REDACTED]	2010	Trifluoroacetic acid: Embryo-fetal oral gavage toxicity study in rats [REDACTED] [REDACTED] [REDACTED], Report No.: 09-4352, Edition Number: M-411209-01-1 Date: 2010-11-08 GLP/GEP: yes, unpublished	Y	Y	Completion of metabolite data package; Required to test the toxicological relevance of groundwater metabolite	Ishihara
KCA 5.8.1 /29	Buerkle, L.; Hartmann, K.; Weile, M.	2014	Flufenacet - Toxicological profile and exposure assessment of the plant metabolites Bayer CropScience, Report No.: M-476535-01-1, Edition Number: M-476535-01-1 Date: 2014-03-07 GLP/GEP: n.a., unpublished	N	N		Bayer CropScience
KCA 5.8.1 /30	Hartmann, K.; Semino, G.; Hamm, M.	2014	Trifluoroacetate (TFA) - Waiver of an acute reference dose (ARfD) Bayer CropScience, Report No.: M-480037-01-1, Edition Number: M-480037-01-1 Date: 2014-03-12 GLP/GEP: n.a., unpublished	N	N		Bayer CropScience

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KCA 5.8.2 /01	Christens on, W. R.; Wahle, B. S.	1995	Technical grade FOE 5043: Evidence for an extrathyroidal mechanism to explain alterations in circulating thyroid hormone concentration following exposure of the male rat to the experimental acetanilide Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: BC7685, Edition Number: M-004982-03-1 EPA MRID No.: 43850041 Date: 1995-07-21 ...Amended: 1996-11-19 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 5.8.2 /02	Jones, R. D.; Lake, S. G.	1995	Method development to establish michaelis-menten conditions for the punitive neurotoxin thiadone in the beagle dog Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: BC7675, Edition Number: M-004978-01-1 Date: 1995-06-07 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience



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KCA 5.8.2 /03	Christenson, W. R.; Becker, B. D.; Wahle, B. S.; Moore, K. D.; Dass, P. D.; Lake, S. G.; Stuart, B. P.; Goethem, D. L. van; Sangha, G. K.; Thyssen, J. H.	1996	Evidence of chemical stimulation of hepatic metabolism by an experimental acetanilide (FOE 5043) indirectly mediating reductions in circulating thyroid hormone levels in the male rat Publisher:Society of Toxicology, Location:Dallas, USA, Journal:Fundamental and Applied Toxicology, Volume:29, Pages:251-259, Year:1996, Report No.: M-012231-01-2, Edition Number: M-012231-01-2 GLP/GEP: n.a., published	Y	N		
KCA 5.8.2 /04	Christenson, W. R.; Becker, B. D.; Hoang, H. D.; Wahle, B. S.; Moore, K. D.; Dass, P. D.; Lake, S. G.; Stuart, B. P.; Goethem, D. L. van; Sangha, G. K.; Thyssen, J. H.	1995	Extrathyroidally mediated changes in circulating thyroid hormone concentrations in the male rat following administration of an experimental oxyacetamide (FOE 5043) Publisher:Academic Press, Location:USA, Journal:Toxicology and Applied Pharmacology, Volume:132, Pages:253-262, Year:1995, Report No.: MO-99-006106, Edition Number: M-012226-01-1 GLP/GEP: n.a., published	N			

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KCA 5.8.2 /05	████████	2000	Developmental neurotoxicity study of technical grade Flufenacet administered orally via diet to CrI:CD BR VAF/Plus presumed pregnant rats ██████████ ██████████ ██████████ ██████████, Report No.: BC9333, Edition Number: M-026105-01-1 Date: 2000-09-12 GLP/GEP: yes, unpublished ...also filed: KCA 5.7.1 /03	Y	Y	Requested by non-EU authorities	Bayer CropScience
KCA 5.8.2 /06	████████	2012	FOE 5043 (flufenacet) - A tolerability and pilot study to verify the exposure of offspring during lactation when administered via the diet to Sprague-Dawley rats ██████████ ██████████ ██████████ ██████████, Report No.: SA 10153, Edition Number: M-434509-01-1 EPA MRID No.: 48898901 Date: 2012-07-11 GLP/GEP: no, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience
KCA 5.8.2 /07	████████	2012a	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat (gestational exposure phase) ██████████ ██████████ ██████████ ██████████ Report No.: SA 10154, Edition Number: M-435619-01-1 EPA MRID No.: 48898902 Date: 2012-07-27 GLP/GEP: yes, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience

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KCA 5.8.2 /08	████████	2012b	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat by dietary exposure (gestational and lactational exposure phase) ██████████ ██████████ ██████████ ██████████, Report No.: SA 11052, Edition Number: M-435313-01-1 EPA MRID No.: 48898903 Date: 2012-07-27 GLP/GEP: yes, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience
KCA 5.8.2 /12	████████	2012c	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat complementary assay (gavage exposure of pups) ██████████ ██████████ ██████████ ██████████ Report No.: SA 11167, Edition Number: M-435126-01-1 EPA MRID No.: 48898904 Date: 2012-07-25 GLP/GEP: yes, unpublished	Y	Y	Requested by non-EU authorities, relevant for reference dose derivation	Bayer CropScience
KCA 5.8.2 /15	Herbold, B. A.	1993	FOE 5043-Hydroxy - Salmonella/microsome test plate incorporation and preincubation method Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 22438, Edition Number: M-004586-01-1 Date: 1993-08-05 GLP/GEP: yes, unpublished	N	Y	Supportive information for justification of the active substance specification	Bayer CropScience

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KCA 5.8.2 /16	██████████ ██████████ ██████████	1992a	FOE 5043-Hydroxy - Study of the acute oral toxicity to rats ████████████████████ ██████████ ████████████████████, Report No.: 21889, Edition Number: M-004579-01-1 Date: 1992-12-03 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /17	██████████	1993	FOE 5043-Hydroxy (intermediate for the manufacture of FOE 5043 technical) - Study of the acute inhalation toxicity in rats in accordance with OECD guideline no. 403 ████████████████████ ██████████ ████████████████████, Report No.: 22155, Edition Number: M-004589-01-2 Date: 1993-03-30 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /18	██████████ █	1992b	FOE 5043-Hydroxy - Study for skin and eye irritation/corrosion in rabbits ████████████████████ ██████████ ████████████████████, Report No.: 21257, Edition Number: M-004564-01-1 Date: 1992-04-08 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience

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KCA 5.8.2 /19	██████████	1994	FOE 5043-Hydroxy - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman) ██████████ ██████████ ██████████, Report No.: 22824, Edition Number: M-004614-01-2 Date: 1994-01-24 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /20	Herbold, B. A.	1993	FOE 5043-Sulfon - Salmonella/microsome test plate incorporation and preincubation method Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 22629, Edition Number: M-004606-01-1 Date: 1993-10-22 GLP/GEP: yes, unpublished	N	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /21	██████████ █	1992c	FOE 5043 Sulfon - Study for acute oral toxicity in rats ██████████ ██████████ ██████████, Report No.: 21893, Edition Number: M-004578-01-1 Date: 1992-12-03 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /22	██████████	1992a	FOE 5043-Sulfone - Study of the acute inhalation toxicity to rats in accordance with OECD guideline no. 403 ██████████ ██████████ ██████████, Report No.: 21784, Edition Number: M-004576-01-2 Date: 1992-10-21 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience

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KCA 5.8.2 /23	██████████	1992d	FOE 5043-Sulfon - Study for skin and eye irritation/corrosion in rabbits ██████████ ██████████ ██████████, Report No.: 21156, Edition Number: M-004522-01-1 Date: 1992-03-10 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /24	██████████	1994	FOE 5043-Sulfone - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman) ██████████ ██████████ ██████████, Report No.: 23001, Edition Number: M-004673-01-2 Date: 1994-04-19 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /25	██████████	1993	FOE 5043-Sulfone - Study to assess the sensory irritation potential to mice (RD50 determination) ██████████ ██████████ ██████████, Report No.: 22729, Edition Number: M-004601-01-1 Date: 1993-12-03 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /26	██████████	1992a	FOE 5043-Sulfone - Range-finding study of the subacute inhalation toxicity to rats (exposure: 5x6h) ██████████ ██████████ ██████████, Report No.: 21390, Edition Number: M-004571-01-2 Date: 1992-05-21 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience

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KCA 5.8.2 /27	██████████	1992b	FOE 5043-Sulfone - Study of the subacute inhalation toxicity to rats in according with OECD guideline no. 412 ██████████ ██████████, Report No.: 22918, Edition Number: M-004779-01-1 Date: 1994-03-01 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /28	██████████	1994	FOE 5043 Acetate - Study for acute oral toxicity in rats ██████████ ██████████, Report No.: 23279, Edition Number: M-004640-01-1 Date: 1994-08-24 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /29	██████████	1996	FOE 5043 Acetat (intermediate product of FOE 5043) -Study for acute inhalation toxicity in rats according to OECD no. 403 ██████████ ██████████, Report No.: 25414, Edition Number: M-004734-01-1 Date: 1996-09-09 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience
KCA 5.8.2 /30	██████████ █	1994	FOE 5043 Acetat - Study for skin and eye irritation/corrosion in rabbits ██████████ ██████████, Report No.: 23062, Edition Number: M-004662-01-1 Date: 1994-05-31 GLP/GEP: yes, unpublished	Y	Y	Supportive information for justification of the active substance specification	Bayer CropScience

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KCA 5.9.1 /01	Bloomberg, J. R.	1995	Worker exposure to FOE 5043/Axiom Bayer Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: BC7815, Edition Number: M-005467-01-1 Date: 1995-10-09 GLP/GEP: no, unpublished	N	Y		Bayer CropScience
KCA 5.9.1 /02	Steffens, W.	2014	Occupational medical experiences with flufenacet Bayer CropScience, Report No.: M-475871-01-1, Edition Number: M-475871-01-1 Date: 2014-01-30 GLP/GEP: yes, unpublished	N	Y	Updated information	Bayer CropScience