Bayer CropScience

Document Title

Summary of the toxicological and metabolism studies for Flufenacet

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 5: Toxicological and metabolism studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE STUBSTANCE

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 wherein 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 – 3rd 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.

Comments with respect to the Annex I renewal process

This supplemental dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of flufenacet and were therefore not evaluated during the first EU review of this compound. The summaries on the different toxicological endpoints (information is taken from the Monograph/Review Report (July 2003)/Evaluation table (December 2001)) were supplemented and adapted with the new information. In order to facilitate discrimination between new information and original paragraphs, the new information is written in bold letters. All other 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 5-1: Overview of flufenacet batches used for toxicity studies

Batch Number	Purity (%)	Study type	Reference
17001/90	94.8	Acute inhalation toxicity, rat	[redacted], 1990 M-004844-01-1
17001/90	92.6-93.8	Skin irritation, rabbit	[redacted] & [redacted], 1992 M-004846-01-1
17001/90	92.6-93.8	Eye irritation, rabbit	[redacted] & [redacted], 1992 M-004847-01-1
17001/90	92.6-93.8	Skin sensitization, Guinea pig	[redacted] & [redacted], 1992 M-004845-01-1
17001/90	93.8-94.8	Dog, 13-week oral (diet) toxicity	[redacted] & [redacted], 1995 M-004977-02-1
17001/90	92.6-94.8	Mouse, 13-week oral (diet) (range-finding)	[redacted] & [redacted], 1995 M-004985-01-1
17001/90	92.6-94.8	Rat, 13-week oral (diet)	[redacted] & [redacted], 1995 M-004999-01-1

Batch Number	Purity (%)*	Study type	Reference
17001/90	92.6	In vitro Unscheduled DNA synthesis (UDS), rat hepatocytes	[redacted], 1992 M-004577-01-1
17001/90 FL 0036	92.6-94.8 95.2-99.0	Mechanistic study on thyroid hormone effects,21-day, rat, diet	[redacted] & [redacted], 1995, M-004982-03-1
FL 036	95.0-99	Dog, 12-month chronic oral (diet) toxicity	[redacted] & [redacted], 1995 M-005001-02-2
FL 036	97.0-98.5	Rat, 21-day dermal toxicity	[redacted], 1995, M-004981-01-1
FL 036	97.1-97.5	In vitro mammalian cell gene mutation test (HGPRT), Chinese hamster cells V79	[redacted], 1994 M-004634-01-1
FL 036	97.5	In vitro mammalian chromosome aberration test, Chinese hamster cells	[redacted], 1995 M-004692-01-1
FL 036	97.5	In vivo Micronucleus test, mouse	[redacted], 1993 M-004388-01-1
FL 036	95.2-98.5	Mouse, 18-months feeding (carcinogenicity)	[redacted] & [redacted], 1995 M-005060-02-1
FL 036	95.2-99.0	Rat, 24-month feeding (carcinogenicity and chronic toxicity)	[redacted] & [redacted], 1995 M-005062-02-1
FL 036	95.2-99.0	Rat dietary two-generation reproduction study	[redacted] & [redacted], 1995 M-004984-03-1
FL 036	97.2	Rat, oral developmental	[redacted], 1995, M-004976-02-1
FL 036	98.5	Rabbit, oral developmental	[redacted], 1995, M-004979-01-1
FL 036	97.4-97.8	Rat, acute oral neurotoxicity	[redacted] & [redacted], 1995 M-004986-02-1
FL 036	98.0-98.2	Rat, 90-day neurotoxicity study (diet) (screening study)	[redacted], 1995, M-005014-01-2
FL 036 NLL 3643-5	99.0 98.0	Acute oral toxicity, rat	[redacted] & [redacted], 1993 M-004865-02-1
NLL 3643-5	98.1	Acute oral toxicity, rat	[redacted] & [redacted], 1991 M-004850-01-1
NLL 3643-5	98.0-98.1	Acute oral toxicity, rat	[redacted], 1992 M-004864-01-1
NLL 3643-5	98.0-98.1	Acute dermal toxicity, rat	[redacted] & [redacted], 1992 M-004843-01-1

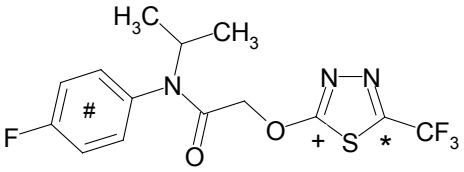
Batch Number	Purity (%)*	Study type	Reference
603-0013	**96.0-96.9**	**Rat, oral developmental neurotoxicity**	**[redacted], 2000 M-026105-01-1**
898313105	96.8	Skin sensitization, Guinea pig	[redacted], 1994 M-004637-01-1
898313105	96.8	Bacterial reverse mutation assay	[redacted], 1995 M-004696-01-1
920902ELB01	**99.5**	**Skin sensitization, Guinea pig**	**[redacted], 1995 M-004677-01-1**
EDHB001715	**97.5**	**Skin sensitization, LLNA (mouse)**	**[redacted], 2004 M-090513-01-1**
EDHB001715	**97.0**	**Rat, 1-week inhalation toxicity**	**[redacted], 2008 M-300005-01-1**
EDHB001715	**97.0**	**Rat, 4-week inhalation toxicity**	**[redacted], 2008 M-302961-01-2**
NK61AX0177	**96.8**	**Bacterial reverse mutation assay**	**[redacted], 2010 M-395211-01-1**
NK61AX0177	**96.8**	**Rat, oral (diet) developmental toxicity (range-finding)**	**[redacted]-[redacted], 2012 M-434509-01-1**
NK61AX0177	**96.8**	**Rat, comparative thyroid sensitivity assay (gestational exposure phase)**	**[redacted], 2012 M-435619-01-1**
NK61AX0177	**96.8**	**Rat, comparative thyroid sensitivity assay (gestational & lactational exposure phase)**	**[redacted], 2012 M-435313-01-1**
NK61AX0177	**96.8**	**Rat, comparative thyroid sensitivity assay (gavage exposure of pups)**	**[redacted], 2012 M-435126-01-1**
NK61CK0650	**98.2**	**Phototoxicity test**	**[redacted], 2013 M-464615-01-1**

* Purity as stated in study reports

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral route

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



\# fluorophenyl-UL-^{14}C
* thiadiazole-2-^{14}C
* thiadiazole-5-^{14}C

A study on absorption, distribution, metabolism and excretion (ADME) of ^{14}C-labelled flufenacet with rats was conducted with all three label positions ([redacted], M. E., [redacted], C. M., [redacted], L. L., [redacted], Y. (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).

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-^{14}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-^{14}C]- and [thiadiazole-2-^{14}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-^{14}C]FOE 5043 and was 92 % on average in the experiments with [thiadiazole-2-^{14}C]FOE 5043. After application of [fluorophenyl-UL-^{14}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-^{14}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-^{14}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-^{14}C]FOE 5043 oxalate as well as [thiadiazole-2-^{14}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 thiadizole moiety of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues."

Toxicokinetics critical end points	
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 $^{14}CO_2$ and $^{14}CH_4$
Distribution	quick redistribution from blood
Potential for accumulation	none
Rate and extent of excretion	[fluorophenyl-4,5-^{14}C]FOE5043: 59 - 79 % with urine, 8 - 30 % with faeces (72h) [thiadiazole-2-^{14}C]FOE5043: 41 - 59 % with urine, 2 - 6 % with faeces (72h), 22 - 32 % $^{14}CO_2$ and 12 - 23 % CH_4 in the expired air [thiadiazole-5-^{14}C]FOE5043: 82 – 89 % with urine, 6 – 7 % with faeces (72h)
Main animal metabolites	N-acetylcysteine conjugate of N-isopropyl-fluorophenyl-acetanilide (M10), glucuronic acid of thiadone (M24), oxalylacetic acid conjugate of thiadone (M26), thiadone (M9)

Toxicokinetic evaluation of the ADME study

An amendment has been prepared to the above mentioned ADME study on the determination of toxicokinetic plasma parameters in the rat. As this amendment was not available at the time of submission of the original submission for Annex I inclusion of EU Directive 91/414. Therefore, it is now presented in the following.

Report:	**KCA 5.1.1/01, [redacted], D. W., [redacted], M. E., 2010**
Title:	Amendment No. 1 to the Final Report: The Metabolism of FOE 5043 in Rats
Document No:	M-384235-01-1
Report No:	Bayer Report No. 106665-1
Guidelines and data requirements:	US EPA Ref.: 85-1, Rat Metabolism
GLP	Yes

Material and Methods

In a basic ADME study[1], groups of each five male and five female rats received a single oral dose of [fluorophenyl-UL-^{14}C] and [thiadiazole-2-^{14}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 5.1.1- 1 for the fluorophenyl label and in Table 5.1.1- 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 - ∞), T_{max} and C_{max} are derived.

The results of this pharmacokinetic evaluation are compiled in Table 5.1.1- 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-^{14}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

[1] [redacted], M. E., [redacted], C. M., [redacted], L. L., [redacted], Y. (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.

contrast, a low dose using the [thiadiazole-2-^{14}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.

Table 5.1.1- 1: Averaged plasma levels in rats following oral administration of [fluorophenyl-UL-^{14}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	**0.312**	**0.361**	7.709	6.554	**0.368**	**0.390**
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	**0.240**	0.163	14.306	19.756	0.251	0.138
8 hr	0.239	**0.193**	22.085	27.874	**0.283**	**0.164**
24 hr	0.132	0.131	**36.833**	37.000	0.155	0.096
32 hr	0.093	0.100	32.549	**39.272**	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 5.1.1- 2: Averaged plasma levels in rats following oral administration of [thiadiazole-2-^{14}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	**3.359**	1.438	165.230
90 min	3.341	1.786	173.790
2 hr	3.332	2.630	180.878
4 hr	2.430	**2.731**	**185.704**
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 5.1.1- 3: Summary of toxicokinetic plasma parameters determined in rats following oral administration of [fluorophenyl-UL-^{14}C] and [thiadiazole-5-^{14}C]flufenacet

	Calculated via Excel				Calculated via TOPFIT model			
	T_{max} (h)	C_{max} (mg/L)*	$T_{1/2}$ (h)	AUC** (mg/L*h)	T_{max} (h)	C_{max} (mg/L)*	$T_{1/2}$ (h)	AUC (mg/L*h)
[fluorophenyl-UL-^{14}C] label								
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
[thiadiazole-2-^{14}C] label								
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-2-14C], low dose, females)

Rat metabolism study with [thiadiazole-5-^{14}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-^{14}C]flufenacet, particularly the formation of trifluoroacetate (TFA).

Report:	**KCA 5.1.1/02, Bongartz, R., 2012**
Title:	[Thiadiazole-5-^{14}C]Flufenacet: Supportive Experiment for Identification of Metabolites in the Urine of the Rat
Document No:	M-441499-01-1
Report No:	EnSa-12-0439
Guidelines:	OECD Guideline 417: Toxicokinetics, adopted 22-July-2010 US EPA OCSPP Health Effects Test Guideline OPPTS 870.7485
GLP	yes

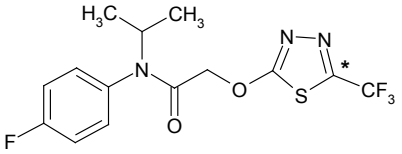
Executive Summary

A supportive study was conducted on the metabolism of [thiadiazole-5-^{14}C] in the rat to investigate polar metabolites. The test substance war 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	 * denotes the ^{14}C label
Chemical name	N-(4-Fluoro-phenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5-^{14}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 (*Rattus norvegicus domesticus*)
Strain	Wistar Unilever HsdCpb: WU
Breeder	Harlan, The Netherlands
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, *ad libitum*; tap water, *ad libitum*

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 anesthezia 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 ≤ -18°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 $^{14}CO_2$ 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 µm 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-^{14}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.[2] In the blood plasma at sacrifice the residue level amounted to 0.224 mg equivalents/kg (mg equ/kg).

Metabolic profile in urine (Table 5.1.1- 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 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 bread 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-^{14}C]flufenacet to rats most of the radioactivity was already excreted within 24 hours with renal excretion being the predominant route of elimination. The

[2] [redacted], M.E., [redacted], C.M., [redacted], L.L., [redacted], Y. (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.

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 5.1.1- 4: Metabolic profile in urine samples collected at different intervals after oral administration of 1 mg/kg bw of [thiadiazole-5-^{14}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[3] (for comparison, pKa of acetic acid: 4.76) indicating a complete dissociation at a higher pH than 1.3

[3] 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

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.

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.

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).

Nevertheless, 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.

Report:	**KCA 5.1.1/03, [redacted], J., 2013**
Title:	[Thiadiazole-5-^{14}C]Flufenacet: Metabolic stability of profiling in liver microsomes from rats and humans for inter-species comparison
Document No:	M-475336-01-1
Report No:	834338 of Harlan Laboratories, Barcelona, Spain, for Bayer CropScience, Germany
Guidelines:	No guideline available
GLP:	yes

Executive Summary

The comparative metabolism of [Thiadiazole-5-^{14}C]Flufenacet (^{14}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.

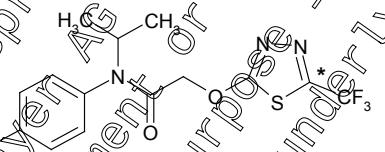
The metabolic activity of the microsomes was clearly demonstrated by determining 6β-hydroxy-testosterone formed from testosterone by testosterone 6β-hydroxylase (positive control). This biochemical reaction is well-known for a CYP3A microsomal enzyme.

^{14}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 ^{14}C-Flufenacet is comparable in rat and human.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluoro-phenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5-^{14}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

^{14}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}C-Flufenacet solutions were incubated separately with rat and human liver microsomes. 50 µM of flufenacet were mixed with 350 µL of 100 mM sodium phosphate buffer (pH 7.4), 100 µL of 100 mM magnesium dichloride and 25 µL of rat liver microsomes (20 mg protein/mL) or 19.5 µL of human liver microsomes (26 mg protein/mL). After 2 minutes pre-heating to 37°C 50 µL of 6.4 mM NADPH (± 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}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 6ß-hydroxytestosterone by the CYP3A microsomal system that is part of the liver microsomes. The quantitative determination of (non-labelled) 6ß-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 µm) 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 6β-hydroxytestosterone from testosterone demonstrated sufficient metabolic capability of the microsome batches used in the study. Testosterone 6 β-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 ^{14}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 ^{14}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 5.4.1- 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

^{14}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 5.1.1- 5: Metabolic profile of [thiadiazole-5-^{14}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 14C-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

CA 5.1.2 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-^{14}C labelled flufenacet.

CA 5.2 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 5.2-1: Summary of acute toxicity studies*

Route/Study	Species	Sex	Results	Reference
Oral[1)]	Rat	M F	LD_{50}: 1617 mg/kg bw 589 mg/kg bw	[redacted] & [redacted], 1993 M-004865-02-1
Oral[2)]	Rat	M	LD_{50}: 683 mg/kg bw	[redacted], 1992 M-004864-01-1
Oral	Mouse	M F	LD_{50}: 1331 mg/kg bw 1736 mg/kg bw	[redacted] & [redacted], 1991 M-004850-01-1
Dermal	Rat	M F	LD_{50}: >2000 mg/kg bw >2000 mg/kg bw	[redacted], 1992 M-004843-01-1
Inhalation (aerosol, 4h)	Rat	M	LC_{50}: >3740 mg/m³ >3740 mg/m³	[redacted], 1990 M-004844-01-1
Skin irritation	Rabbit	M	Not irritating	[redacted] & [redacted], 1992 M-004846-01-1
Eye irritation	Rabbit	M	Not irritating	[redacted] & [redacted], 1992 M-004847-01-1
Skin sensitisation Buehler method	Guinea pig	M	Not sensitizing	[redacted] & [redacted], 1992 M-004845-01-1
Skin sensitisation M&K method	Guinea pig	M	Sensitizing	[redacted], 1994 M-004637-01-1
Skin sensitisation M&K method	**Guinea pig**	**F**	**Sensitizing**	**[redacted], 1995** **M-004677-01-1**
Skin sensitization Local lymph node assay	**Mouse**	**F**	**Not sensitizing**	**[redacted], 2004** **M-090513-01-1**
***In vitro* 3T3 NRU phototoxicity test**	**BALB/c 3T3 cells**		**Not phototoxic**	**[redacted], 2013** **M-464615-01-1**

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

CA 5.2.1 Oral

All necessary acute oral toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.2.2 Dermal

All necessary acute dermal toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.2.3 Inhalation

All necessary acute inhalation toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.2.4 Skin irritation

All necessary skin irritation studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.2.5 Eye irritation

All necessary eye irritation studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.2.6 Skin sensitization

In addition to the skin sensitization studies described in the Monograph and baseline dossier of flufenacet, a Maximization test was conducted using a different (pure) flufenacet batch and a Local Lymph Node Assay according to the new testing guideline.

Report: [redacted], 1995;M-004677-01
Title: FOE 5043 - Study for the skin sensitization effect in guinea pigs (Maximization test Magnusson and Kligman)
Report No: 23924
Document No: M-004677-01-1
Guidelines: OECD 406; EC Guideline 92/69, Method B.6.; US-EPA-FIFRA §81-; Deviations: none
GLP/GEP: yes

I. 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

2. Vehicle: physiological saline solution containing 2% v/v Cremophor EL®

3. Test animals:

Species:	Guinea pig
Strain:	Hsd Win:HD
Age:	5 – 7 weeks
Weight at dosing:	279 – 374 g
Source:	[redacted] Germany
Acclimatisation period:	At least seven days
Diet:	"Altromin®3020 - Maintenance Diet for Guinea Pigs" (Altromin GmbH, Lage, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
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)

B. Study design and methods

1. Animal assignment and treatment

Dose	
Intradermal induction:	5% (= 20 mg test substance/animal)
Topical induction:	50% (= 250 mg test substance/animal)
1st Challenge:	50% (= 250 mg test substance/animal)
	25% (= 125 mg test substance/animal)
2nd Challenge	12% (= 60 mg test substance/animal)
	6% (= 30 mg test substance/animal)
Application route:	Intradermal, dermal
Application volume:	intradermal induction: 0.1 mL/injection
	topical induction, challenge: 0.5 mL/patch
Duration:	topical induction: 48 hours, challenge: 24 hours
Group size:	50 males (test item: 20, control: 2x10, dose-range finding: 10)
Observations:	mortality, clinical signs, skin effects, body weight (at beginning and termination of study)

II. Results and discussion

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

After the 2nd 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 1st 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 2nd 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 5.2.6/03-1: 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	48	72	Total	48	72	48	72	Total	48	72
1st Challenge										
50%	6	5	6	0	0	0	0	0	0	0
25%	6	6	7	0	0	0	0	0	0	0
2nd Challenge										
12%	5	3	5	0	0	0	0	0	0	0
6%	6	4	6	0	0	0	0	0	0	0

III. Conclusions

After the 1st 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 2nd challenge the 12% and 6% test substance formulations led to skin redness in 25% and 30% of the test animals.
Thus, flufenacet exhibits a skin-sensitization potential under the conditions of the maximization test.

Report: [redacted]0;[redacted];2004;M-090513-01
Title: FOE 5043 - Local lymph node assay in mice (LLNA/IMDS)
Report No: AT01491
Document No: M-090513-01-1
Guidelines: OECD 406; Guideline 96/54/EC, Method B.6; US-EPA 712-C-03-197, OPPTS 870.2600;
Deviations: none
GLP/GEP: yes

I. 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] Germany
Acclimatisation period:	At least seven days
Diet:	"PROVIMI KLIBA SA 3883 maintenance diet for rats and mice (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
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)

II. Results and discussion

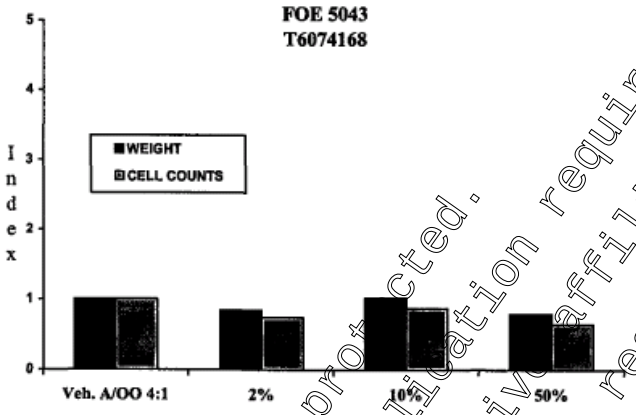
The body weights of the animals were not affected by any treatment.

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 $2x10^{-2}$ 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 5.2.6/04-1: Bar charts (weight and cell count) for the LLNA



III. 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.

CA 5.2.7 Phototoxicity

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.

The Circumstances in which a phototoxicity study, according to the new data requirements is required is "*where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol-1 x cm-1, no toxicity testing is required.*"

As the Ultraviolet/visible molar extinction/absorption coefficient of the active substance exceeds the trigger of 10 L x mol-1 x cm-1 a cytotoxicity assay in vitro with BALB/c 3T3 cells has been performed.

Report: KCA 5.2.7 /01; [redacted], A.;2013;M-464615-01
Title: Flufenacet TC: Cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight
Report No: 1561200
Document No: M-464615-03-1
Guidelines: **Commission Regulation (EC) No. 440/2008, B41; Committee for Proprietary Medicinal Products (CPMP) CPMP/SWP/398/01; OECD 432; Deviations: none**
GLP/GEP: **yes**

I. 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], Germany).
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 ± 1.5 °C in 75 cm^2 plastic flasks. Seeding was done with about 1 x 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 ± 1.5 °C in a 7.5 ± 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 x 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^2 (7.65 - 8.1 J/cm^2) for 50 min ± 2 min at 20-30 °C, the other plate was stored for 50 min ± 2 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 ± 1.5 °C and 7.5 ± 0.5 % CO_2.
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°, 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

II. 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 5.2.7/01-1: OD_{540} values Neutral Red assay of the main experiment

Con-centration [µg/mL]	OD_{540} with artificial sunlight Mean	SD	% of solvent control	Con-centration [µg/mL]	OD_{540} without artificial sunlight 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	0.9879	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	0.0506	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_{540} out of 12 wells

Table 5.2.7/01-2: OD_{540} values Neutral Red assay of the confirmatory experiment

Con-centration [µg/mL]	OD_{540} with artificial sunlight Mean	SD	% of solvent control	Con-centration [µg/mL]	OD_{540} without artificial sunlight 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	0.0305	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*	0.0298	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_{540} out of 12 wells

Table 5.2.7/01-3: Summary of results of the Neutral Red assay

	Substance	ED_{50} (+UV) [µg/mL]	ED_{50} (-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

III. Conclusions

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

CA 5.3 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 5.3-1: 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.	[redacted] & [redacted], 1995 M-004981-01-1
Rat 1-week inhalation	**M, F**	**~14 48 mg/m³**	**~66 225 mg/m³**	**T4 ↓ Liver: rel. weight ↑**	**[redacted], 2008 M-300005-01-1**
Rat 4-week inhalation	**M, F**	**~7 19 mg/m³**	**~81 220 mg/m³**	**HB ↓, HCT ↓, RETI ↑, HEINZ ↑, AP ↓, TG ↓, Liver: enzymes ↑, rel. weight ↑, spleen: weight ↑, histopathological changes in nasal cavity and larynx, spleen, testes, thyroid, liver**	**[redacted], 2008 M-302961-01-1**
Rat 90-day feeding	M F	-- a) 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	[redacted] & [redacted], 1995 M-004999-01-1
Mouse 90-day feeding	M F	18 25	64 91	T4 ↓ Liver: rel. weight ↑	[redacted] & [redacted], 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 ↑	[redacted] & [redacted], 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 ↑	[redacted], [redacted], 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

CA 5.3.1 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. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.3.2 Oral 90-day study

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

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.

Report:	[redacted]::[redacted];2012;M-430840-02; Amended: 2012-07-04
Title:	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
Report No:	EnSa-12/0266
Document No:	M-430840-02-1
Guidelines:	**not applicable;** **Deviations: not appplicable**
GLP/GEP:	**no**

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.

CA 5.3.3 Other routes

Report:	[redacted];[redacted];2008;M-300005-01
Title:	Flufenacet (FOE 5043) - 1-week inhalation pilot study in Wistar rats (exposure 6h/day, 5 days/week)
Report No:	AT04505
Document No:	M-300005-01-1
Guidelines:	**OECD 412; Directive 88/302/EEC, Annex V, Method B.29.; US-EPA 712C-98-193, OPPTS 870.3465;** **Deviations: 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.**
GLP/GEP:	**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], Germany
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), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	singly in conventional Makrolon® Type III_H cages; bedding: Litalabo (S.P.P.S., Frasne, 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^3 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/m³)	control	40	200	800
Nominal concentration (mg/m³)	0	51.8	315	1091
Gravimetric concentration (mg/m³)*	--	47.9	225	846
Temperature (mean, °C)	20.8	21.7	231.8	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/m³)	--	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/m³ air did not show any signs.
In rats of the 800 mg/m³ air group the following signs were observed: piloerection, bradypnea, labored and irregular breathing patterns.

Table 5.3.3/02-1: Summary of sub-acute inhalation toxicity

Sex	Target concentration (mg/m³)	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	--

* 1st number = number of dead animals, 2nd number = number of animals with signs; 3rd 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/m³ group.

D. Laboratory investigations

Haematology

Haematology revealed in female rats exposed at 800 mg/m³ 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 5.3.3/02-2: Summary of haematology

	ERY (10^{12}/L)	**HB** (g/L)	**HCT** (L/L)	**MCV** (fl)	**MCH** (pg)	**MCHC** (g/L ERY)
Dose (mg/m³)	Males					
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
	ERY (10^{12}/L)	**HB** (g/L)	**HCT** (L/L)	**MCV** (fl)	**MCH** (pg)	**MCHC** (g/L ERY)
Dose (mg/m³)	Females					
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^{+}	125^{++}	0.398^{+}	60.2	19.0	316

	LEUKO (10^{9}/L)	**RETI** (‰)	**THRO** (10^{9}/L)	**HEINZ** (‰)
Dose (mg/m³)	Males			
0	2.89	27	1190	22
40	3.67	29	1206	31
200	3.14	33	1209	36^{+}
800	4.81	39	1231	38
Dose (mg/m³)	Females			
0	2.48	25	1142	16
40	2.61	22	1029	28^{+}
200	3.44	26	995	27
800	3.01	34	980^{+}	18

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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/m³ decreased concentrations of T4 and increased concentrations of TSH existed. At 200 mg/m³ the decrease of T4 gained statistical significance.

Table 5.3.3/02-3: Summary of clinical chemistry

Dose (mg/m³)	Males T3 (nmol/L)	Males T4 (nmol/L)	Males TSH (µg/L)	Females T3 (nmol/L)	Females T4 (nmol/L)	Females 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⁺	6.87	1.14	30	6.50
800	1.28	32	10.21⁺	1.19	22	7.87

⁺ Statistically significant at $p<0.05$, ⁺⁺ 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/m³ and above. Kidney weights were increased in the female rats exposed at 800 mg/m³ only.

Table 5.3.3/02-4: Summary of absolute organ weight data

Dose (mg/m³)	Absolute organ weight (mg) Males Lung	Heart	Liver	Spleen	Kidneys	Females 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⁺⁺	369	1309
800	933	663	10140⁺⁺	387	1534	872	624	9334⁺⁺	408	1432⁺⁺

⁺ Statistically significant at $p<0.05$, ⁺⁺ statistically significant at $p<0.01$

Table 5.3.3/02-5: Summary of relative organ weight data

Dose (mg/m³)	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++	198	791
800	194	480	341	5211++	196	788
	Females					
0	161	530	393	4015	200	765
40	164	517	388	4228	245	798
200	163	553	377	4837+	226	801
800	166	526	376	5625++	247	863+

+ Statistically significant at p<0.05, ++ 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/m³ air group an enlarged liver was noticed.

III. Conclusions

The derived NOAEC based on the actual gravimetric concentration is 48 mg/m³ (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/m³ (ca. 66 mg/kg bw/day) and above.

Report: [redacted] ;2008,M-302961-01
Title: Flufenacet (FOE 5043) - 4-week subacute inhalation study in Wistar rats (exposure 6h/day, 5 days/week on four consecutive weeks)
Report No: AT04589
Document No: M-302961-01-2
Guidelines: OECD 412, Directive 67/302/EEC, Annex V, Method B.29.; US-EPA 712C-98-193, OPPTS 870.3465;
Deviations: none
GLP/GEP: yes

I. 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], Germany
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), *ad libitum*
Water: Tap water, *ad libitum*
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/m³ 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/m³)	control	20	220	400
Nominal concentration (mg/m³)	0	22	314	513
Gravimetric concentration (mg/m³)*	--	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/m³)	--	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.

II. 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/m³) 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/m³ group and flaccidity was observed in one male rat of the 220 mg/m³ 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/m³ 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 5.3.3/03-1: Summary of body weights

Dose (mg/m³)	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^{+}	242.70	255.55	259.01	271.80
220	221.08	212.46	224.98	218.89^{++}	230.62^{++}	231.31^{++}	244.91^{++}	246.50^{++}	259.34^{++}
400	223.54	216.24	229.53	223.84^{+}	235.29^{++}	238.06	252.28^{+}	255.00	267.48^{+}

Dose (mg/m³)	Day 1	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21	Day 25	Day 28
	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^{+}	177.36^{+}	182.45	187.96^{+}	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

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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/m³ (most changes significant in males and trend in females) and 400 mg/m³ (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/m³.

Table 5.3.3/03-2: Summary of haematological examinations

	ERY (10^{12}/L)	HB (g/L)	HCT (L/L)	MCV (fl)	MCH (pg)	MCHC (g/L ERY)
Dose (mg/m³)	Males					
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	0.418^{++}	57.2	17.6	309
400	6.87^{++}	122^{++}	0.407^{++}	59.5	17.8	300^{++}
Dose (mg/m³)	Females					
0	7.33	133	0.419	57.1	18.2	318
20	7.49	134	0.411	54.9^{+}	17.9	325^{+}
220	7.09	127	0.399	56.3	17.9	318
400	7.16	131	0.426	59.4^{++}	18.3	308^{++}

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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 5.3.3/03-3: Summary of haematological examinations

	LEUKO (10^9/L)	RETI (‰)	THRO (10^9/L)	HEINZ (‰)	HQUICK (sec)
Dose (mg/m³)	Males				
0	3.83	21	1019	0	42.6
20	3.48	19	983	0	43.6
220	4.77	37^{++}	1025	11^{++}	44.0
400	3.74	51^{++}	1066	16^{++}	42.9
Dose (mg/m³)	Females				
0	3.29	25	1069	0	38.2
20	3.78	16^{++}	1070	1	36.8
220	3.24	25	1043	4	37.1
400	3.62	33^{++}	1031	6^{++}	38.2

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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/m³ showed decreased serum alkaline phosphatase and triglyceride activities/concentrations. The latter was already significantly decreased at 20 mg/m³. T3 and T4 were significantly decreased at 400 mg/m³. 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 monoxigenase and cytochrome P450 activities were increased at 20 mg/m³ and above (changes in cytochrome P450 > O-demethylase > N-demethylase activities).

Table 5.3.3/03-4: 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	63.7	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^{++}	4.4	313	107	5.05	1.3	55.5
400	89.1	56.0	170^{++}	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^{+}	56.6^{+}
220	126.6	59.5	131	5.8	525	225	5.21	1.0^{+}	57.6
400	97.5	56.0	135	10.6	380	179	4.90	1.1^{+}	59.3

Dose (mg/m^3)	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^{+}	7.31	56	144	5.5	2.48	99
220	30.5	1.24^{+}	0.34^{++}	7.90	55^{+}	144	5.5	2.52	99
400	30.7	1.31	0.24^{++}	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^{++}	8.10	56	143	5.1	2.47	99
220	31.6	1.29	0.78^{+}	7.65	53	143	5.0	2.48	102^{++}
400	32.7	1.28	0.61	7.57	61	145	5.2	2.52	99

Dose (mg/m^3)	Mg	P	T3	T4	TSH
	(mmol/L)		(nmol/L)		(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^{+}	39^{+}	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^{+}	6.85

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ statistically significant at $p<0.01$
ALAT (GPT) = Alanine aminotransferase, ALP = Alkaline phoshatase, ASAT (GOT) = Aspartate aminotransferase, CK = Creatine kinase, PAC, GLDH = Glutamate dehydroenase, 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 5.3.3/03-5: Summary of clinical chemistry determinations in liver tissue

	males				females			
Dose (mg/m^3)	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	42.0	5.75	65.0	9.2	36.0	5.61
20	138.1	12.7	55.7^{++}	6.18	77.8	10.3	40.7^{++}	6.18
220	179.3	22.3^{++}	72.6^{++}	6.51	116.6^{++}	17.3^{++}	51.4^{++}	6.47^{+}
400	235.1^{++}	25.5^{++}	73.7^{++}	6.32	131.0^{++}	19.3^{++}	55.2^{++}	6.41

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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/m³ 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/m³ air exposure groups, spleen weights were significantly increased in a concentration-dependent manner. At 400 mg/m³ liver and kidney weights were increased in addition.

Table 5.3.3/03-6: Absolute organ weights

Dose (mg/m³)	Absolute organ weight (mg)								
	Brain	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+	550	2038	53	2792	423
220	1737	1118	883+	10095+	635	1993	46	2729	416
400	1776	1174	932	11168	764++	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

+ Statistically significant at $p<0.05$, ++ statistically significant at $p<0.01$

Table 5.3.3/03-7: Relative organ weights versus body weights

Dose (mg/m³)	BW (g)	Relative organ weight (mg/100g) vs. body weight								
		Brain	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++	684	438	343	2916	249++	777	18	1064	160
400	268	667	439	349	4186	288++	789+	18	1068	150
Females										
0	207	854	481	361	3871	220	741	32	67	184
20	195+	872	483	370	3957	218	746	33	64	164
220	198	877	514	388	4295++	241	775	31	65	172
400	199	857	512	366	4339++	255++	766	34	68	168

+ Statistically significant at $p<0.05$, ++ statistically significant at $p<0.01$

Table 5.3.3/03-8: Relative organ weights versus brain weights

Dose (mg/m^3)	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^{+}	115146	2641	157454	24028
400	1776	66123	52438	628809	42142^{++}	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^{+}	29784^{+}	89408	3913	7933	19606

$^{+}$ Statistically significant at $p<0.05$, $^{++}$ 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^3. Beginning epithelial alterations, including atrophy or degenerative changes, occurred at the 400 mg/m^3 exposure level only. In the larynx, epithelial alteration and concomitant increased inflammatory infiltrates epithelial metaplasia occurred at 220 and 400 mg/m^3. Minimal epithelial effects were already observed at 20 mg/m^3; 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, spermatic debris in the testes and epididymides and oligospermia occurred at all exposure levels. Retinal atrophy and/or degeneration occurred at 20 mg/m^3 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^3. 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^3 and above, in some rats associated with increased blood congestion. Prussian Blue stained sections revealed a concentration-dependent increase at 220 mg/m^3 and above.

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

III. Conclusions

The derived NOAEC based on the actual gravimetric concentration is 19 mg/m^3 (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^3 (ca. 81 mg/kg bw/day) and above.

CA 5.4 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.
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.
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 L x mol-1 x 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 L x mol-1 x cm-1. Therefore photomutagenicity testing is not required.

Table 5.4-1: Summary of genotoxicity testing*

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

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

CA 5.4.1 In vitro studies

In addition to the *in vitro* studies already available in the Monograph and baseline dossier, a new bacterial reverse mutation assay was conducted in 2010 for the registration of flufenacet in Japan.

Report: [redacted]k;[redacted];2010;M-395211-01
Title: Salmonella typhimurium reverse mutation assay with flufenacet techn.
Report No: 1370100
Document No: M-395211-01-1
Guidelines: **OECD 471; Commission Regulation (EC) No. 440/2008, Method B13/14; US-EPA 712-C-98-247;**
Deviations: none
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test material: 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

2. Vehicle and/or positive control: DMSO
Sodium azide (Na-azide), 4-nitro-o-phenylene diamine (4-NOPD), methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)

3. Test system: *Salmonella typhimurium* strains TA1535, TA1537, TA100,

	TA98, TA102
Metabolic activation:	S9 mix

B. Study design and methods

Dose:	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
Application volume:	0.1 mL
Incubation time /temperature:	Pre-incubation: 60 minutes, 37°C 48 hours, 37°C

II. 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 P	8 ± 1 P	35 ± 1 P	199 ± 15 P	355 ± 31 P
		2500	6 ± 2 P	8 ± 3 P	38 ± 9 P	198 ± 12 P	300 ± 34 P
		5000	8 ± 3 P	4 ± 2 P	40 ± 2 P	203 ± 12 P	318 ± 49 P
	NaN3	10	1675 ± 199			1632 ± 187	
	4-NOPD	10			206 ± 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 P	9 ± 0 P	48 ± 7 P	173 ± 8 P	420 ± 104 P
		2500	12 ± 3 P	6 ± 3 P	37 ± 4 P	176 ± 16 P	451 ± 26 P
		5000	12 ± 3 PM	6 ± 3 P	45 ± 9 P	129 ± 16 P	221 ± 7 P
	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	101 ± 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 P	11 ± 3 P	24 ± 5 P	117 ± 12 P	229 ± 27 P
		2500	8 ± 1 P	9 ± 4 P	24 ± 3 P	86 ± 22 P	210 ± 16 P
		5000	6 ± 1 P	8 ± 3 P	14 ± 5 P	65 ± 8 P M	135 ± 3 P
	NaN3	10	1639 ± 236			1730 ± 203	
	4-NOPD	10			371 ± 43		
	4-NOPD	50		37 ± 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 P	12 ± 3 P	37 ± 4 P	99 ± 15 P	170 ± 16 P
		2500	19 ± 3 P	10 ± 4 P	30 ± 3 P	93 ± 1 P	169 ± 27 P
		5000	10 ± 5 PM	5 ± 1 P M	12 ± 1 PM	62 ± 9 P M	77 ± 11 PM
	2-AA	2.5	347 ± 29	207 ± 28	1742 ± 49	1737 ± 118	
	2-AA	10.0					2434 ± 485

NaN_3 = sodium azide; 2-AA = 2-aminoanthracene, MMS = methyl methane sulfonate, 4-NOPD = 4-nitro-o-phenylene-diamine; P = Precipitate, M = Manual count

III. Conclusion

Flufenacet is considered to be non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

CA 5.4.2 In vivo studies in somatic cells

All necessary *in vivo* genotoxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.4.3 In vivo studies in germ cells

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. Therefore, an *in vivo* study in germ cells is not regarded necessary.

CA 5.5 Long-term toxicity and carcinogenicity

Summary of long-term studies

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	NO(A)EL mg/kg bw/day	LO(A)EL mg/kg bw/day	Main findings seen at LO(A)EL	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.	[redacted] & [redacted], 1995, M-005062-02-1
Mouse 20-month feeding	M F	1.4 9.4	30 77	MetHB ↑ Ocular cataracts ↑	[redacted] & [redacted], 1995 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 – 3rd 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 by the study pathologists. Therefore, a re-assessment of the study endpoint might be considered.

CA 5.6 Reproductive toxicity

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 NOELs 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 NOEL 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 5.6-1: Summary of reproductive and developmental toxicity studies

Study	Sex	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	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.	[redacted], 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	[redacted] et al, 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 ↑	[redacted] et al., 1995 M-004979-01-1

M = male, F= female, D = dam, Fet = fetus, BW = body weight
↓ = decrease, ↑ = increase

CA 5.6.1 Generational studies

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.6.2 Developmental toxicity studies

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of flufenacet.

CA 5.7 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.

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).

Table 5.7-1: Summary of neurotoxicity studies

Study	Sex	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Main effects seen at LOAEL	Reference
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).	[redacted], 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)	[redacted] et al., 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)**	**[redacted], 2000 M-026105-01-1**

CA 5.7.1 Neurotoxicity studies in rodents

Report: [redacted];2000;M-026105-01-1
Title: Developmental neurotoxicity study of technical grade Flufenacet administered orally via diet to Crl:CD BR VAF/Plus presumed pregnant rats
Report No: BC9333
Document No: M-026105-01-1
Guidelines: **US-EPA guideline 83-3; US-EPA OPPTS 870.6300; PMRA DACO:4.5.12;**
Deviations: none
GLP/GEP: **yes**

I. 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:	[redacted], USA
Acclimatization period:	6 days
Diet:	Purina Mills Rodent Lab Chow® #5001-4 in "etts" Form (PMI Nutrition International Inc., St. Louis, Missouri,

	USA), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	individually in stainless-steel wire-bottomed cages; bedding: Bed-o'cobs® (The Andersons Industrial Products Group Maumee, Ohio, USA)

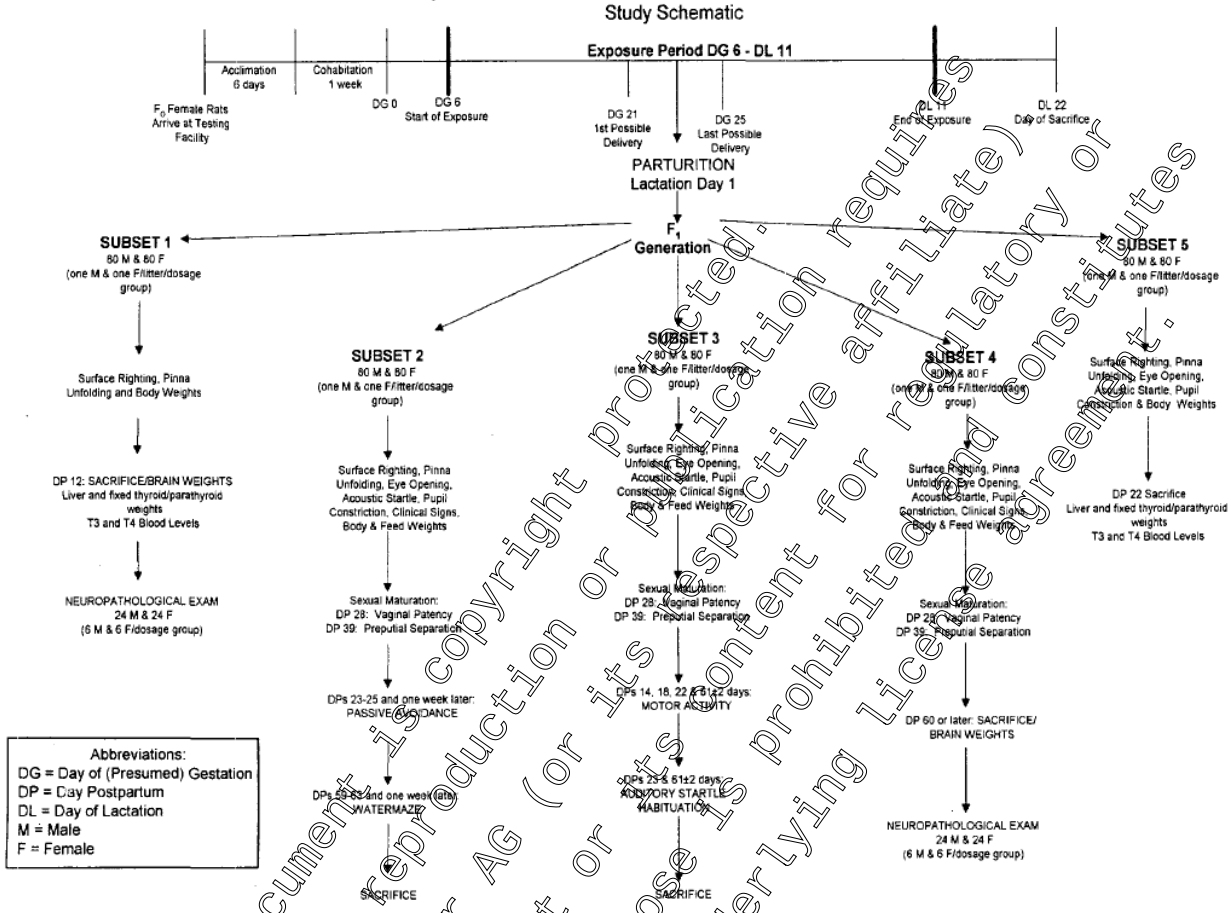
B. Study design and methods

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 14 (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



II. Results and discussion

A. F0 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 5.7.1/03-1: 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 21and DLs 1 to 11).

Table 5.7.1/03-2: 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 5.7.1/03-3: 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 5.7.1/03-4: 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 pupps	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 5.7.1/03-5: Litter observations

Litter observations					
Dose (ppm)		0	20	100	500
Pups delivered		375	338 [1)]	350	332
Liveborn	mean	15.2	13.9 [1)]	13.9	15.0
	N (%)	97.3	98.8 [1)] **	99.4 **	99.7 **
Stillborn	mean	0.4	0.1	0.1	0.0
	N (%)	2.7	0.9 **	0.6 **	0.0 **
Unknown vital status		0	1 [1)]	0	1
Viability index	(%)	97.5	96.7 [1,2,5)]	95.5 [3)]	96.7
Lactation index	(%)	62.5	65.8	67.2	70.0
Mean pup weights/litter (g)					
	DP 1	6.6	6.2 [1)]	6.4	6.1
Preculling	DP 5	8.8	8.8 [2)]	9.0 [2)]	8.4
Postculling	DP 5	9.0	8.9	9.1 [3)]	8.4
Surviving pups/litter	DP 1	15.2	13.9	13.9	15.0
Preculling	DP 5	14.8	13.5	13.3	14.5
Postculling	DP 5	10.0	9.6	9.8	10.0
	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
	DP 18	7.5	7.9	7.7	7.7
	DP 22	7.5	7.9	7.7	7.7

[1)] excluded values for 1 litter as dam delivered one additional pup on DP 2
[2)] excluded values for 2 litters as 1 pup each was culled on DP 5
[3)] 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 postparturn 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.

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 5.7.1/03-5.

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 5.7.1/03-6: Summary of body weights in F1-generation

	Body weights– means (g)							
	Males				Females			
Dose (ppm)	**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 5.7.1/03-7: 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**
	Body weight changes– means (g) of F1-females			
Dose	**Study days**			
(ppm)	**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

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 5.7.1/03-8: Summary of relative feed consumption in F1-generation

	Feed consumption– means (g/kg/day) Males				Females			
Dose (ppm)	**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 5.7.1/03-9: 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 5.7.1/03-10: Summary of terminal body and organ weights in F1-generation

Dose (ppm)	F1-males on DP 12						
	Terminal body weight (g)	Absolute organ weight (g)			Relative organ weights (%)[1)]		
		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)	**F1-females on DP 12**						
	Terminal body weight (g)	**Absolute organ weight (g)**			**Relative organ weights (%)[1)]**		
		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
[1)] Ratio (%) = (organ weight/terminal body weight) x 100
* statistically significant at $p<0.05$; ** statistically significant at $p<0.01$
# 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 5.7.1/03-11: Summary of brain weights and morphometry data in F1-generation

Brain weight and morphometry data (mean values)									
	Males				Females				
Postpartum day	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	1923
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

The following table summarizes the substance-related effects observed in F0-dams and F1-pups (F1-offspring).

Table 5.7.1/03-12: Summary of test-substance-related effects

Dose (ppm)	F0 (dams)	F1 (offspring)
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)

III. Conclusions

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.

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.

Thus, the **NOEL for dams and offspring is 20 ppm** based on effects of the body weight, feed consumption and a slight developmental delay at 100 ppm.

CA 5.7.2 Delayed polyneuropathy studies

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.

CA 5.8 Other toxicological studies

CA 5.8.1 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 5.8.1- 1: Summary of genotoxicity studies with FOE-oxalate (M01)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium*, TA1535, TA1537, TA98, TA100, TA102)	**16 - 5000 µg/plate (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2009 M-358953-01-1**
Mammalian cell gene mutation test (Chinese hamster V79 cells)	**150 - 2400 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2010 M-361724-01-1**
Mammalian chromosome aberration test (Chinese Hamster Ovary (CHO) cells)	**600 - 2400 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2009 M-358043-01-1**
Bioavailability study in rats [Fluorophenyl-UL-^{14}C] FOE5043-oxalate	1 mg/kg bw	Excretion of unchanged FOE-oxalate 70% faeces, 28% urine	[redacted], [redacted], 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 consider 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 5.8.1- 2: Summary of studies with FOE-sulfonic acid (M02)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA100, TA1537, TA98, TA102)	16-5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	[redacted], 2000 M-019064-01-1
Mammalian cell gene mutation test (Chinese hamster V79 cells)	**202-3230 µg/mL (+ S9 mix) 101-808 µg/mL (- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2009 M-361158-01-1**
Mammalian chromosome aberration test (Chinese hamster V79 cells)	**250-3000 µg/mL (+ S9 mix) 200-1000 µg/mL (- S9 mix)**	**Negative (+ S9 mix) Positive (- S9 mix)**	**[redacted], 2010 M-366380-01-1**
***In vivo* Micronucleus test (Mouse bone marrow)**	**500-2000 mg/kg bw (2x intraperitoneal)**	**Negative**	**[redacted], 2010 M-368627-01-1**
***In vivo* Unscheduled DNA synthesis (UDS) assay (rat primary hepatocytes)**	**1000-2000 mg/kg bw (oral)**	**Negative**	**[redacted], 2010 M-397810-01-1**
Rat Acute oral (fasted)	500-2000 mg/kg bw/day	LD_{50} > 2000 mg/kg bw	[redacted]; 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: $t_{1/2}$ ≈ 30 min)	[redacted] & [redacted], 2000 M-042251-01-1

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

<u>FOE-thioglycolate sulfoxide (M04)</u>

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 5.8.1- 3: Summary of studies with FOE-thioglycolate sulfoxide (M04)

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA100, TA1537, TA98, TA102)	16 - 5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	[redacted], 2000 M-032500-01-1

<u>FOE-methylsulfone (M07)</u>

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 5.8.1- 4: Summary of studies with FOE-methylsulfone (M07)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA1537, TA98, TA100, TA102)	**3 - 5000 µg/plate (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2012 M-422370-01-1**
Mammalian cell gene mutation test (Chinese hamster V79 cells)	**43.3 - 2800 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2012 M-430571-01-1**
Mammalian chromosome aberration test (Chinese Hamster V79 cells)	**170.6 - 2730.0 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2012 M-437250-01-1**

* 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_{50} 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 5.8.1- 5: Summary of studies with FOE-thiadone (M09)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA1537, TA98, TA100, TA102)	**3 - 5000 µg/plate (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2011 M-413989-01-1**
Rat acute oral	1650 mg/kg bw (males) 600 mg/kg bw (females)	LD_{50} <1650 /<600 mg/kg bw (males/females):	[redacted] & [redacted], 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 5.8.1- 6: Summary of studies with FOE 5043-trifluoroethanesulfonic acid Na-salt (M44)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA1537, TA98, TA100, TA102)	**3-5000 µg/plate (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2012 M-434728-01-1**
Mammalian cell gene mutation test (Chinese hamster V79 cells)	**125-2000 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2013 M-446033-01-1**
Mammalian chromosome aberration test (Chinese Hamster V79 cells)	**465-1860 µg/mL (+/- S9 mix)**	**Negative (+/- S9 mix)**	**[redacted], 2013 M-447404-01-1**

* 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_{50} 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 5.8.1- 7: Summary of acute and genotoxicity studies with trifluoroacetate (TFA) (M45)*

Study	Dose	Result	Reference
Bacterial reverse mutation assay (*S. typhimurium* TA1535, TA1537, TA98, TA100, TA102)	**16-5000 µg/plate (+/- S9 mix**	**Negative (+/- S9 mix)**	**[redacted], 2005 M-256628-01-1**
Mammalian cell gene mutation test (mouse lymphoma L5178Y cells)	**360-1360 µg/mL (+/- S9 mix**	**Negative (+/- S9 mix)**	**[redacted], 2005 M-260699-01-1**
Mammalian chromosome aberration test (human lymphocytes)	**85-1360 µg/mL (+/- S9 mix**	**Negative (+/- S9 mix)**	**[redacted], 2005 M-260807-01-1**
Rat acute oral (fasted)	**2000 mg/kg bw**	**LD_{50}: >2000 mg/kg bw**	**[redacted], 2013 M-444479-01-1**

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

Table 5.8.1- 8: Summary of repeated toxicity studies with trifluoroacetate (TFA) (M45)*

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 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).**	**[redacted], 2001 M-202165-01-1**
Rat 28-day feeding	**M F**	**1315 1344**	**-- --**	**No adverse effects observed. (liver weight changes without histopathological correlates)**	**[redacted], 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**	**[redacted], 2007 M-283994-01-1**
Rat developmental toxicity gavage	**D Fet**	**150 150**	**-- --**	**No adverse effects at 150 mg/kg bw/d**	**[redacted], 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 <u>not</u> triggered for this compound. The rational 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)").

<u>Acceptable Daily Intake (ADI) derivation for TFA</u>

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)

Report:	[redacted]b; [redacted];2009;M-358953-01
Title:	FOE 5043-Oxalate (Project: FOE 5043 (Flufenacet/AE F133402)) - Salmonella/microsome test - Plate incorporation and preincubation method
Report No:	AT05640
Document No:	M-358953-01-1
Guidelines:	**OECD 471; Council Regulation 440/2008/EEC, Method B.13/14.; US-EPA712-C-98-247, OPPTS 870.5100;** **Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. 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
2. 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)
3. 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

II. 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	**S9**	**Strain**				
Dose (µg/plate)	**mix**	**TA1535**	**TA100**	**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

Mean revertants per plate							
Substance	Dose (µg/plate)	S9 mix	Strain				
			TA1535	TA100	TA1537	TA98	TA102
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	1525	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	–	764				
	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	+	11	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	+	106	2254	275	1534	488
	6	+	62	2023	196	2180	801

III. Conclusion

FOE 5043-oxalate has to be regarded as non-mutagenic.

Report:	[redacted]ü;[redacted];2002;M-361724-01
Title:	FOE 5043-Oxalate - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report No:	1277301
Document No:	M-361724-01-1
Guidelines:	**OECD 476; Commission Regulation (EC) No. 440/2008, B17; US-EPA 712-C-98-221, OPPTS870.5300;** **Deviations: none**
GLP/GEP:	**yes**

I. 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:	Chinese hamster V79 cells
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

II. 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/10^6 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 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table 5.8.1/08- 1: Summary of results

	concentration	S9	relative cloning efficiency I	relative cloning efficiency II	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I	relative cloning efficiency II	mutant colonies/10^6 cells	induction factor
	µg/mL		%	%			%	%		
Experiment I / 5 h treatment			**culture I**				**culture II**			
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	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/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor
Experiment II / 5 h treatment			**culture I**				**culture II**			
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	15.7	0.9	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	1322	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	82.1	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

III. Conclusion

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.

Report:	[redacted]o;[redacted];2009;M-358043-01
Title:	FOE 5043-oxalate (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
Report No:	AT05598
Document No:	M-358043-01-1
Guidelines:	**Directive 2000/32/EC, Method B.10; OECD 473; US-EPA 712-C-98-223, OPPTS 870.5375;** **Deviations: none**
GLP/GEP:	**yes**

I. 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

II. 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 5.8.1/09-1: Summary of cells with structural aberrations

Substance Dose (µg/mL)		+/- S9	Cells scored	Metaphases with aberrations (%) Including gaps	 Excluding gaps	Mitotic Index (%)
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[a]	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	75.3	75.5[a]	39.9[a]
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

[a] statistical significant at $p \leq 0.01$

Table 5.8.1/09-2: 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	

III. Conclusion

FOE 5043-oxalate is considered not to be clastogenic for mammalian cells *in vitro*.

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.

Report: [redacted]; [redacted];2009;M-361158-01
Title: FOE 5043-Sulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report No: 1277302
Document No: M-361158-01-1
Guidelines: **OECD 476; Commission Regulation 440/2008/EC, Method B.17; US-EPA 712-C-98-221, OPPTS 870.5300;**
Deviations: none
GLP/GEP: **yes**

I. 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

II. 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 5.8.1/10-1: Summary of results

	concen-tration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 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	50.5	–	93.0	culture not continued#			90.8	culture not continued#		
Na-salt	101.0	–	96.6	57.2	10.0	0.5	98.5	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	101.0	+	100.9	culture not continued#			94.3	culture not continued#		
Na-salt	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

	concen-tration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 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	13.5	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

III. Conclusion

Based on the study results FOE 5043-sulfonic acid Na-salt is considered to be non-mutagenic in this HPRT assay.

Report: [redacted] ; [redacted];2010;M-366380-01
Title: FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
Report No: AT05870
Document No: M-366380-01-1
Guidelines: **OECD 473; Directive 2000/32/EC, Method B.10; US-EPA 712-C-98-223, OPPTS 870.5375;none**
GLP/GEP: **yes**

I. 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

II. 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 5.8.1/11-1: Summary of cells with structural aberrations

Substance Dose (µg/mL)	+/- S9	Cells scored	Metaphases with aberrations (%)		Mitotic Index (%)
			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 $p < 0.05$; ** statistical significant at $p < 0.01$

Table 5.8.1/11-2: 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	2 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 5.8.1/11-3: 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

III. Conclusion

FOE 5043-sulfonic acid Na-salt is considered to be clastogenic without S9 mix for mammalian cells in vitro.

Report:	[redacted]1;[redacted];2010;M-368627-01
Title:	FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse
Report No:	AT05913
Document No:	M-368627-01-1
Guidelines:	**OECD 474; Council Regulation 440/2008, Method B.12.; US-EPA 712-C-98-226, OPPTS 870.5395;** **Deviations: none**
GLP/GEP:	**yes**

I. 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
Content:	92.4 %
Stability of test compound:	guaranteed for study duration; expiry date: 2010-07-08
2. Vehicle / positive control:	deionised water, phys. saline solution (positive control)/ cyclophosphamide
3. Test animals	
Species:	mouse
Strain:	NMRI BR
Age:	approx. 6 - 12 weeks
Weight at dosing:	39 g - 47 g
Source:	[redacted]Germany
Acclimatisation period:	at least five days
Diet:	fixed-formula feed 3883 (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	tap water , *ad libitum*
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

II. 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 5.8.1/12-1: 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

III. Conclusion

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 in vivo.

Report:	[redacted]; [redacted];2010;M-397810-01
Title:	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - Unscheduled DNA synthesis test with male rat liver cells in vivo
Report No:	AT06167
Document No:	M-397810-01-1
Guidelines:	**Council Regulation No. 440/2008, B.39.; OECD 486; Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: 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

2. Vehicle / positive control: deionised water, corn oil, phys. saline solution / 2-Acetylaminofluorene (2-AAF), 1,2-Dimethylhydrazine (DMH)

3. Test animals

Species:	Wistar rat
Strain:	Crl:(WI)BR
Age:	approx. 6 - 7 weeks
Weight at dosing:	146 g - 183 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	Fixed-formula diet 3883 (10 mm cubes) (Provimi Kliba SA. Switzerland), *ad libitum*
Water:	tap water, *ad libitum*
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

II. 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 5.8.1/13-1: Mean grain values per dose group

Dose group	Mean NNG + SD	Mean NG + SD	Mean CG + SD
Sacrifice interval 16 hours			
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
Sacrifice interval 4 hours			
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

III. Conclusion

FOE 5043-sulfonic acid Na-salt is considered negative in the *in vivo* UDS Assay with rat liver cells.

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.

FOE-methylsulfone (M07)

Report:	[redacted]i; [redacted];2012;M-422370-01
Title:	Salmonella typhimurium reverse mutation assay with FOE 5043-methylsulfone
Report No:	1454201
Document No:	M-422370-01-1
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/GEP:	**yes**

I. 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_3), 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

II. 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 5.8.1-14-1: Summary of results of the pre-experiment and experiment I

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD) TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	Acetone		13 ± 1	12 ± 5	26 ± 2	111 ± 16	492 ± 8
	Untreated		17 ± 6	14 ± 4	24 ± 4	113 ± 2	458 ± 38
	FOE 5043-methyl-sulfone	3 µg	13 ± 4	11 ± 3	27 ± 7	112 ± 4	460 ± 47
		10 µg	17 ± 2	13 ± 1	23 ± 4	111 ± 22	490 ± 24
		33 µg	21 ± 9	10 ± 3	29 ± 2	119 ± 11	499 ± 13
		100 µg	17 ± 3	11 ± 4	26 ± 3	117 ± 2	479 ± 10
		333 µg	13 ± 7	14 ± 4	32 ± 8	110 ± 12	437 ± 8
		1000 µg	12 ± 5	8 ± 2	23 ± 5	95 ± 9	424 ± 6
		2500 µg	17 ± 1	17 ± 6	25 ± 4	100 ± 13	382 ± 32
		5000 µg	17 ± 4	8 ± 4	26 ± 5	87 ± 1	296 ± 21
	NaN_3	10 µg	1880 ± 145			2233 ± 73	
	4-NOPD	10 µg			286 ± 12		
	4-NOPD	50 µg		72 ± 11			
	MMS	3.0 µL					5294 ± 106
With Activation	Acetone		19 ± 3	20 ± 0	34 ± 4	131 ± 3	611 ± 12
	Untreated		16 ± 6	17 ± 3	31 ± 6	116 ± 17	624 ± 21
	FOE 5043-methyl-sulfone	3 µg	19 ± 2	21 ± 4	30 ± 2	123 ± 8	596 ± 18
		10 µg	23 ± 8	20 ± 3	42 ± 3	136 ± 14	633 ± 17
		33 µg	19 ± 3	23 ± 4	40 ± 9	135 ± 9	656 ± 13
		100 µg	21 ± 3	20 ± 6	43 ± 4	123 ± 5	637 ± 15
		333 µg	18 ± 7	19 ± 4	35 ± 6	135 ± 9	573 ± 28
		1000 µg	17 ± 6	21 ± 5	36 ± 3	122 ± 10	533 ± 33
		2500 µg	14 ± 1	19 ± 4	29 ± 8	99 ± 9	481 ± 9
		5000 µg	14 ± 2	12 ± 3	33 ± 5	93 ± 10	353 ± 76
	2-AA	2.5 µg	484 ± 24	358 ± 21	2621 ± 162	2940 ± 32	
	2-AA	10.0 µg					2946 ± 105

NaN_3 = sodium azide
MMS = methyl methane sulfonate
2-AA= 2-aminoanthracene
4-NOPD = 4-nitro-o-phenylene-diamine

Table 5.8.1-14-2: 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 M R	2 ± 1 M R	9 ± 2 M R	17 ± 2 M R	2 ± 2 M R
	NaN_3	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 R M	4 ± 1 R	13 ± 3 M R	54 ± 7 M R	121 ± 15 M R
	2-AA	2.5 µg	368 ± 14	243 ± 25	1751 ± 466	1756 ± 125	
	2-AA	10.0 µg					2957 ± 115

NaN_3 = sodium azide
MMS = methyl methane sulfonate
M = Manual count
2-AA = 2-aminoanthracene
4-NOPD = 4-nitro-o-phenylene-diamine
R = Reduced background growth

III. Conclusion

FOE 5043-methylsulfone is considered to be non-mutagenic in the *Salmonella typhimurium* reverse mutation assay.

Report: [redacted]y: [redacted];2012;M-430571-01
Title: FOE 5043-methylsulfone - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report No: 1454202
Document No: M-430571-01-1
Guidelines: OECD 476; Commission Regulation (EC) No. 440/2008, B.17; US-EPA 712-C-98-221;
Deviations: none
GLP/GEP: yes

I. 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

II. 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 5.8.1/15-1: Summary of results of experiment I and II

	Concen-tration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor
Experiment I / 4 hr treatment										
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
	30.0	–	88.9	111.0	11.6	1.1	71.6	124.0	11.6	0.4
	700.0	–	26.0	122.2	17.9	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
	30.0	+	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/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 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	17.0	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
	30.0	+	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

III. Conclusion

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.

Report:	[redacted]h; [redacted];2012;M-437250-01
Title:	In vitro chromosome aberration test in Chinese hamster V79 cells with FOE 5043-methylsulfone
Report No:	1454203
Document No:	M-437250-01-1
Guidelines:	**OECD 473; Commission Regulation (EC) No. 440/2008, B10; US-EPA 712-C-98-223, OPPTS 870.5375; Deviations: none**
GLP/GEP:	**yes**

I. 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

II. 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 5.8.1/16-1: Summary of cells with structural aberrations

Substance Dose (µg/mL)	+/– S9 mix	Cells scored	Metaphases with aberrations (%) Including gaps*	Metaphases with aberrations (%) Excluding gaps*	Cell numbers % of control	Mitotic Index (%)
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[s]	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[s]	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[s]	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[s]	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[s]	90.4	107.0
2000.0#	–	400	5.8	4.5[s]	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 (%) Including gaps*	 Excluding gaps*	Cell numbers % of control	Mitotic Index (%)
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[s]	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	2.0	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[s]	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[s]	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

III. Conclusion

The test item did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) *in vitro*.
Therefore, FOE 5043-methylsulfone is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation.

FOE-thiadone (M09)

Report:	[redacted]§;[redacted];2011;M-413989-01
Title:	Salmonella typhimurium reverse mutation assay with FOE 5043-Thiadone
Report No:	1423000
Document No:	M-413989-01-1
Guidelines:	**OECD 471; Commission Regulation (EC) No. 440/008, Method B13/14; US-EPA 712-C-98-227, OPPTS 870.5100;** **Deviations: none**
GLP/GEP:	**yes**

I. 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 (NaN3); 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 (NaN3):	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	

II. 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 in-crease of induced revertant colonies.

Table 5.8.1/17-1: Summary of results for pre-experiment and experiment I

Metabolic Activation	Test Group	Dose	Revertant Colony Counts (Mean ±SD)				
			Strain				
		(per plate)	TA1535	TA1537	TA98	TA100	TA102
Without Activation	DMSO	--	12 ± 2 BM	12 ± 5	29 ± 8	126 ± 4	399 ± 6
	Untreated	--	14 ± 3 BM	12 ± 5	25 ± 1	117 ± 22	389 ± 23
	FOE 5043-Thiadone	3 µg	11 ± 3 BM	12 ± 3	27 ± 3	117 ± 2	362 ± 40
		10 µg	11 ± 1 BM	15 ± 5	29 ± 4	119 ± 3	400 ± 9
		33 µg	12 ± 2 BM	14 ± 1	27 ± 7	116 ± 17	374 ± 23
		100 µg	12 ± 2 BM	13 ± 1	26 ± 4	121 ± 2	365 ± 39
		333 µg	11 ± 3 BM	13 ± 2	27 ± 8	136 ± 18	308 ± 33
		1000 µg	9 ± 2 BM	14 ± 1	29 ± 6	120 ± 15	97 ± 17
		2500 µg	8 ± 2 BM	11 ± 3	31 ± 3	92 ± 6	9 ± 3
		5000 µg	6 ± 2 BM	5 ± 2	13 ± 3 UM	76 ± 13	2 ± 1
	NaN3	10 µg	1822 ± 44			1743 ± 210	
	4-NOPD	10 µg			286 ± 34		
	4-NOPD	50 µg		76 ± 10			
	MMS	3.0 µL					4674 ± 98
With Activation	DMSO	--	18 ± 4 BM	18 ± 3	37 ± 13	138 ± 4	522 ± 22
	Untreated	--	19 ± 4 BM	16 ± 1	42 ± 13	140 ± 4	502 ± 60
	FOE 5043-Thiadone	3 µg	17 ± 3 BM	14 ± 2	41 ± 5	116 ± 3	492 ± 43
		10 µg	16 ± 4 BM	18 ± 4	41 ± 8	141 ± 13	510 ± 23
		33 µg	17 ± 2 BM	16 ± 5	34 ± 11	140 ± 13	492 ± 1
		100 µg	18 ± 3 BM	19 ± 3	43 ± 1	138 ± 8	407 ± 18
		333 µg	16 ± 3 BM	14 ± 5	35 ± 4	131 ± 17	353 ± 50
		1000 µg	13 ± 4 BM	16 ± 1	32 ± 5	129 ± 10	144 ± 10
		2500 µg	11 ± 4 BM	15 ± 2	36 ± 2	120 ± 19	15 ± 5
		5000 µg	9 ± 1 BM	6 ± 3	23 ± 3	84 ± 13	5 ± 2
	2-AA	2.5 µg	300 ± 60	224 ± 10	2112 ± 88	2400 ± 76	
	2-AA	10.0 µg					2944 ± 55

Table 5.8.1/17-2: 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	119 ± 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		
4-NOPD	4-nitro-o-phenylene-diamine		

III. Conclusion

FOE 5043-thiadone is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

FOE-trifluoroethanesulfonic acid Na-salt (M44)

Report: [redacted]n;[redacted];2012;M-434728-01
Title: Salmonella typhimurium reverse mutation assay with FOE 5043-trifluoroethanesulfonic acid Na-salt
Report No: 1486601
Document No: M-434728-01-1
Guidelines: **OECD 471; Commission Regulation (EC) No. 440/2008, Method B.13/14; US-EPA 712-C98-247. OPPTS 870.5100;**
Deviations: none
GLP/GEP: **yes**

I. 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_3), 4-nitro-o-phenylene-diamine (4-NOPD), methyl methane sulfonate (MMS), 2-aminoanthracene (2-AA)

3. Test system: Salmonella typhimurium TA1535, TA1537, TA98, TA100, TA102
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_3: 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

II. 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 5.8.1/18-1: Revertant counts (mean ±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_3 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_3	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_3 sodium azide 2-AA 2-aminoanthracene
MMS methyl methane sulfonate 4-NOPD 4-nitro-o-phenylene-diamine

III. Conclusion

FOE 5043-trifluoroethanesulfonic acid Na-salt is non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

Report: [redacted]g; [redacted];2013;M-446033-01
Title: FOE 5043-trifluoroethanesulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report No: 1486603
Document No: M-446033-01-1
Guidelines: **OECD 476; Commission Regulation (EC) No. 440/2008, B17; US-EPA 712-C-98-221, OPPTS 870.5300;**
Deviations: none
GLP/GEP: **yes**

I. 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

II. 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/10^6 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/10^6 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/10^6 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/10^6 cells) was slightly exceeded at 1000 µg/mL (41.5 mutant colonies/10^6 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 5.8.1/19-1: Summary of results for experiment I and II

			relative	relative	relative	mutant		relative	relative	relative	mutant	
	conc.	S9	cloning	cell	cloning	colonies/	induction	cloning	cell	cloning	colonies/	induction
	µg/mL	mix	efficiency I	density	efficiency II	10^6 cells	factor	efficiency I	density	efficiency II	10^6 cells	factor
			%	%	%			%	%	%		
Column	1	2	3	4	5	6	7	8	9	10	11	12
Experiment I / 4 h treatment			culture I					culture II				
Solvent control with water		-	100.0	100.0	100.0	23.0	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#				93.4	culture was not continued#			
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	-	108.4	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#				91.9	culture was not continued#			
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	83.8	21.5	1.5	94.4	80.3	83.6	15.2	0.4
Experiment II / 24 h treatment			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#				102.1	culture was not continued#			
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
Experiment II / 4 h treatment												
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#				81.5	culture was not continued#			
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

III. Conclusion

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.

Report:	[redacted]; [redacted];2013;M-447404-01
Title:	In vitro chromosome aberration test in Chinese hamster V79 cells with FOE 5043-trifluoroethanesulfonic acid Na-salt
Report No:	1486602
Document No:	M-447404-01-1
Guidelines:	**OECD 473; Commission Regulation No. 440/2008, B10; US-EPA 712-C-98-223;** **Deviations: none**
GLP/GEP:	**yes**

I. 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

II. 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 5.8.1/20-1: Summary of results

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 IA; Exposure period 4 hrs without S9 mix; preparation interval 18 hrs							
Solvent control[1]	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	**14.5**[S]	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[1]	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**[S]	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[1]	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**[S]	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[1]	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**[S]	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**[S]	1.3
Experiment IIB: Exposure period 4 hrs with S9 mix; preparation interval 18 hrs							
Solvent control[1]	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**[S]	11.0
930.0	2.6	0.2	65.4	116.7	4.0	**3.5**[S]	1.5
1395.0	4.4	1.2	72.9	110.0	4.0	**3.0**[S]	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**[S]	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

[1] Deionised water 10.0 % (v/v)

III. Conclusion

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.

Trifluoroacetate (TFA) (M45)

Report:	[redacted]d;[redacted];2005;M-256628-01
Title:	Trifluoroacetate (TFA): reverse mutation in five histidine-requiring strains of Salmonella typhimurium
Report No:	2014/82
Document No:	M-256628-01-1
Guidelines:	**OECD 471; EEC Annex V, B13/14; UKEMS Guidelines; Japanese MOHW; JMAFF; ICH Harmonised Tripartite Guideline; US-EPA OPPTS 870.5100;** **Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	Trifluoroacetate (TFA)
Description:	white powder
Lot/Batch no:	01691I/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_3), 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_3: 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

II. 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

Dose (µg/plate)	S-9 mix (-/+)	*Salmonella typhimurium* strains TA98	TA100	TA1535	TA1537	TA102
		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 ± 5	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	13 ± 2	17 ± 7	211 ± 27
Positive controls						
2NF: 5.0	–	1192 ± 121				
NaN_3: 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				
NaN_3: 2.0	–		438 ± 30	438 ± 30		
AAC: 50.0					75 ± 12	
MMC: 0.2	–					620 ± 8

Dose (µg/plate)	S-9 mix (-/+)	*Salmonella typhimurium* strains TA98	TA100	TA1535	TA1537	TA102
		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 ± 0	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 ± 3	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	200 ± 72	124 ± 16	
AAN: 20.0	+					796 ± 246

* Dunnett's test, significam at 1% level

TFA = trifluoro acetate; 2NF = 2-Nitrofluorene (2NF); NaN_3 = sodium azide; AAC = 9-Aminoacridine; MMC = mitomycin C; B[a]P = Benzo[a]pyrene; AAN = 2-Aminoanthracene

III. Conclusion

Trifluoroacetate (TFA) did not induce mutation in five histidine-requiring strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA 102) when tested under the conditions of this study.

Report: [redacted]K; [redacted];2005;M-260699-01
Title: Trifluoroacetate (TFA) - Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique
Report No: 2014/84-D6173
Document No: M-260699-01-1
Guidelines: **OECD 476; UKEMS Guidelines; US-EPA OPPTS 870.5300; Deviations: none**
GLP/GEP: **yes**

I. 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$^{+/-}$-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

II. 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§	% rel. total growth	mutant frequency§
Experiment 1 (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	85.40
NQO	0.15	57	314.40		
	3	42	435.99		
BP	2			46	648.58
	3			30	975.59
Experiment 2 (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

§ 5-TFT (5-trifluorothymidine) resistant mutants 10^6 viable cells 2 days after treatment
* Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level

III. Conclusion

Trifluoroacetate (TFA) did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells in the absence and presence of a rat liver metabolic activation system.

Report: [redacted]6;[redacted];2005;M-260807-01
Title: Trifluoroacetate (TFA) - Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
Report No: 2014/83-D6172
Document No: M-260807-01-1
Guidelines: **OECD 473; EEC Annex V, B.10; Japanese MOHW (1999); JMAFF; ICH Harmonised Tripartite Guideline; US-EPA-OPPTS Guideline 870.5375;**
Deviations: none
GLP/GEP: **yes**

I. 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)

II. 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 5.8.1/23-1: Summary of cells with structural aberrations

Substance Dose (µg/mL)		+/- S9	Cells scored	Cells with aberrations Including gaps	Cells with aberrations Excluding gaps	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[a]	
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[a]	
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[a]	
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[a]	

[a] statistical significance $p < 0.001$

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 hyperdiploid	endoreduplicated	polyploid	Total abs	% with num abs
Experiment 1 (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
Experiment 2 (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
Experiment 2 (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	-	202	0	0	0	0	0

abs = aberrations, num = numerical

III. Conclusion

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).

Report: [redacted]; [redacted];2013;M-444479-01
Title: Sodium Trifluoroacetate - Acute oral toxicity study in rats
Report No: 12/333-001P
Document No: M-444479-01-1
Guidelines: **OECD 425; Commission Regulation (EC) No 440/2008; B.1.TRIS; US-EPA 712-C-98-190 , OPPTS 870.1100;**
Deviations: none
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test material: 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

2. Vehicle: distilled water

3. Test animals
Species: Wistar rat
Strain: CRL:(WI)
Age: 8 - 9 weeks
Weight at dosing: 190 g - 220 g
Source: [redacted] Germany
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) *ad libitum*
Water: tap water *ad libitum*
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

II. 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.

III. Conclusion

Sodium trifluoroacetate is non-toxic after acute oral administration with an LD_{50} value above 2000 mg/kg bw in female rats.

Report: [redacted];[redacted];2001;M-202165-01
Title: Trifluoroacetate - Exploratory 14-day toxicity study in the rat by dietary administration
Report No: C016316
Document No(s): M-202165-01-1
Guidelines: not applicable
GLP/GEP: no

I. Materials and methods

A. Materials

1. Test material: Trifluoroacetate
- Description: white powder
- Lot/Batch no: 129113458
- Purity: 98.7% (Sigma-Aldrich)

2. Positive control: clofibric acid (positive control for peroxisomal proliferation)

3. Test animals
- Species: Wistar (HAN) rats
- Strain: RJ: Wl (TOPS HAN)
- Age: 8 weeks
- Weight at dosing: males: 304 g - 355 g; females: 212 g - 231 g
- Source: [redacted] France
- 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

II. 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 5.8.1/25-1: Summary of mean body weights

Dose (ppm)	Trifluoroacetate 0	600	1200	2400	Pos. Contr.	Trifluoroacetate 0	600	1200	2400	Pos. 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 5.8.1/25-2: Summary of mean body weight gain

Dose (ppm)	Trifluoroacetate 0	600	1200	2400	Pos. Contr.	Trifluoroacetate 0	600	1200	2400	Pos. 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 5.8.1/25-3: Summary of mean food consumption

Dose (ppm)	**Trifluoroacetate 0**	**600**	**1200**	**2400**	**Pos. Contr.**	**Trifluoroacetate 0**	**600**	**1200**	**2400**	**Pos. Contr.**
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 5.8.1/25-4: Summary of haematology

Dose (ppm)	**Trifluoroacetate 0**	**600**	**1200**	**2400**	**Pos. Contr.**	**Trifluoroacetate 0**	**600**	**1200**	**2400**	**Pos. Contr.**
Parameter (unit)	males					females				
White blood cell count (10^9/L)	15.1	12.8	13.9	14.4	17.3	11.9	10.9	9.9	8.3*	11.7
Neutrophil count (10^9/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 (10^9/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 5.8.1/25-5: Summary of clinical chemistry

	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
Dose (ppm)	**0**	**600**	**1200**	**2400**	**Contr.**	**0**	**600**	**1200**	**2400**	**Contr.**
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 ≥ 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 5.8.1/25-6: Summary of hepatotoxicity assessment

	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
Dose (ppm)	**0**	**600**	**1200**	**2400**	**Contr.**	**0**	**600**	**1200**	**2400**	**Contr.**
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 ≥ 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 5.8.1/25-7: Summary of organ weights

	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
Dose (ppm)	**0**	**600**	**1200**	**2400**	**Contr.**	**0**	**600**	**1200**	**2400**	**Contr.**
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**
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. Comtr. = 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.

III. Conclusion

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 ≥ 1200 ppm (85 mg/kg bw/day).

Report:	[redacted]5;[redacted];2005;M-259106-01
Title:	Sodium trifluoroacetate (TFA) - 28-day toxicity study in the rat by dietary administration
Report No:	SA05054
Document No:	M-259106-01-1
Guidelines:	**OECD 407; Directive 96/54/EC, Method B.7; Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	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
2. Vehicle:	none
3. Test animals	
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], France
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) *ad libitum*
Water:	tap water *ad libitum*
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

II. 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 ≥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 5.8.1/26-1: Summary of clinical chemistry

Parameter	Dose group (ppm) males					Dose group (ppm) females				
means (unit)	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 5.8.1/26-2: 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 5.8.1/26-3: 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≤0.05	NC	NC	+7% NS	+15% p≤0.05
Mean liver to body weight ratio	NC	+15% p≤0.01	+19% p≤0.01	+33% p≤0.01	NC	+7% NS	+13% p≤0.05	+18% p≤0.01
Mean liver to brain weight ratio	NC	NC	+12% NS	+27% p≤0.01	NC	+10% NS	+12% NS	+24% p≤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.

III. Conclusion

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.

Report:	[redacted]2;[redacted];2007;M-283994-01
Title:	Sodium trifloroacetate (TFA) 90-day toxicity study in the rat by dietary administration
Report No:	SA06080
Document No:	M-283994-01-1
Guidelines:	**OECD 408; Directive 2001/59/EC , Method B.26; US-EPA OPPTS 870.3100; JMAFF 12 Nousan n°8147;** **Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: 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

2. Vehicle: none

3.Test animals

Species:	Wistar rat
Strain:	Rj:WI(IOPS.HAN)
Age:	7 weeks
Weight at dosing:	males: 226 g - 259 g, females: 165 g - 200 g
Source:	[redacted], France
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

II. 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 5.8.1/27-1: 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+	378+	404+	421+	439+	450+	465+	471+	482+	490+	496+
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 ($p < 0.05$)
+ 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≤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≤0.01) was noted at 16000 ppm in females only. This slight change was associated with lower mean corpuscular volume (−6%, p≤0.01), mean corpuscular haemoglobin (−7%, p≤0.01) and haematocrit (−6%, p≤0.01).

At 1600 ppm, lower mean haemoglobin concentration (−4%, p≤0.05), essentially due to low values noted in two animals, and lower mean corpuscular haemoglobin (−3%, p≤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 5.8.1/27-2: 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≤0.05); ** = statistically significant different from control (p≤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 5.8.1/27-3: Summary of clinical chemistry parameter changes in males and females

Parameter	Mean ± SD (% change when compared to control) Bili (mmol/L)	Gluc (mmol/L)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Dose (ppm)	**males**				
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%)
Dose (ppm)	**females**				
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≤0.05); ** = statistically significant different from control (p≤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 5.8.1/27-4: Semi-quantitative urinalysis- incidence summary table

Dose (ppm)		0	160	1600	16000	0	160	1600	16000
Grade		males				females			
n° samples examined		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	2	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 5.8.1/27-5: Summary of liver weight data at terminal sacrifice

Parameter	Mean (% change when compared to control)					
	Absolute liver weight (g)		Liver to body weight ratio		Liver to brain weight ratio	
Dose (ppm)	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≤0.05); ** = statistically significant different from control (p≤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 5.8.1/27-6: 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
Centrilobular to panlobular hepatocellular hypertrophy, diffuse								
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
Periportal hepatocellular vacuolation, diffuse								
Minimal	4	3	0	0	5	6	7	0
Total	4	3	0	0	5	6	7	0
Hepatocellular necrotic focus (i), focal/multifocal								
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

III. Conclusion

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.

Report:	[redacted]h;[redacted];2010;M-411209-01
Title:	Trifluoroacetic acid: Embryo-fetal oral gavage toxicity study in rats
Report No:	09-4352
Document No:	M-411209-01-1
Guidelines:	**US-EPA OPPTS 870.3700; OECD 414; Deviations: none**
GLP/GEP:	**yes**

CA 5.8.2 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/secreting 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/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 21 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 5.8.2-1: Summary of additional studies on the active substance

Study	Sex	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Main effects seen at LOAEL	Reference
Rat developmental neurotoxicity diet	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)	[redacted], 2000 M-026105-01-1 also cited MCA 5.7.1
Rat range-finder diet	Dam Pup	Na (DG 6-DL 10 or 16)	Na	500 ppm maternal and pup toxicity	[redacted], 2012 M-434509-01-1
Rat mechanistic study thyroid effects, diet	Dam Fet	35 (500 ppm) (DG 6-20)	--	Slight changes in T4 without correlating changes in T3 and TSH, as well as histopathological changes in the thyroid	[redacted], 2012 M-435619-01-1
Rat mechanistic study thyroid effects, diet	Dam Pup	17 (100 ppm) (DG 6-DL21)	84 (500 ppm)	Dams: BW ↓, T4 ↓ (-70%), T3 ↓ (-19%), rel. liver weight ↑, thyroid follicular cell hypertrophy 2 cases Pups: BW/gain ↓, T3 ↓ (-24 %)	[redacted], 2012 M-435313-01-1
Rat mechanistic study thyroid effects, gavage	Pup	1.7 (DL 10-21)	--	No adverse effects.	[redacted], 2012 M-435313-01-1

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 slightly irritating to eyes of rabbits, and showed no skin sensitizing potential under the conditions of the Magnusson-Kligman test.

Table 5.8.2- 2: Summary of studies with FOE 5043-hydroxy

Study	Dose	Result	Reference
Bacterial reverse mutation assay	8-5000 mg/plate (+/- S9 mix)	Negative (+/- S9 mix)	[redacted], 1993 M-004586-01-1
Acute oral, rat	500-800-1000 mg/kg bw (m) 200-400-500 mg/kg bw (f)	LD_{50} approx. 726 mg/kg bw (m) LD_{50} approx. 474 mg/kg bw (f)	[redacted], 1992 M-004579-01-1
Acute inhalation, rat (4 hours)	0-301-6802 mg/m³	LC_{50} > 6802 mg/m³ (males) LC_{50} ≈ 6800 mg/m³ (females)	[redacted], 1993 M-004589-01-2
Skin irritation, rabbit	0.5 g/patch (undiluted)	Not irritating	[redacted], 1992 M-004564-01-1
Eye irritation, rabbit	0.1 mL/animal (undiluted)	Slightly irritating (classification not triggered)	[redacted], 1992 M-004564-01-1
Skin sensitization, Guinea pig (MKT*)	Intradermal: 5% Topical: 50% Challenge: 25%	Not sensitizing	[redacted], 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/m³ was observed. Signs of respiratory tract irritation were observed at concentrations of ≥0.5 mg/m³.

Table 5.8.2- 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)	[redacted], 1993 M-004606-01-1
Acute oral, rat	50-100-150-170-200-300-1000 mg/kg bw	LD_{50} > 150 - < 2000 mg/kg bw	[redacted], 1992 M-004578-01-1
Acute inhalation, rat (4 hours)	Dust: 0-35.3-122.7 mg/m³ Aerosol: 0-8.2-52.6-89.8-146.3 mg/m³	LC_{50} ≈ 69 mg/m³ (males) LC_{50} > 146.3 mg/m³ (females)	[redacted], 1992 M-004576-01-1
Skin irritation, rabbit	0.5 g/patch (undiluted)	Irritating	[redacted], 1992 M-004522-01-1
Eye irritation, rabbit	0.1 mL/animal (undiluted)	Severely irritating	[redacted], 1992 M-004522-01-1
Skin sensitization, Guinea pig (MKT**)	Intradermal: 5% Topical: 6% Challenge: 1 and 0.5%	Sensitizing	[redacted], 1994 M-004673-01-1
Sensory irritation, mice (45 min)	0-4.3-9.8-13.3 mg/m³	Severe sensory irritation potential non-irritant threshold concentration 0.3 mg/m³	[redacted], 1993 M-004601-01-1
Sub-acute inhalation, range finder, rat (5 x 6h/day)	0-0.5-3.5-16.3 mg/m³	NOAEC: 0.5 mg/m³ LOAEC: 3.5 mg/m³ (slight body weight changes, respiratory tract irritation, hypothermia caused by irritation) Mortality occurred at 16.3 mg/m³	[redacted], 1992 M-004571-01-2
Sub-acute inhalation, rat (5 x 6h/day) 28-days	0-0.47-2.04-7.63 mg/m³	LOAEC: 0.47 mg/m³ Inflammatory changes in the upper respiratory tract, sensory irritation, effect on body weight, clinical signs	[redacted], 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 5.8.2- 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_{50} > 1000 mg/kg bw (m) LD_{50} > 200 < 1000 mg/kg bw (f)	[redacted], 1994 M-004640-01-1
Acute inhalation, rat (4 hours)	0-2350 mg/m³	LC_{50} > 2350 mg/m³ (maximum technically attainable concentration)	[redacted], 1996 M-004734-01-1
Skin irritation, rabbit	0.5 g/patch	Not irritating	[redacted], 1994 M-004662-01-1
Eye irritation, rabbit	0.1 mL/animal	Not irritating	[redacted], 1994 M-004662-01-1

Flufenacet

Report: [redacted]y;[redacted];2012;M-434509-01
Title: 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
Document No: M-434509-01-1
Guidelines: **not applicable;**
Deviations: not applicable
GLP/GEP: **No**

I. 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: 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], France
Acclimatisation period:	At least 5 days
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 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)
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

II. 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 5.8.2/06-1: Summary of maternal body weight and body weight changes (mean ± standard deviation)

Dose (ppm)	Body weight (g) GD 13	GD 20	Body weight change (g) GD 13-20
	(mean ± standard deviation)		
0	35.5 ± 9.14	125.3 ± 16.01	89.9 ± 9.20
500	32.9 ± 11.99	111.7 ± 15.33	78.8 ± 9.09*T
Dose (ppm)	**Body weight (g) LD 4**	**LD 7**	**LD 14**
	(mean ± standard deviation)		
0	13.7 ± 13.21	28.2 ± 13.06	34.9 ± 19.46
500	14.8 ± 11.01	24.4 ± 10.77	38.2 ± 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 5.8.2/06-2: Summary of maternal food consumption

Dose	Mean achieved dietary intake of flufenacet (mg/kg bw/day)						
	GD 6-13	GD 13-20	Mean gestation phase	LD 0-4	LD 4-7	LD 7-14	Mean lactation phase
500 ppm	35.2	36.2	**35.7**	50.2	69.1	77.2	**67.7**

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 5.8.2/06-3: Summary of pup body weights and litter weights

Dose (ppm)	Pup weight (g) PND 4	PND 7	PND 14
	(mean ± standard deviation)		
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**	**PND 14**
	(mean ± standard deviation)		
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 5.8.2/06-4: 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 T4-levels.

Hormone analyses data are summarized in the following tables.

Table 5.8.2/06-5: Summary of TSH hormone analyses in pubs

	TSH concentration (ng/mL) mean ± standard deviation (% change when compared to controls)			
	PND 10 pubs		**PND 16 pubs**	
Dose (ppm)	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 5.8.2/06-6: Summary of T4 hormone analyses in pubs

	T4 concentration (µg/mL) mean ± standard deviation			
	PND 10 pubs		**PND 16 pubs**	
Dose (ppm)	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 pubs 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 5.8.2/06-7: Summary of analyses in liver tissue

	Concentration in liver (mean ± standard deviation)			
	Flufenacet		**Thiadione**	
Flufenacet dose (ppm)	Extract (µg/L)	Tissue (µg/g)	Extract (µg/L)	Tissue (µg/g)
	PND 10			
0	ND	ND	<50	--
500	ND	ND	0.56 ± 0.11	1.82 ± 0.42
	PND 16			
0	<10	--	<50	
500	<10	--	137 ± 41	1.13 ± 0.31

III. Conclusion

Based on the study results flufenacet at dietary exposure of 500 ppm induced maternal and pup toxicity. Thus, 500 ppm was considered to be an appropriate dose level for subsequent toxicity assays. 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.

Report: [redacted]2;[redacted];2012;M-435619-01
Title: Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat (gestational exposure phase)
Report No: SA 10154
Document No: M-435619-01-1
Guidelines: **US E.P.A. OCSPP 870.SUPP;**
Deviations: not specified
GLP/GEP: **yes**

I. 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 / 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:	232 - 375 g
Source:	[redacted] France
Acclimatisation period:	At least 3 days
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 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)
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)

II. 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 5.8.2/07-1: Summary of maternal body weight

Dose (ppm)	Mean body weight ± standard deviation (g) Gestation day			
	3	6	13	20
0	278.2 ± 27.7	293.3 ± 26.0	340.2 ± 33.2	420.3 ± 43.7
20	276.0 ± 36.4	293.5 ± 35.6	336.7 ± 41.8	421.7 ± 47.3
100	272.8 ± 36.3	289.9 ± 37.5	336.9 ± 40.7	426.9 ± 46.3
500	272.7 ± 31.0	294.2 ± 30.99	337.1 ± 33.8	413.9 ± 38.3

15 (PTU)	276.1 ± 33.0	291.0 ± 34.0	333.7 ± 38.9	406.0 ± 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

	Mean achieved dietary intake (mg/kg bw/day)			
	PTU	Flufenacet		
Dose (ppm)	**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	Terminal Body weight	Organ weights (mean ± standard deviation) (% change when compared to controls)			
		Liver weight		Thyroid weight	
(ppm)	(g)	Absolute (g)	Relative (%)	Absolute (mg)	Relative (%)
0	418.4 ± 43.3	14.85 ± 1.49	3.553 ± 0.155	19.5 ± 6.0	0.0046 ± 0.0011
20	420.9 ± 47.4	14.87 ± 1.85	3.534 ± 0.202	17.1 ± 3.7	0.0040 ± 0.0006
100	426.6 ± 45.4	15.22 ± 1.75	3.573 ± 0.264	18.7 ± 6.8	0.0043± 0.0011
500	413.5 ± 38.0	15.46 ± 1.57	3.741 ± 0.206	15.46 ± 1.57	0.0043 ± 0.0009
15 (PTU)	405.9 ± 46.8	13.58 ± 1.71	3.343 ± 0.152**	37.2 ± 3.9**	0.0092 ± 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 ± standard deviation (% change when compared to controls)		
	T3 (ng/ml)	**T4 (µg/dL)**	**TSH (ng/mL)**
0	1.08 ± 0.111	1.90 ± 0.837	2.200 ± 1.6260
20	1.07 ± 0.120 (–1%)	1.62 ± 0.593 (–15%)	1.926 ± 0.5525 (–12%)
100	1.05 ± 0.224 (–3%)	1.66 ± 0.529 (–13%)	1.823 ± 0.6124 (–17%)
500	0.98 ± 0.088 (–9%)	1.20 ± 0.653 (–37%)	1.691 ± 0.5493 (–23%)
15 (PTU)	0.63 ± 0.13** (–42%)	0.34 ± 0.24** (–82%)	9.49 ± 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	Terminal Body weight	Organ weights (mean per litter per group ± standard deviation) (% change when compared to controls)			
		Liver weight		Thyroid weight	
(ppm)	(g)	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 ± standard deviation (% change when compared to controls)		
	T3	**T4**	**TSH**
0	0.55 ± 0.058	0.70 ± 0.397	4.493 ± 1.2687
20	0.57 ± 0.051 (+4%)	0.55 ± 0.308 (–21%)	3.645 ± 0.4770 (–19%)
100	0.55 ± 0.057 (0%)	0.48 ± 0.186 (–31%)	3.174 ± 0.6148** (–29%)
500	0.54 ± 0.040 (–2%)	0.36 ± 0.259 (–49%)	2.474 ± 0.5043** (–45%)
15 (PTU)	0.51 ± 0.04	0.15 ± 0.15** (–79%)	11.67 ± 1.90** (+160%)

* Statistically significantly different from controls ($p \leq 0.05$)
** Statistically significantly different from controls ($p \leq 0.01$)

III. Conclusion

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.

Thus, a dose level of 500 ppm (equating to 34.8 mg/kg/day) is considered a No Observed Adverse Effect Level (NOAEL) for both dams and fetuses.

Report: [redacted]t;[redacted];2012;M-435313-01
Title: Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat by dietary exposure (gestational and lactational exposure phase)
Report No: SA 11052
Document No: M-435313-01-1
Guidelines: **US E.P.A. OCSPP 870.SUPP;**
Deviations: not specified
GLP/GEP: **yes**

I. 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 / 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] France
Acclimatisation period:	At least 4 days
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	Flufenacet: 0, 20, 100, 500 ppm corresponding to : 0-1.3-6.6-35.2 mg/kg bw/day (gestation) corresponding to : 0-3.4-16.7-84.2 mg/kg bw/day (lactation) Positive control PTU: 15 ppm corresponding to approx. 0.9 and 1.9 mg/kg bw/day during gestation and lactation, respectively
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)

II. 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 5.8.2/08-1: 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 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 weight

Flufenacet

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 LD21) 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 5.8.2/08-2: Summary of maternal body weight

Dose (ppm)	Mean body weight (g)							
	Gestation			Lactation				
	GD6	GD13	GD20	LD0	LD4	LD7	LD14	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 intake

Flufenacet

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/day received by the animals during the study are summarized in the following table.

Table 5.8.2/08-3: Summary of test compound intake

Dose level	Mean achieved test compound intake (mg/kg bw/day)			
	PTU	Flufenacet		
	15 ppm	20 ppm	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 analyses

Flufenacet

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 5.8.2/08-4: Summary of hormone analyses in dams on LD 21

Dose group (ppm)	**Hormone analysis** mean ± standard deviation (% change when compared to controls)		
	T3 (ng/mL)	**T4 (µg/dL)**	**TSH (ng/mL)**
0	0.73 ± 0.088	2.29 ± 0.428	1.365 ± 1.0147
20	0.71 ± 0.159 (-3%)	2.36 ± 0.442 (+3%)	2.277 ± 1.7483 (+67%)
100	0.67 ± 0.144 (–8%)	1.69 ± 0.395** (–26%)	1.890 ± 1.1473 (+38%)
500	0.59 ± 0.112* (–19%)	0.68 ± 0.245** (–70%)	2.669 ± 2.6633 (+96%)
15 (PTU)	0.44 ± 0.090#T	0.38 ± 0.103#T	18.530 ± 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 weights

Flufenacet

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 5.8.2/08-5: Summary of dam organ weights

Dose group (ppm)	Terminal Body weight (g)	Organ weights (mean ± standard deviation) (% change when compared to controls) Liver weight Absolute (g)	Liver weight Relative (%)	Thyroid weight Absolute (g)	Thyroid weight 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 5.8.2/08-6: 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	**0**
20	14	0	0	**0**
100	14	0	1	**1**
500	13	2	0	**2**
15 (PTU)	12	No data	No data	**12#**

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 5.8.2/08-7: 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 weights

Flufenacet

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 5.8.2/08-8: Summary of pup body weight

Dose (ppm)	Mean body weight (g) Males PND4	PND7	PND14	PND21	Females 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 5.8.2/08-9: 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≤0.001) and mean TSH concentration was markedly higher (+721%; p≤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≤0.001), mean T4 concentrations were markedly lower (-93% in males and -94% in females; p≤0.001) and mean TSH concentrations were markedly higher (+697% in males; p≤0.001 and +429% in females; p≤0.001), when compared to the controls.

Thyroid hormone data are summarized in the following tables.

Table 5.8.2/08-10: Summary of hormone analyses in pups on PND 4 (pooled per litter)

Dose group (ppm)	**Hormone analysis** mean ± standard deviation (% change when compared to controls) T3 (ng/mL)	T4 (µg/dL)	TSH (ng/mL)
0	0.71 ± 0.141	1.00 ± 0.226	1.505 ± 0.5547
20	0.72 ± 0.137 (+1%)	1.00 ± 0.217 (0%)	1.521 ± 0.7668 (+1%)
100	0.76 ± 0.135 (+7%)	0.97 ± 0.223 (–3%)	2.963 ± 4.1301 (+97%)
500	0.72 ± 0.157 (+1%)	1.05 ± 0.363 (+5%)	1.237 ± 0.4397 (–18%)
15 (PTU)	0.44 ± 0.070#T	0.12 ± 0.120#W	12.361 ± 3.2925#T

* Statistically significantly different from controls (p ≤ 0.05)
** Statistically significantly different from controls (p ≤ 0.01)
#T Statistically significantly different from controls (p ≤ 0.001) Student T test
#W Statistically significantly different from controls (p ≤ 0.001) adjusted Welch test

Table 5.8.2/08-11: Summary of hormone analyses in pups on PND 21 (1 pup/sex/litter)

Dose group (ppm)	**Hormone analysis** mean ± standard deviation (% change when compared to controls) Males T3 (ng/mL)	Males T4 (µg/dL)	Males TSH (ng/mL)	Females T3 (ng/mL)	Females T4 (µg/dL)	Females TSH (ng/mL)
0	1.10 ± 0.196	3.18 ± 0.458	0.779 ± 0.506	1.24 ± 0.179	3.09 ± 0.667	1.236 ± 0.423
20	0.94 ± 0.217 (–15%)	3.30 ± 0.975 (+4%)	0.856 ± 0.423 (+10%)	1.14 ± 0.191 (–8%)	3.36 ± 1.091 (+9%)	1.395 ± 0.763 (+13%)
100	0.92 ± 0.152* (–16%)	3.16 ± 0.673 (–1%)	1.172 ± 1.208 (+50%)	1.16 ± 0.145 (–6%)	3.03 ± 0.554 (–2%)	1.624 ± 1.086 (+31%)
500	0.84 ± 0.139** (–24%)	3.41 ± 0.864 (+7%)	0.790 ± 0.559 (+1%)	1.04 ± 0.178* (–16%)	3.33 ± 0.535 (+8%)	1.218 ± 0.589 (–1%)
15 (PTU)	0.14 ± 0.053#W	0.23 ± 0.166#W	6.208 ± 1.9850#W	0.21 ± 0.078#W	0.18 ± 0.136#W	6.541 ± 2.2392#T

* Statistically significantly different from controls (p ≤ 0.05)
** Statistically significantly different from controls (p ≤ 0.01)
#T Statistically significantly different from controls (p ≤ 0.001) Student T test
#W Statistically significantly different from controls (p ≤ 0.001) adjusted Welch test

Gross pathology, terminal body and organ weights

Flufenacet

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 5.8.2/08-12: 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 (%)
	PND 4 - Males			**PND 4 - Females**		
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**
	PND 21 - Males			**PND 21 - Females**		
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

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 (%)
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 pathology

Flufenacet

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.

III. Conclusion

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.

Analytical methods

A method for the determination of Flufenacet by HPLC analysis in rodent diet was developed. The reference of the study report is presented in the following.

Report: [redacted]4;[redacted];[redacted];2010;M-393212-01
Title: Flufenacet - Determination by high performance liquid chromatography analysis in ground rodent diet
Report No: SA 10292
Document No: M-393212-01-1
Guidelines: **not applicable**
GLP/GEP: **Yes**

Report:	[redacted]d;[redacted];[redacted];2011;M-426082-01
Title:	Flufenacet - Determination by high performance liquid chromatography analysis in ground rodent diet
Report No:	SA 11357
Document No:	M-426082-01-1
Guidelines:	**O.E.C.D. Principles of Good Laboratory Practice, 1997 (January 26, 1998) and Article Annexe II à l'article D523-8 du Code de l'Environnement du 16 octobre 2007 (French GLP Legislation)** **US EPA OCSPP 870.SUPP;Not specified**
GLP/GEP:	**Yes**

The stability of flufenacet in ground rodent diet was evaluated. The reference of the study report is presented in the following.

Report:	[redacted]v;[redacted];2012;M-435625-01
Title:	Flufenacet - Stability in ground rodent diet
Report No:	SA 11175
Document No:	M-435625-01-1
Guidelines:	**OECD, 1997;not specified**
GLP/GEP:	**Yes**

Report:	[redacted];2012;M-435126-01
Title:	Flufenacet (FOE5043) - Comparative thyroid sensitivity assay in the rat complementary assay (gavage exposure of pups)
Report No:	SA 11167
Document No:	M-435126-01-1
Guidelines:	**US-EPA OCSPP 870.SUPP;none**
GLP/GEP:	**Yes**

I. Materials and methods

A. Materials

1. Test material: Flufenacet

Description:	Beige solid
Lot/Batch no:	NK01AX0177
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], France
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

II. 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 5.8.2/12-1: 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)	**Males**										
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
	Females										
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 pubs

	Hormone analysis mean ± standard deviation (% change when compared to controls)			
	Males		**Females**	
Dose (mg/kg bw/day)	0	1.7	0	1.7
T3 (ng/mL)	1.14 ± 0.16	1.07 ± 0.16 (–6%)	1.09 ± 0.16	1.05 ± 0.15 (–4%)
T4 (µg/dL)	3.40 ± 0.51	3.19 ± 0.42 (–6%)	3.13 ± 0.74	3.24 ± 0.51 (+4%)
TSH (ng/mL)	1.66 ± 0.80	1.30 ± 0.79 (–22%)	1.65 ± 0.59	1.63 ± 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 ± standard deviation)	Relative thyroid weight
males			
0	50.0 ± 4.8	0.0045 ± 0.00155	0.0087 ± 0.00269
1.7	52.0 ± 5.8	0.0052 ± 0.00120	0.0102 ± 0.00300
females			
0	49.0 ± 4.2	0.0047 ± 0.00118	0.0096 ± 0.00263
1.7	49.0 ± 4.6	0.0051 ± 0.00160	0.0105 ± 0.00371

Gross pathology
There were no treatment-related effects observed.

Microscopic pathology
No treatment-related effect on the thyroid was observed.

III. Conclusion

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).

Analytical methods
A method for the determination of Flufenacet by HPLC analysis in vehicle was developed. The reference of the study report is presented in the following.

Report: [redacted]0; [redacted]; [redacted];2011;M-411290-01
Title: Flufenacet - Determination by high performance liquid chromatography analysis in aqueous 0.5 percent methylcellulose 400
Report No: SA 11176
Document No: M-411290-01-1
Guidelines: OECD, 1997; not specified
GLP/GEP: yes

The stability of flufenacet in vehicle was evaluated. The reference of the study report is presented in the following.

Report:	[redacted] ;[redacted];2011;M-418123-01
Title:	Flufenacet - Stability in aqueous 0.5 percent methylcellulose 400
Report No:	SA 11177
Document No:	M-418123-01-1
Guidelines:	**(O.E.C.D. Principles of Good Laboratory Practice, 1997 (January 26, 1998) and Article Annexe II à l'article D523-8 du Code de l'Environnement du 16 octobre 2007 (French GLP Legislation)** **US EPA OCSPP 870.SUPP;not specified**
GLP/GEP:	**yes**

FOE 5043-hydroxy

Report:	[redacted]∃ ;[redacted];1993;M-004586-01
Title:	FOE 5043-Hydroxy - Salmonella/microsome test plate incorporation and preincubation method
Report No:	22438
Document No:	M-004586-01-1
Guidelines:	**OECD 471 (1983), EEC Directive 84/449/EEC Method B.14**
	Deviations:
GLP/GEP:	**yes**

I. 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)
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 5.8.2/15-1: 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_3	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_3	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_3 = sodium azide; NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene
= bacteriotoxic effect; * = mutagenic effect

III. Conclusion

FOE 5043-Hydroxy is considered to be non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

Report: [redacted] 1992;M-004579-01
Title: FOE 5043-Hydroxy - Study of the acute oral toxicity to rats
Report No: 21889
Document No: M-004579-01-1
Guidelines: OECD 401 (1987), US-EPA Pesticide assessment Guidelines, Series 81-1 (1984)
Deviations: none
GLP/GEP: yes

I. 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:	[redacted] Germany
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 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

II. 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
Approximate LD_{50} = 726 mg/kg bw							

Animal Nos.	Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
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_{50} = 474 mg/kg bw							

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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 rad 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.

III. Conclusion

FOE 5043-hydroxy is considered to be moderately toxic after acute oral administration. The estimated acute oral LD_{50} values of male and female rats were approximately 726 mg/kg bw and approximately 474 mg/kg bw, respectively.

Report:	[redacted]9; [redacted];1993;M-004589-01
Title:	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
Document No:	M-004589-01-2
Guidelines:	**OECD 403 (1981), EC guideline 84/449/EEC B.2, US-EPA TSCA guideline 798.1150 (1985)** **Deviations: none**
GLP/GEP:	**yes**

I. 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:	[redacted]Germany
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula diet (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*

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/m³)	Control (air)	Control (vehicle)	2500	--
Analytical concentration (mg/m³)	-	--	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) GSD Aerosol mass < 3 µm (%)	-- - -	1.63 1.51 93	1.44 1.83 89	2.25 1,78 69

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation; - = not applicable.

II. 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 5.8.2/17-1: Result summary

Dose (mg/m³)	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_{50} > 6802 mg/m³						

Dose (mg/m^3)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
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_{50} approximate 6800 mg/m³						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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^3): There were no signs of toxicity observed in males and females.

Group 4 (6802 mg/m^3): 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, sereous nasal discharge, bloody incrustrations 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^3).

On day 1 of the recovery period, pinnal 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.

III. Conclusion

FOE 5043-hydroxy is considered to be slightly toxic after acute inhalation exposure. The determined acute LC_{50} values of male and female rats were > 6802 mg/m³ and approximately 6800 mg/m³.

Report: [redacted]d; [redacted];1992;M-004564-01
Title: FOE 5043-Hydroxy - Study for skin and eye irritation/corrosion in rabbits
Report No: 21257
Document No: M-004564-01-1
Guidelines: **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
GLP/GEP: **yes**

I. 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:	[redacted], England
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, *ad libitum*
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^2)
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)

II. 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 5.8.2/18-1.

Table 5.8.2/18-1: 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
Mean 24-72 h	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

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 5.8.2/18-2.

Table 5.8.2/18-2: 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	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					

III. Conclusion

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.

Report: [redacted]5; [redacted];1994;M-004614-01
Title: FOE 5043-Hydroxy - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman)
Report No: 22824
Document No: M-004614-01-2
Guidelines: OECD 406, Directive 84/449/EEC B.6 (1984)
Deviations: none
GLP/GEP: yes

I. 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
2. Vehicle:	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] Germany
Acclimatisation period:	At least 7 days
Diet:	Altromin® 3020 maintenace diet for guinea pigs (ALTROMIN GmbH, Lage, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
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)

II. 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 5.8.2/19-1.

Table 5.8.2/19-1: 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.

III. Conclusions

Based on the study results FOE 5043-hydroxy does not possess a skin sensitizing potential.

FOE 5043-TDA sulfone

Report: [redacted]y;[redacted];1993;M-004606-01
Title: FOE 5043-Sulfon - Salmonella/microsome test plate incorporation and preincubation method
Report No: 22629
Document No: M-004606-01-1
Guidelines: **EEC Directive 84/449/EEC B.14; OECD 471 (1983); US-EPA New and revised health effects test guidelines (1984)**
Deviations: none
GLP/GEP: **yes**

I. 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	

II. 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 5.8.2/20-1: 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[$]	FOE 5043-Hydroxy	0	10 ± 2	8 ± 3	20 ± 4	66 ± 14
		8	4 ± 2	5 ± 2	13 ± 6	45 ± 8
		40	0 ± 1[B]#	0 ± 0[B]#	0 ± 0[B]#	0 ± 0[B]#
		200	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
		1000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
		5000	0 ± 0#	0 ± 0#	0 ± 0#	0 ± 0#
	NaN_3	10	445± 36*			
	NF	0.2				336 ± 16*
	4-NPDA	10		49 ± 7*		
	4-NPDA	0.5			105 ± 32*	
With Activation[$]	FOE 5043-Hydroxy	0	17 ± 5	9 ± 2	24 ± 7	72 ± 10
		8	11 ± 1	8 ± 2	24 ± 6	89 ± 9
		40	5 ± 1[B]#	4 ± 3 [B]#	14 ± 6#	53 ± 17 [B]#
		200	0 ± 0#	0 ± 0#	0 ± 0[B]#	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
		4	14 ± 5	5 ± 2	23 ± 6	99 ± 19
		8	12 ± 1	5 ± 3#	25 ± 7	100 ± 6
		16	10 ± 3	1 ± 2[B]#	19 ± 3[B]	103 ± 18
		32	8 ± 3[B]#	1 ± 1[B]#	3 ± 2[B]#	76 ± 11[B]#
	NaN_3	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 [B]#	5 ± 1[B]#	23 ± 9[B]#	80 ± 17[B]#
	2-AA	3	130 ± 10*#	50 ± 15*#	901 ± 212*#	1200 ± 221*#

NaN_3 = sodium azide, NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene

[$] = not used for assessment due to increased toxicity. Results were used only for assessment of bacteriotoxicity.

[B] = background lawn reduced, # = bacteriotoxic effect; * = mutagenic effect

Table 5.8.2/20-2: 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 [B]
		8	4 ± 1	0 ± 0[B]	8 ± 4 [B]	47 ± 17 [B]
		16	2 ± 1	0 ± 0[B]	3 ± 1[B]	15 ± 6 [B]
		32	0 ± 0[B]#	0 ± 0[B]	0 ± 0[B]#	0 ± 0[B]#
	NaN_3	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	21 ± 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[B]#	7 ± 2[B]#	10 ± 4[B]#	64 ± 5[B]#
	2-AA	3	149 ± 3*	188 ± 9*	492 ± 47*	729 ± 41*

NaN_3 = sodium azide; NF = nitrofurantoin (NF), 4-NPDA = 4-nitro-1,2-phenylene diamine, 2-AA = 2-aminoanthracene
[B] = background lawn reduced, # = bacteriotoxic effect; * = mutagenic effect

III. Conclusion

FOE 5043-Sulfon is considered to be non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

Report: [redacted];1992;M-004578-01
Title: FOE 5043 Sulfon - Study for acute oral toxicity in rats
Report No: 21893
Document No: M-004578-01-1
Guidelines: OECD 401 (1987); US-EPA Pesticide assessment guidelines, Series 81-1 (1984)
Deviations: none
GLP/GEP: yes

I. 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] Germany
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:	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 ± 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 5.8.2/21-1: 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_{50} > 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_{50} > 150 < 200 mg/kg bw							

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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 fait, 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.

III. Conclusion

FOE 5043-Sulfon is considered to be toxic after acute oral administration. The estimated acute oral LD_{50} for male and female rats is > 150 and < 200 mg/kg bw.

Report:	[redacted]1;[redacted];1992;M-004576-01
Title:	FOE 5043-Sulfone - Study of the acute inhalation toxicity to rats in accordance with OECD guideline no. 403
Report No:	21784
Document No:	M-004576-01-2
Guidelines:	**OECD 403 (1981), EC guideline 84/449/EEC B.2, US FIFRA guideline § 81-3 (1984)** **Deviations: none**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	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
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:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*
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/m³ air (actual concentration) Dust: 0-35.3-122.7 mg/m³ (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/m³)	0	--	--	0	50	500	1000	1500
Analytical concentration (mg/m³)	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

II. 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/m3 dust. One male rat each died at 35.3 and 122.7 mg/m³.

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/m³.

Four males and one female each died at concentrations of 89 and 146.3 mg/m³.

Table 5.8.2/21-2: Result summary

Dose (mg/m³)	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
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_{50} approximately 69 mg/m³						

Dose (mg/m^3)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
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_{50} > 146.3 mg/m^3						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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/m3 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^3.

At higher concentrations all male and female rats exhibited clinical signs. The clinical signs at 52.6 mg/m3 consisted of dyspnea and bradypnea, respiratory sounds, difficult breathing, rhinarium reddened and bloody, serous nasal discharge, reduced activity, atony, piloerection.

At 89.8 mg/m3 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^3 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^3 dust reduced grip strength and reduced reaction to external stimuli were observed I 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/^{3}. At 89.8 and 146.3 mg/m^3 dust reduced grip strength and reduced reaction to external stimuli were observed I 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^3 dust.

Aerosol exposure
There was a treatment-related and toxicologically significant reduction on body weight gain at 52.6 mg/m^3 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^3 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^3 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.

III. Conclusion

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_{50} values of male and female rats were approximately 69 mg/m^3 and > 146 mg/m^3 after aerosol exposure.

Report:	[redacted]b;[redacted];1992;M-004522-01
Title:	FOE 5043-Sulfon - Study for skin and eye irritation/corrosion in rabbits
Report No:	21156
Document No:	M-004522-01-1
Guidelines:	**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**
GLP/GEP:	**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;

2. Vehicle: 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], England
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, *ad libitum*
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^2)
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)

II. 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 5.8.2/23-1.

Table 5.8.2/23-1: 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
Mean 24-72 h	**0.0**	**0.0**	**2.0**	**0.3**	**2.0**	**0.0**
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
Mean 24-72 h	**1.0**	**0.0**	**1.0**	**0.0**	**1.0**	**0.0**
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 5.8.2/23-2.

Table 5.8.2/23-2: 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	**2.3**	Conjunctivea whitish colored, hemorrhage, conjunctivae and eyelids: partly black colored Conjunctivae and nictitating membrane: strong formation of vessels	++
	Iris	ne*	2	2	ne#	**x**		
	Redness conjunctivae	2	3	3	ne	**x**		
	Chemosis conjunctivae	3	3	3	3	**3.0**		
2s	Corneal opacity	ne*	2	2	3	**2.3**		++
	Iris	ne*	2	2	ne#	**x**		
	Redness conjunctivae	2	3	3	ne	**x**		
	Chemosis conjunctivae	4	3	3	3	**3.0**		
3s	Corneal opacity	ne*	2	2	3	**2.3**		++
	Iris	ne*	2	2	ne#	**x**		
	Redness conjunctivae	2	3	3	ne	**x**		
	Chemosis conjunctivae	4	3	3	3	**3.0**		

*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 5.8.2/23-3: 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	**1.0**	7 days	Conjunctivea: whitish colored Conjunctivae and nictitating membrane: strong formation of vessels	+
	Iris	0	0	0	0	**0.0**	na		
	Redness conjunctivae	2	2	2	3	**2.3**	14 days		+
	Chemosis conjunctivae	3	3	3	3	**3.0**	21 days		+
5	Corneal opacity	ne*	1	1	1	**1.0**	14 days	Conjunctivea: whitish colored Conjunctivae and nictitating membrane: strong formation of vessels; vascularization	+
	Iris	ne*	1	1	1	**1.0**	7 days		+
	Redness conjunctivae	2	2	2	3	**2.3**	14 days		+
	Chemosis conjunctivae	3	3	3	3	**3.0**	21 days		+
6	Corneal opacity	1	0	0	0	**0.0**	1 day	Conjunctivae and nictitating membrane: strong formation of vessels;	–
	Iris	0	0	0	0	**0.0**	na		–
	Redness conjunctivae	2	2	1	1	**1.3**	21		–
	Chemosis conjunctivae	3	2	1	1	**1.3**	7 days		–

*ne = no evaluation possible due to the chemosis of the conjunctivae

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 serious damage	≥3	≥1.5			(Regulation (EC) No 1272/2008 and GHS category 1) (Directive 1999/45/EC as amended)
na	not applicable					

III. Conclusion

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.

Report: [redacted]0;[redacted];1994;M-004673-01
Title: FOE 5043-Sulfone - Study of the skin sensitization effect on guinea pigs (Maximization test of Magnusson and Kligman)
Report No: 23001
Document No: M-004673-01-2
Guidelines: **OECD 406; Directive 84/449/EEC (1984)**
Deviations: none
GLP/GEP: **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], Germany
Acclimatization period:	At least 7 days
Diet:	Altromin® 3020 maintenace diet for guinea pigs (ALTROMIN GmbH, Lage, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
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)

II. 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*

	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 1%	20/20	20/20	20/20	0/20	0/20	2/10	2/10	2/10	0/10	0/10
Challenge 0.5%	19/20	18/20	19/20	0/20	0/20	1/10	1/10	1/10	0/10	0/10

* After patch removal

The last two reliability checks performed in the laboratory with 2-mercaptobenzothiazole confirmed the sensitivity and reliability of the test method.

III. Conclusions

Based on the study results FOE 5043-Sulfon does possess a skin sensitizing potential.

Report: [redacted];1993;M-004601-01
Title: FOE 5043-Sulfone - Study to assess the sensory irritation potential to mice (RD50 determination)
Report No: 22729
Document No: M-004601-01-1
Guidelines: ASTM E981-84
(Exposure technique in accordance with OECd 403 and EC guideline 84/449/EEC B.2)
Deviations: not specified
GLP/GEP: yes

I. Materials and methods

A. Materials

1. Test material:	FOE 5043-Sulfon
Description:	white crystals
Lot/Batch no:	17004+5/91
Purity:	99.2-99.9%
Stability of test compound:	guaranteed for study duration; expiry date: 1993-02-03
2. Vehicle:	None
3. Test animals	
Species:	Mouse
Strain:	OF1 (SPF-bred)
Age:	approximately5 to 7 weeks
Weight at dosing:	Mean: 29 g
Source:	[redacted] France
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*
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/m³ 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_{50} (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/m³)	0	7.6	16.1	23.3
Analytical concentration (mg/m³)	Air control	4.3	9.8	13.3
Temperature (mean, °C)	22	42	50	54

II. Results and discussion

A. Mortality

One rat at 4.3 mg/m³ died on study day 4.

Table 5.8.2/25-1: Result summary

Dose (mg/m³)	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

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd number = number of animals used

\# air control

B. Clinical observations

Except for the one mouse that died, no clinical signs were observed.

The one rat 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 RD50 was calculated to be **8.9 mg/m³ air**.

Table 5.8.2/25-2: Results for respiratory decrease

Concentration (mg/m³ 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.

III. Conclusion

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.

Based on the most sensitive parameter (i.e. respiratory rate), 0.3 mg FOE 5043-Sulfon /m³ is regarded as the non-irritant threshold concentration.

Report:	[redacted];[redacted];1992;M-004571-01
Title:	FOE 5043-Sulfone - Range-finding study of the subacute inhalation toxicity to rats (exposure: 5x6h)
Report No:	21390
Document No:	M-004571-01-2
Guidelines:	**EC Guideline 84/449/EEC; OECD 403 and 412**
	Deviations:
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	FOE 5043-Sulfon
Description:	colourless crystals
Lot/Batch no:	TE 86005, 17004+5/91
Purity:	99.2%
Stability of test compound:	guaranteed for study duration; expiry date:
2. Vehicle:	None
3. Test animals	
Species:	Rat
Strain:	Wistar Bor:WISW (SPF-Cpb)
Age:	Approximately2 to 3 months
Weight at dosing:	Mean males: 210 ± 9 g; mean females: 185 ± 7 g
Source:	[redacted], Germany
Acclimatisation period:	at least 1 week
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*
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/m³ 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/m³)	Air control	0.5	4.0	15.0
Analytical concentration (mg/m³)	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

II. 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 5.8.2/26-1: Result summary

Animal Nos.	Concentration (mg/m³)	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

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd number = number of animals used

** All rats were sacrificed on day 7 (1st 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/m³ 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/m³ 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/m³. At 16.3 mg/m³ reduced reaction to touch were observed in the rats.

Table 5.8.2/26-2: Summary of effects observed during reflex tests

Concentration (mg/m³)	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

* 1st number = number of rats with abnormal reflexes, 2nd 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/m³.
A statistically significant effect on rectal temperature was determined in the 15 mg/m³ group (see Table below).

Table 5.8.2/26-3: summary of rectal temperature measurements

Concentration (mg/m³)	Males Rectal temperature on day			Females 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/m³ and above (see Table below).

Table 5.8.2/26-4: Summary of rectal temperature measurements

Concentration (mg/m³)	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 $p < 0.05$
++ = significant different from control $p < 0.01$

F. Organ weights

Up to and including 3.5 mg/m³, 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/m³ group, the relative organ to brain weights of heart, kidney and spleen weights were reduced.

Table 5.8.2/26-5: Summary of absolute organ weights on day 7

Concentration (mg/m³)	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 $p < 0.05$
++ = significant different from control $p < 0.01$

Table 5.8.2/26-6: Summary of relative organ weights on day 7

Concentration (mg/m³)	Absolute organ weighs (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 $p < 0.05$
++ = significant different from control $p < 0.01$

Table 5.8.2/26-7: Summary of relative organ to brain weights on day 7

Concentration (mg/m³)	Relative organ to brain weighs (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 $p < 0.05$
++ = significant different from control $p < 0.01$

G. Necropsy

Animals that died during the study:

The three males of the 16.3 mg/m³ 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/m³.
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 ling (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/m³ sacrificed at termination.

III. Conclusion

Based on the study results the NOEC for FOE 5043-Sulfon was determined to be 0.5 mg/m³.
Significant clinical findings were observed starting at 3.5 mg/m³. The most prominent changes (irritation of the respiratory tract mucosae and hypothermia caused by irritation) were observed in rats exposed to 16.3 mg/m³. This concentration was within the lethal range. The cause of death is considered to be causally related to lung damage resulting from irritation.

Report: [redacted]h; [redacted];1994;M-004779-01
Title: FOE 5043-Sulfone - Study of the subacute inhalation toxicity to rats in according with OECD guideline no. 412
Report No: 22918
Document No: M-004779-01-1
Guidelines: **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.
GLP/GEP: **yes**

1. Materials and methods

A. Materials

1. Test material: FOE 5043-Sulfon
Description: colourless crystals
Lot/Batch no: 17004+5/91
Purity: 99.2%
Stability of test compound: guaranteed for study duration; expiry date: 1993-01-02

2. Vehicle: None

3. Test animals

Species: Rat
Strain: Wistar Bor:WISW (SPF-Cpb)
Age: Approximately2 to 3 months

Weight at dosing:	Mean males: 190 g; mean females: 170 g
Source:	[redacted], Germany
Acclimatisation period:	Approximately 2 weeks
Diet:	Standard fixed-formula standard diet (maintenance diet for rats and mice) (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*
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/m³)	Air control	0.5	2	8
Analytical concentration (mg/m³)	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) GSD Aerosol mass < 3 µm (%)	Not reported	Not reported	Not reported	Not reported

II. Results and discussion

A. Mortality

No mortalities were observed in any dose group.

Table 5.8.2/27-1: Result summary

Animal Nos.	Concentration (mg/m^3)	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

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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^3 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^3 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^3. 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^3, the reflex tests did not reveal any abnormal findings that would indicate specific neurological changes. Individual animals of the 8 mg/m^3 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^3 group.

Table 5.8.2/27-2: Summary of effects observed during reflex tests

Concentration (mg/m^3)	Type of reflex (study day) Reduced righting response (day 3)	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

* 1st number = number of rats with abnormal reflexes, 2nd number = total number of animals examined

D. Rectal temperature

When compared to control, there was a slight hypothermia noted at 2 and 8 mg/m³.

Table 5.8.2/27-3: summary of rectal temperature measurements

Concentration (mg/m^3)	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/m³ and above (see Table below). As can be seen in the table below, the animals clearly gained weight during the exposure-free weekends.

Table 5.8.2/27-4: Summary of body weight measurements

Concentration (mg/m^3)	Mean body weight (g)								
	0	4	7	11	14	18	21	25	28
	Males								
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++
	Females								
Air control	171	167	171	167	172	174	174	180	186
0.5	170	165	168	167	170	172	172	176	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^3 and in females at 8 mg/m^3. 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^3 and above (i.e. a relative increase in the segmented leukocytes and monocytes but no effect on the absolute leukocyte count).

Table 5.8.2/27-5: summary of haematology and leucocyte differential count

Concentration (mg/m^3)	LEU ($10^{-9}/L$)	MCHC (g/L ERY)	THRO ($10^{-9}/L$)	HQUICK (sec)	LYM (%)	SEGM (%)	MONO (%)
	Males						
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
Historical control data - males							
HCD: ± 2S	3.0-9.8	288-323	801-1547	25.1-39.0	78-97	1-18	Up to 7
HCD: ± 3S	1.3-11.5	279-332	614-1733	21.7-42.5	73-100	Up to 22	Up to 10
	Females						
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++
Historical control data - females							
HCD: ± 2S	2.5-8.6	287-318	883-1475	24.2-33.2	81-98	1-16	Up to 5
HCD: ± 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^3 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^3. No toxicologically relevant changes were observed in female rats of the 2 mg/m^3 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 5.8.2/27-6: Summary of clinical chemistry

Concentration (mg/m^3)	ASAT (U/L)	ALAT (U/L)	Total BILI (mmol/L)	Cholesterol (mmol/L)	Chloride (mmol/L)
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
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 $p \leq 0.05$

++ Statistically significant different from control $p \leq 0.01$

Protein electrophoresis

In both sexes at 8 mg/m^3 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^3 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^3 group.

Table 5.8.2/27-7: Summary of examinations in liver tissue

Concentration (mg/m³)	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≤ 0.05 (U-test)
++ Statistically significant different from control p≤ 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. Ophthamology
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/m³. In males there was also a treatment-related reduction in spleen weights observed at that concentration.
Up to and including 3.5 mg/m³, 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/m³ group, the relative organ to brain weights of heart, kidney and spleen weights were reduced.
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/m³ group.
However, most of the organ weight changes summarised in the tables below are considered to be due to the body weight changes.

Table 5.8.2/27-8: Summary of absolute organ weights

Concentration (mg/m^3)	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
3.5	234	7993+	1808	1499	1168	782	211	8
16.3	209++	7309+	1749	1387+	1068++	731+	177++	9
		Spleen	**Adrenals**	**Testes**				
Air control	240	478	49	2811				
0.5	235	489	50	3043				
3.5	234	470	47	2946				
16.3	209++	388	55	2773				
	Females							
Air control	186	6933	1788	1189	994	669	241	8
0.5	183	6515	1763	1174	977	642	231	8
3.5	177	6167	1737	1184	949	626	198	8
16.3	182	6430	1742	1245	1075	718	166+	7
		Spleen	**Adrenals**	**Ovaries**				
Air control	186	447	65	136				
0.5	183	432	65	120				
3.5	177	389	66	128				
16.3	182	414	70	129				

\+ = significant different from control $p < 0.05$ (ANOVA)
\+ = significant different from control $p < 0.01$ (ANOVA)

Table 5.8.2/27-9: Summary of relative organ weights

Concentration (mg/m^3)	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
3.5	3489	791	656	510	342	92+	3
16.3	3637	886	697	541	369	87+	5
	Spleen	**Adrenals**	**Testes**				
Air control	337	20	1163				
0.5	329	21	1285				
3.5	342	21	1292				
16.3	369	28+	1388++				
Concentration (mg/m^3)	**Relative organ weighs (mg/100 g bodyweight)**						
	Liver	**Brain**	**Kidneys**	**Lung**	**Heart**	**Thymus**	**Thyroid**
	Females						
Air control	3703	956	655	530	357	128	4
0.5	3558	963	640	533	351	126	4
3.5	3532	998	679	546	361	113	5
16.3	3706	1010	721++	622++	410++	95+	4
	Spleen	**Adrenals**	**Ovaries**				
Air control	239	35	72				
0.5	236	36	65				
3.5	224	38	73				
16.3	239	40	73				

\+ = significant different from control $p < 0.05$ (ANOVA)
++ = significant different from control $p < 0.01$ (ANOVA)

Table 5.8.2/27-10: Summary of relative organ to brain weights

Concentration (mg/m^3)	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
3.5	444	83	65	43	12+	0.4
16.3	418	79	61	42	10++	0.5
	Spleen	**Adrenals**	**Testes**			
Air control	25	3	149			
0.5	27	3	168			
3.5	26	3	163			
16.3	22	3	159			
Concentration (mg/m^3)	**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
3.5	354	68	55	36	11	0.5
16.3	369	72	62+	41	10+	0.4
	Spleen	**Adrenals**	**Ovaries**			
Air control	25	4	4			
0.5	25	4	4			
3.5	22	4	4			
16.3	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^3 sacrificed at termination.

M. Histopathology

Irritation-induced morphological changes in the turbinates, nasopharynx and larynx were determined at 0.5 mg/m^3.

At 2 mg/m^3 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^3 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^3 and females at 2 and 8 mg/m^3 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.

III. Conclusion

Based on the most sensitive end point (inflammatory changes in the upper respiratory tract, sensory irritation), 0.5 mg/m^3 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^3.

FOE 5043-acetate

Report: [redacted]o;[redacted];1994;M-004640-01
Title: FOE 5043 Acetate - Study for acute oral toxicity in rats
Report No: 23279
Document No: M-004640-01-1
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/GEP: **yes**

I. 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:	[redacted]Germany
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 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

II. 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 5.8.2/28-1: 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_{50} > 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_{50} > 200 - < 1000 mg/kg bw							

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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 incrusted.

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.

III. Conclusion

FOE 5043-Acetate is considered to be moderately toxic after acute oral administration. The determined acute oral LD_{50} values of male and female rats were > 1000 mg/kg bw and > 200 - < 1000 mg/kg bw, respectively.

Report:	[redacted]d;[redacted];1996;M-004734-01
Title:	FOE 5043 Acetat (intermediate product of FOE 5043) - Study for acute inhalation toxicity in rats according to OECD no. 403
Report No:	25414
Document No:	M-004734-01-1
Guidelines:	**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**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: FOE 5043-Acetat

Description:	Beige, crystalline, solid
Lot/Batch no:	17025/93
Purity:	96.9%
Stability of test compound:	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:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	Standard fixed-formula diet (Altromin ® 1324; Altromin GmbH, Germany), *ad libitum*
Water:	tap water, *ad libitum*
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/m³ 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/m³)	control (vehicle)	100000
Analytical concentration (mg/m³)	0	2350
Test substance concentration in vehicle (%, w/v)	--	30
Temperature (mean, °C)	24	24
Relative humidity (mean, %)	32	32
MMAD (µm) GSD Aerosol mass < 3 µm (%)	1.6 2.0 83	1.5 2.0 84

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation, - = not applicable.

A. Mortality
There were no mortalities observed during the study.

Table 5.8.2/29-1: Result summary

Dose (mg/m³)	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_{50} > 2350 mg/m³						
Female rats						
0	0	0	5	--	--	0
2350	0	0	5	--	--	0
LC_{50} > 2350 mg/m³						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd 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.

III. Conclusion

FOE 5043-Acetat is considered to be non-toxic after acute inhalation exposure. The determined acute LC_{50} values of male and female rats were > 2350 mg/m³, the maximum technically attainable concentration.

Report: [redacted]7; [redacted];1994;M-004662-01
Title: FOE 5043 Acetat - Study for skin and eye irritation/corrosion in rabbits
Report No: 23062
Document No: M-004662-01-1
Guidelines: **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)**
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.
GLP/GEP: **yes**

A. Materials
1. Test material:
Name: FOE 5043-Acetat
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], England
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, *ad libitum*
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^2)
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
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 5.8.2/30-1.

Table 5.8.2/30-1: 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
Mean 24-72 h	**0.0**	**0.0**	**0.0**	**0.3**	**0.0**	**0.0**
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 5.8.2/30-2.

Table 5.8.2/30-2: 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	**0.0**	–	na
	Iris	0	0	0	0	**0.0**	–	na
	Redness conjunctivae	1	0	0	0	**0.0**	–	na
	Chemosis conjunctivae	1	0	0	0	**0.0**	–	na
2s	Corneal opacity	0	0	0	0	**0.0**	–	na
	Iris	0	0	0	0	**0.0**	–	na
	Redness conjunctivae	1	0	0	0	**0.0**	–	na
	Chemosis conjunctivae	1	0	0	0	**0.0**	–	na
3s	Corneal opacity	0	0	0	0	**0.0**	–	na
	Iris	0	0	0	0	**0.0**	–	na
	Redness conjunctivae	1	0	0	0	**0.0**	–	na
	Chemosis conjunctivae	1	0	0	0	**0.0**	–	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					

III. Conclusion

The test item FOE 5043-Sulfon was not irritating to the skin.
Based on the study results the test substance FOE 5043-Acetat is not irritating to eyes of rabbits.

CA 5.8.3 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.

So, after a 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.

CA 5.9 Medical data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Report: [redacted]4;[redacted];2014;M-475871-01
Title: Occupational Medical Experiences with Flufenacet
Report No: --
Document No: M-475871-01-1
Guidelines: **Not applicable**
Deviations: not applicable
GLP/GEP: **No (Not applicable)**

I. 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, googles, 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

II. Results and discussion

A. In-company experience:
There were no unusual occurrences or complaints recorded.

B. Medical assessment:
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. No further consultations of the Medical Service due to work or contact with Flufenacet were required.

CA 5.9.2 Data collected on humans

No cases of human poisoning have been reported up to now.

CA 5.9.3 Direct observations

Up to now there are no direct observations available.

CA 5.9.4 Epidemiological studies

Up to now there are no epidemiological studies available.

CA 5.9.5 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++ in hemoglobin to Fe+++, 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.

CA 5.9.6 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+++ to Fe++.

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 b.w.) 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 b.w.
  - **Toluidine Blue:**
    - 3% solution (30 mg/ml) intravenously at 0.07 to 0.13 ml/kg b.w. (2-4 mg/kg b.w.).
    - 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.

CA 5.9.7 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-^{14}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-^{14}C]- and [thiadiazole-2-^{14}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-^{14}C]FOE 5043 and was 92% on average in the experiments with [thiadiazole-2-^{14}C]FOE 5043. After application of [fluorophenyl-UL-^{14}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-^{14}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-^{14}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-^{14}C]FOE 5043-oxalate as well as [thiadiazole-2-^{14}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 thiadizole moiety of the molecule from dietary exposure of livestock to FOE 5043-derived crop residues.

An additional metabolism study with [thiadiazole-5-^{14}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^3 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[1)]	Rat	M F	LD_{50}: 1617 mg/kg bw 589 mg/kg bw	[redacted] & [redacted], 1993 M-004865-02-1
Oral[2)]	Rat	M	LD_{50}: 683 mg/kg bw	[redacted], 1992 M-004864-01-1
Oral	Mouse	M F	LD_{50}: 1331 mg/kg bw 1756 mg/kg bw	[redacted] & [redacted], 1991 M-004850-01-1
Dermal	Rat	M F	LD_{50}: >2000 mg/kg bw >2000 mg/kg bw	[redacted], 1992 M-004843-01-1
Inhalation (aerosol, 4h)	Rat	M F	LC_{50}: >3740 mg/m³ >3740 mg/m³	[redacted], 1990 M-004844-01-1
Skin irritation	Rabbit	M	Not irritating	[redacted] & [redacted], 1992 M-004846-01-1
Eye irritation	Rabbit	M	Not irritating	[redacted] & [redacted], 1992 M-004847-01-1
Skin sensitisation Buehler method	Guinea pig	M	Not sensitizing	[redacted] & [redacted], 1992 M-004845-01-1
Skin sensitisation M&K method	Guinea pig	M	Sensitizing	[redacted], 1994 M-004637-01-1
Skin sensitisation M&K method	**Guinea pig**	**F**	**Sensitizing**	**[redacted], 1995** **M-004677-01-1**
Skin sensitization Local lymph node assay	**Mouse**	**F**	**Not sensitizing**	**[redacted], 2004** **M-090513-01-1**
***In vitro* 3T3 NRU phototoxicity test**	**BALB/c 3T3 cells**		**Not phototoxic**	**[redacted], 2013** **M-464615-01-1**

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

M = male, F= female; [1)] animals were fasted (overnight); [2)] 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	
In-vitro				
Bacterial reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	negative	negative	[redacted], 1995 M-004696-01-1
Bacterial reverse mutation assay	***S. typhimurium*** **TA98, TA100, TA102, TA1535, TA1537**	**negative**	**negative**	[redacted]**, 2010 M-395211-01-1**
Mammalian cell gene mutation test (HGPRT)	Chinese hamster lung fibroblasts V79	negative	negative	[redacted], 1994 M-004634-01-1
Mammalian chromosome aberration test	Chinese hamster ovary cells CHO	negative	negative	[redacted], 1995 M-004692-01-1
Unscheduled DNA synthesis (UDS) assay	Primary rat hepatocytes	negative	negative	[redacted], 1992 M-004577-01-1
In-vivo				
Micronucleus test	Mouse bone marrow	negative		[redacted], 1993 M-004588-01-1

* 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 10-21) 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 NO(A)ELs and main findings at LO(A)EL in toxicity studies relevant for setting reference values

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.	[redacted] & [redacted], 1995 M-004981-01-1
Rat 1-week (5x6h) inhalation	**M, F**	**~14 48 mg/m³**	**~66 225 mg/m³**	**T4 ↓ Liver: rel. weight ↑**	**[redacted], 2008 M-300005-01-1**
Rat 4-week (20x6h) inhalation	**M, F**	**~7 19 mg/m³**	**~81 220 mg/m³**	**HB ↓, HCT ↓, RETI ↑, HEINZ ↑, AP ↓, TG ↓, Liver: enzymes ↑, rel. weight ↑, spleen: weight ↑, histopathological changes in nasal cavity and larynx, spleen, testes, thyroid, liver**	**[redacted], 2008 M-302961-01-1**
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).	[redacted], 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) NOEL neurobehavioral effects: 38/43 mg/kg bw/d	[redacted] et al., 1995 M-005014-01-2
Rat 90-day feeding	M F	-- 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	[redacted] & [redacted], 1995 M-004999-01-1
Rat 2-year feeding	M F	12 1.5	19 24	BWG↓, structural and/or functional alterations in liver-, kidney-, haematopoietic-, thyroid-related endpoints.	[redacted] & [redacted], 1995, M-005062-02-1
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	[redacted] et al, 1995 M-004976-02-1
Rabbit oral (gavage) developmental	Dam Fetal	5 25	25 125	Maternal: soft stool, BW gain ↓ during treatment, histopathological changes of the liver Fetal: skeletal variation ↑	[redacted] et al., 1995 M-004979-01-1

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 2-generation feeding	M F	7.4 8.2	37 41	BW ↓ in P females during pre-mating No reproductive effects observed at any dose level.	[redacted], 1995 M-004984-03-1
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)**	[redacted] **2000 M-026105-01-1**
Rat, mechanistic study thyroid feeding	**Dam Fetal**	**35 35 (DG 6-20)**	**-- --**	**No adverse effects observed at any dose level.**	[redacted], **2012 M-435619-01-1**
Rat, mechanistic study thyroid feeding	**Dam Pup**	**13 13 (DG 6 - DL 4/DL 21)**	**65 65**	**Dam: BW gain ↓, T4/T3 ↓, Liver: rel. weight ↑, thyroid: follicular cell hypertrophy in 2 of 13 dams Pup: BW ↓**	[redacted], **2012 M-435313-01-1**
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 ↑	[redacted] & [redacted], 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 ↑	[redacted], [redacted], 1995, 1997 M-005001-02-2
Mouse 90-day feeding	M F	18 25	64 91	T4 ↓ Liver: rel. weight ↑	[redacted] & [redacted], 1995 M-004985-01-1
Mouse 20-month feeding	M F	7.4 9.4	30 77	MetHb ↑ Ocular cataracts ↑	[redacted] & [redacted], 1995 M-005060-02-1

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, 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 renal pelvic mineralization 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).

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 mg/kg bw/day 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 thyroxin (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.