Bayer CropScience

Document Title

Summary of the toxicological and metabolism studies for Flurtamone

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

Date

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Author(s)

[redacted]

BayerCropScience

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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 SUBSTANCE

INTRODUCTION

Flutamone is a herbicidal active substance and was included into Annex I of Directive 91/414 in 2003 (Directive 2003/84/EC, dated 25th of September 2003, Entry into Force 1st of January 2004).

This Supplemental Dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of flurtamone and were, therefore, not evaluated during the first EU review of this compound. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph, its Addenda and are included in the Baseline dossier provided by Bayer CropScience. These old studies are not summarized in detail again. For all new studies detailed summaries are provided with this Supplemental Dossier. The new studies were a bile excretion study and a phototoxicity study in-vitro in BALB/c 3T3 cells.

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

Flurtamone was rapidly absorbed following oral dosing with a high absorption rate of > 90% at the 5 mg/kg bw dose level. After the maxima had been reached there was a moderately rapid elimination from the blood with elimination half-live times of approximately 66 hours for the males and 78 hours for the females. The comparison of the estimated areas under the curves indicated that bioavailability was slightly higher in males than in females in both blood and plasma.
Total elimination of orally-dosed material was rather rapid after both, single or repeated oral dosing, with excretion of the majority of the dose in the first 48 hours after administration.
A wide distribution of radioactivity in the tissues was seen with predominance in the liver, kidney, skin plus fur and blood. The tissue levels were low and no accumulation in any specific tissue was evident.
Flurtamone was extensively metabolised by phase I reactions (hydroxylation, methylation, ring hydrolysis) and phase II reactions (conjugations). Only very small amounts of unchanged parent compound were recovered, except in the faeces of high dose group animals. A variety of polar metabolites were found in urine, the majority of them being conjugates. Many of the faecal metabolites were not conjugated. The data did not show significant sex differences in absorption, metabolism or excretion.

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

Report:	KCA 5.1.1 /01;[redacted];1992;M-162239-01
Title:	The in vivo metabolism of Trifluoromethyl[U-14C] phenyl RE-40885 (Flurtamone) in rats
Report No:	R002231
Document No:	M-162239-01-1
Guidelines:	**USEPA (=EPA): 85-1;not specified**
GLP/GEP:	**yes**

Report:	KCA 5.1.1 /02;[redacted]; [redacted];1994;M-162895-01
Title:	Rat Metabolism Study Flurtamone
Report No:	R002557

Document No(s): Report includes Trial Nos.:
92131
EC-92-217
M-162895-01-1

Guidelines: **Deviation not specified**
GLP/GEP: **yes**

Report: KCA 5.1.1 /03; [redacted];2000;M-229664-01
Title: Rat supplemental A.D.M.E and blood kinetics study
Report No: C031454
Document No: M-229664-01-1
Guidelines: **EU (=EEC): 87/302/EEC Part B (1994); SANCO: 59, Nohsan 4200 (1985); USEPA (=EPA): OPPTS 870.7485;Deviation not specified**
GLP/GEP: **yes**

The following study is new and was not submitted during the first Annex I inclusion.

It was designed to determine the elimination of flurtamone *via* the bile following single low and high oral administration of [phenyl UL-^{14}C]flurtamone at nominal dose levels of 5 mg/kg and 500 mg/kg to the male and female rat. The information obtained will be supplementary to the results already reported in the rat ADME studies ([redacted];1994 and [redacted];2000) and consequently will allow estimation of the level of flurtamone absorbed following oral administration.

Report: KCA 5.1.1 /04; [redacted];2000;M-276270-01-1
Title: Rat bile excretion study
Report No: SA 99026/604887
Document No: M-276270-01-1
Guidelines: **EU (=EEC): 87/302/EEC Part B (1994); SANCO: 59, Nohsan 4200 (1985); USEPA (=EPA): OPPTS 870.7485;Deviation not specified**
GLP/GEP: **yes**

Executive Summary:

[Phenyl UL-^{14}C]-flurtamone was administered to male and female *Sprague Dawley* rats at nominal dose rates of 5 mg/kg bw (low dose) and 500 mg/kg bw (high dose). Urine and bile were collected at 0-6 hours, 6-24 hours and 24-48 hours; faeces were collected at twenty-four hour intervals following dosing. Cage washes were performed just after the collection of the samples. After sacrifice also the residues in the intestinal tract, stomach, caracass, blood and plasma were investigated.
48 hours post administration the recovery of radioactivity was found to be almost quantitative. In the low dose group the elimination of radiolabel *via* the bile was greater than that *via* the urine followed by faeces in both sexes, with the males presenting slightly higher levels for urine and faeces.
In contrast to that the elimination of radiolabel in the high dose group was highest *via* the faeces subsequently followed by bile and urine in both sexes. In this group, the elimination of the radiolabel occurred principally *via* the faeces. The residue levels found in bile, faeces and urine were again higher in males than in females.

Table 5.1.1-1: Total radioactive residues of [phenyl UL-^{14}C]-flurtamone in different compartments 48 hours post dosing

Matrix	Low dose		High dose	
	male	female	male	female
Urine	24.54	19.61	10.13	9.79

Faeces	1.54	1.01	49.33	41.87
Bile	67.45	68.01	27.19	13.23
Cage wash	1.08	3.69	1.19	2.12
Tissues	0.82	3.29	10.55	26.88
Total	95.42	95.61	98.38	93.89

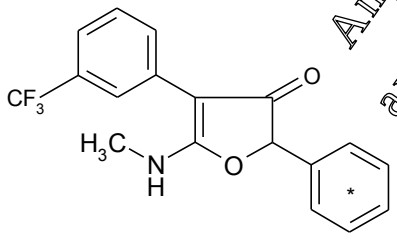
Comparison of these results with those of the regulatory rat A.D.M.E. studies ([redacted], 1994 and [redacted], 2000) suggests that entero-hepatic recirculation normally occurs with radioactivity that had been eliminated in the bile being reabsorbed and consequently eliminated *via* the urine in both low and high dose groups.
Tissue results from the biliary excretion study low dose group showed that highest levels of radioactivity were found in the carcass, intestinal contents and stomach contents (for the females). For the high dose group, highest levels were found in the intestinal contents for both sexes, and in the carcass from the females. These results showed that, at low dosages, flurtamone was practically completely eliminated at 48 hours post-dose and no tissues presented significant levels of radioactivity. Also at high dose levels a predominant part of flurtamone was eliminated or still present in the intestinal content and only very minor portions of radioactivity could be found in rat tissues.
The degree of absorption of flurtamone following a single oral dose can be evaluated from the recoveries obtained in urine (plus cage wash), bile and tissues. Consequently, the degree of absorption of flurtamone at low and high doses can be determined as:

Table 5.1.1-2: Estimated level of oral absorption for flurtamone in % of the administered dose

	Low dose (3 mg/kg) [%]	High dose (High dose) [%]
Male Rats	93.0	40.0
Females Rats	92.0	29.0
Mean	**92.5**	**34.5**

I Materials and Methods

Phenyl-[UL-^{14}C]-flurtamone (Batch No: JXM549A) was obtained from Laboratory [redacted], England. The radiopurity of the test materials were checked just before each dose preparation and were determined by TLC and linear analysis to be 98 %.



phenyl-[U-^{14}C]-flurtamone
**denotes the position of the uniformly-labelled phenyl ring*

The initial specific activity was 35.0 mCi/mmole (1.30 GBq/mmole). This material was stored at -20°C. Non-isotopically-labelled flurtamone (Batch No.: 206994), used for dilution of the radiolabelled material, was obtained from [redacted] and had a purity of >99.5% .

Animals

The rats used in this study were males and females of the Sprague Dawley CD strain (range 290-300 g body weight at their arrival) as supplied by Charles River France. On receipt each animal was identified by ear-tattoo.

Diet
Certified rodent diet M20 ([redacted], France) and filtered and softened water from the municipal water supply were available *ad libitum* except before administration when animals were fasted overnight.

Housing and maintanance of animals
The in-life phase of the study occurred in room 3460, [redacted], France. The laboratory conditions in the study room were controlled and monitored manually. The target specifications were:
Temperature: 22 ± 2°C
Humidity: 55 ± 15%
Lighting: 12-hour light, 12-hour dark cycles
Ventilation: 15 air changes per hour (average, not monitored)
The animals were initially housed in metal, wire-mesh bottomed cages placed in metal trays containing absorbent paper. After surgery the animals were housed individually in glass metabolism cages (Jencon's Metabowl Mk HI)

Dose preparation
Just before the preparation of each dose suspension, the radiochemical purity of [^{14}C]flurtamone in the stock solution was checked.
The calculated volume of the phenyl-[U-^{14}C]-flurtamone solution in acetonitrile was added to a weighted portion non-radioactive material which dissolved in the acetonitrile to provide a homogeneous solution at the desired specific activity.
This solution was evaporated under a gentle stream of nitrogen. Appropriate weights of dose vehicle (PEG 200) were added and the mixture was ground with a pestle to produce a homogenous suspension. Each dose suspension was prepared on the day of dosing and was assayed for active ingredient concentration (by HPLC) and for radioactivity content (by LSC) both before, mid-way through and after the dosing procedure.

Dosing and in life phase
Surgery was performed twenty-four hours before the administration of the radio label. Male and female rats were anaesthetized and the bile ducts were cannulated through a mid-line incision. The cannulae were exteriorized dorsally and the body wall and skin separately sutured. After surgery, the animals were housed individually in glass metabolism cages and the cannula of each animal led through the roof of the cage to permit bile collection into a weighed container. Before the administration of the radioactive dose, four animals of each sex were selected by a normal flow of the bile. The rodent diet was removed approximately 16 hours before administration of the radio labelled test material. After administration the diet was available *ad libitum.* The animals were weighed immediately prior to dosing and the dose required for each animal was calculated and administered by gavage. Mean actual dose rates were 4.7069 mg/kg bw (male), 4.7718 mg/kg bw (female) and 475.1956 mg/kg bw (male), 473.1309 mg/kg bw (female) for the low and high dose groups respectively. The animals remained in the metabolism units for 48 hours post dosing.

Sample Collection
Urine and bile were collected at 0-6 hours, 6-24 hours and 24-48 hours; faeces were collected at twenty-four hour intervals following dosing. Metabowl cage washes with distilled water were performed at the end of each 24 hours period, just after the collection of the samples. Additionally there was a cage wash with acetonitrile for the final cage rinse. All animals were exsanguinated whilst

under anesthesia, 48 hours after administration of the dose. The intestinal tract, intestinal tract contents, stomach and stomach contents were removed from each animal after exsanguination. Also the residual carcass was retained for analysis. Plasma was prepared from cardiac blood samples by centrifugation. Whenever possible samples were processed as they were collected. Remaining samples and samples waiting to be processed were stored at -20°C in the dark until required.

Quantitative assay of radioactivity
The amounts of radioactivity in the various samples were determined by liquid scintillation counting. Samples were counted in an appropriate scintillation cocktail using a Packard 1900 TR counter. For each scintillation cocktail efficiency correlation curves were prepared and regularly checked by the use of [^{14}C]-n-hexadecane standards.
The limit of detection was taken to be twice the background values of blank samples in appropriate scintillation cockails.

Liquid Samples
Aliquots of urine, bile, plasma and cage washings were counted in a liquid scintillation cocktail after being allowed to stabilize to ambient temperature and reduced light intensity.

Solid Samples
The collected faeces (0-24 hours and 24-48 h hours) were homogenized with a portion of water using an Ultra-Turrax. Samples were combusted in a sample oxidiser (Packard Model 387 Tri-Carb). The carbon dioxide generated by combustion was absorbed in a trapping agent, mixed with a scintillation cocktail and then radioassayed.

Tissues and Blood
The intestinal tract, stomach and residual carcass were solubilized prior to radio measurement. The intestinal tract contents and stomach contents were homogenized in appropriate volumes of deionised water using an Ultra-Turrax. Aliquots of these homogenates were combusted using a sample oxidiser. The carbon dioxide generated by the combustion was absorbed by a trapping agent, mixed with an appropriate scintillation cocktail and then radioassayed. Blood samples were combusted directly after drying of weighed aliquots.

II Results and Discussion

The amounts of radioactivity recovered in the urine, faeces, bile, cage wash and tissues of the male and female rats for both dose levels were expressed as percentages of administered dose and are given in Table 5.1.1-3.

Table 5.1.1-3: Recovered radioactivity of [phenyl-UL-^{14}C] flurtamone after single oral high and low dose bile kinetic experiment.

Sampling interval (h)	**Recovered radioactivity (%)**					
	Urine	**Faeces**	**Bile**	**Cage wash**	**Tissues**	**Total**
Low dose (5 mg/kg) **Male**						
0 – 6	15.03	-	46.39			
0 – 24	23.10	0.27	66.08			
0 – 48	24.54	1.54	67.45	1.08	0.82	**95.42**
Low dose (5 mg/kg) **Female**						
0 – 6	9.03	-	30.08			
0 – 24	16.14	0.45	60.20			
0 – 48	19.61	1.01	68.01	3.69	3.29	**95.61**
High dose (500 mg/kg) **Male**						
0 – 6	3.25	-	4.37			

0 – 24	7.72	15.63	19.70			
0 – 48	10.13	49.33	27.19	1.19	10.55	**98.38**
High dose (500 mg/kg) **Female**						
0 – 6	2.7	-	0.63			
0 – 24	7.05	11.91	8.06			
0 – 48	9.79	41.87	13.23	2.12	26.88	**93.89**

The total recovered radioactivity, expressed as percentage of administered radioactivity in the low dose and high dose experiments, were determined at 95.42 – 95.61% and 93.89 – 98.38% respectively. In the low dose group highest elimination rates were observed *via* the bile, subsequently followed by urine and faeces in both sexes. The elimination of [phenyl UL-^{14}C]-flurtamone and/or its metabolites appeared to be slightly higher in males than in females. In contrast, in the high dose group, the greatest amount of radiolabel was eliminated *via* the faeces subsequently followed by bile and urine in both sexes. In this group, the elimination of the radiolabel occurred principally via the faeces in both sexes, and the rates found in bile, faeces and urine were again higher in males than in females.
The recoveries obtained for urine and faeces in the previous A.D.M.E. ([redacted], 1994) and in the supplemental A.D.M.E ([redacted]2000) presented for both doses and sexes showed higher levels of radioactivity in urine than those observed in this biliary excretion study.
This apparent discrepancy suggests that a part of the radioactivity excreted via the bile could be reabsorbed (entherohepatic circulation) and subsequently re-excreted *via* the urine in both, low and high dose groups.

<u>Tissue Distribution of Administered Radioactivity</u>
Radioactivity in the sampled tissues is given as µg [^{14}C]-flurtamone equivalents per g tissue (equivalent to mg/kg). These values are shown in Table 5.1.1-4.

Table 5.1.1-4: Mean tissue concentrations from the biliary excretion study given as µg ^{14}C-flurtamone equivalents/g

Matrix	**Low dose (5 mg/kg)**				**High dose (500 mg/kg)**			
	Male		**Female**		**Male**		**Female**	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Intestinal contens	0.010	0.04	0.257	0.96	267.95	9.23	131.13	22.56
Intestinal tract	0.023	*	0.110	*	152.11	*	155.59	*
Stomach contens	0.000	0.001	0.730	1.19	2.35	*	0.54	*
Stomach	0.050	*	1.557	*	5.13	*	0.69	*
Residual caracass	0.040	0.74	0.049	0.87	2.71	*	0.49	2.64
Blood	0.048	*	0.065	*	5.03	*	1.58	*
Plasma	0.052	*	0.084	*	7.10	*	2.71	*
Total	**-**	**0.82**	**-**	**3.29**	**-**	**10.55**	**-**	**26.88**

*not given by the report

Tissue results from the biliary excretion study showed that highest levels of radioactivity were found in the carcass, intestinal contents and stomach contents (for the females) in the low dose group. For the high dose group, highest levels were found in the intestinal contents for both sexes, and in the carcass from the females.
These results show that, at low dosages, flurtamone was practically completely eliminated within 48 hours after administration and levels of radioactivity found in tissues were insignificant. Also at high dose levels a predominant part of flurtamone was eliminated, or still present in the intestinal content and only very minor portions of radioactivity could be found in rat tissues.

III Conclusion

The absorption level of flurtamone can be evaluated from the recoveries obtained in urine (plus cage wash), bile and tissues. In the tissues, intestinal and stomach contents were excluded because it was not possible to say whether the radioactivity present was due to passive diffusion across the gut wall or had simply not been absorbed. Consequently, the absorption level of flurtamone at low and high doses, can be summarized as follows:

Table 5.1.1-5: Estimated level of oral absorption for flurtamone in % of the administered dose

	Low dose (5mg/kg) [%]	High dose (500 mg/kg) [%]
Male Rats	93.0	40.0
Females Rats	92.0	29.0
Mean	**92.5**	**34.5**

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Not applicable, the toxicokinetic studies have been fully summarised and described under point 5.1.1

CA 5.2 Acute toxicity

No new studies on the acute toxicity of flurtamone were performed, the studies below were already submitted at the time of the first Annex I inclusion.

CA 5.2.1 Oral

Flurtamone shows a low toxicity in rats after oral and inhalation administration and to rabbits after dermal administration. The acute oral LD_{50} in the rat was > 5000 mg/kg bodyweight in males and females. No deaths occurred in the treated animals, and there was no significant difference in body weights between control and treated groups. As clinical signs, yellow anogenital discharge and no faeces in some of the treated animals was seen. No compound-related histopathological findings were noted.

Report: KCA 5.2.1/01; [redacted];1989;M-160698-01
Title: The Acute Oral Toxicity of RE-40885 Technical (SX-1802) in Adult Male and Female Rats
Report No: R001511
Document No: M-160698-01-1
Guidelines: **USEPA (=EPA): 81-1;Deviation not specified**
GLP/GEP: **no**

Report: KCA 5.2.1 /02; [redacted];1989;M-264626-01
Title: The acute oral toxicity of RE-40885 technical (SX-1802) in adult male and female mice
Report No: CEHG2987
Document No: M-264626-01-1
Guidelines: **Not specified;Not specified**
GLP/GEP: **yes**

CA 5.2.2 Dermal

The dermal LD_{50} in rabbits was > 5000 mg/kg bw. No deaths, no signs of toxicity, no body weight effect and no compound-related skin irritation were observed.

Report:	KCA 5.2.2 /01; [redacted];1989;M-160669-01
Title:	The acute dermal toxicity of RE-40885 technical (SX-1802) in adult male and female rabbits
Report No:	R001499
Document No:	M-160669-01-1
Guidelines:	**USEPA (=EPA): 81-2;not specified**
GLP/GEP:	**no**

CA 5.2.3 Inhalation

The inhalation LC_{50} was > 2.2 mg/L. No deaths occurred. The main clinical signs were a decreased motor activity and one day following the exposure, slight abnormal respiratory sounds in some animals of both sexes. All exposed animals appeared normal by the fifth day of the 14-day post-exposure observation period. No macroscopic or microscopic changes related to the exposure were observed at necropsy.

Report:	KCA 5.2.3 /01; [redacted]; [redacted];1991;M-203245-01
Title:	The acute inhalation toxicity of RE-40885 technical (SX-1802) in rats
Report No:	C016891
Document No(s):	Report includes Trial Nos.: CEHC88-32 S-3124 M-203245-01-1
Guidelines:	**USEPA: 81-3;not specified**
GLP/GEP:	**yes**

CA 5.2.4 Skin irritation

Flurtamone was not a skin irritant.

Report:	KCA 5.2.4 /01; [redacted];1989;M-160701-01
Title:	The four-hour skin Irritation Potential of RE-40885 Technical (SX-1802) in Adult Albinos Rabbits
Report No:	R001513
Document No:	M-160701-01-1
Guidelines:	**USEPA (=EPA): 81-5;Deviation not specified**
GLP/GEP:	**yes**

CA 5.2.5 Eye irritation

Contact with the eyes of rabbits produced a mild transient irritation, which did not trigger classification.

Report:	KCA 5.2.5 /01; [redacted];1989;M-160699-01
Title:	The Acute Eye Irritation Potential of RE-40885 Technical (SX-1802) in Adult Albino Rabbits
Report No:	R001512
Document No:	M-160699-01-1
Guidelines:	**USEPA (=EPA): 81-4;Deviation not specified**
GLP/GEP:	**no**

CA 5.2.6 Skin sensitization

Flurtamone was not a skin sensitizing agent in either a Magnusson and Kligman or a modified Buehler test.

Report:	KCA 5.2.6 /01; [redacted];1989;M-160693-01
Title:	Modified Buehler Test for the Skin Sensitization Potential of RE-40885 Technical (SX-1802)
Report No:	R001509
Document No:	M-160693-01-1
Guidelines:	**USEPA (=EPA): 81-6;Deviation not specified**
GLP/GEP:	**no**

Report:	KCA 5.2.6 /02; [redacted];1993;M-203230-01
Title:	Skin sensitization test in guinea-pigs (Maximization method of Magnusson, B. and Kligman, A.M.) Flurtamone
Report No:	C042421
Document No:	M-203230-01-2
Guidelines:	**OECD: 406;Deviation not specified**
GLP/GEP:	**yes**

CA 5.2.7 Phototoxicity

Due to the new data requirements a phototoxicity study is required if the molar extinction coefficient is higher than 10 L x mol^{-1} x cm^{-1}. This is the case for flurtamone so that a phototoxicity study has been conducted in BALB/c 3T3 c31 cells which is described in the following summary.

Report:	KCA 5.2.7 /01; [redacted]; 2014;M-476227-01-1
Title:	Flurtamone (AE B107597), technical material: CYTOTOXICITY ASSAY IN VITRO WITH BALB/c 3T3 c31 CELLS: NEUTRAL RED (NR) TEST DURING SIMULTANEOUS IRRADIATION WITH ARTIFICIAL SUNLIGHT
Report No:	Harlan study no.: 1592400
Document No:	M-476227-01-1
Guidelines:	**OECD: 432;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The phototoxic potential of Flurtamone (AE B107597), technical material, was tested in this assay using BALB/c 3T3 cells. The experiment was performed twice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME). The following

concentrations of the test item solved in DMSO (further diluted in EBSS, final concentration of DMSO in EBSS was 1% (v/v); dose calculation was adjusted to purity) were tested in the presence and in the absence of irradiation in both experiments: 0.48, 0.95, 1.90, 3.80, 7.60, 15.2, 30.4, 60.8 µg/mL. As solvent control EBSS containing 1% (v/v) DMSO was used. Chlorpromazine was used as positive control.
One test group of cells treated with the test item was irradiated with artificial sunlight for 50 minutes with 1.65 mW/cm² UVA, resulting in an irradiation dose of 4.95 J/cm² UVA. Another test group of test item treated cells were kept in the dark for 50 minutes.

I. Material and methods

A. Materials

1. Test material:

Identification:	Flurtamone (AE B107597) technical material
Batch code:	AE B107587-01-10
Origin Batch No.:	CGS 17424-1-1
Purity:	97,3 % w/w (dose calculation was adjusted to purity)
Expiry Date:	29 October 2014
Storage conditions:	At room temperature, protected from light
Stability in Solvent:	Stable for at least 24 hours in organic solvents and aqueous solution

2. Vehicle: DMSO (further diluted in EBSS, final concentration of DMSO in EBSS was 1% (v/v).

3. Test system:

Cells: BALB/c 3T3 cells clone
Source: Large stocks (Master Cell Stock) of the BALB/c 3T3 c31 cell line (supplied by [redacted], Germany) are stored in liquid nitrogen in the cell bank of Harlan CCR. The master cell stock has been characterised by Harlan CCR. A working cell stock is produced by multiplying from the master cell stock.

B. Study design and methods

1. Preparation of the Test Item Solution
The experiment was performed twice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME). The test item was dissolved in DMSO (purity: 99.9%). The final concentration of the solvent in EBSS was 1% (v/v). The highest applied concentration of the test item was 60.8 µg/mL due to the limited solubility of the test item. According to the OECD guideline no test item precipitation should occur in the irradiated cultures. The test item's purity was taken into account when calculating the concentrations.
The following concentrations were tested (each concentration of the test item was measured 6 times):

	µg/mL of the test item							
with and without irradiation	0.48	0.95	1.90	3.80	7.70	15.2	30.4	60.8

2. Controls
2.1 Solvent controls:
The solvent control for the positive control was EBSS. The solvent control for the test item was EBSS containing 1% (v/v) DMSO.

2.2 Positive Control:
Chlorpromazine (Sigma) dissolved in EBSS was used as positive control. The following concentrations were applied:

	µg/mL chlorpromazine
Absence of Irradiation	6.25, 12.5, 25, 37.5, 50, 75, 100, 200
Presence of Irradiation	0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0

3. Solar Simulator
The irradiation was performed with a Dr. Hönle Sol 500 solar simulator. The filter UV 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 heterogenous distribution of irradiation intensity the UVA intensity was measured for the complete area with a UV-meter. The homogeneous irradiation 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 maxima between 274.9 to 311.9 nm.

4. Experimental Performance
4.1 Seeding of the Cultures
About 2 x 10^4 cells per well were seeded in 100 µL culture medium (two plates, one was exposed to artificial sunlight, one was kept in the dark).

4.2 Treatment
24 hours after seeding the cultures were treated with the test item. The treatment was performed according to the OECD guideline as follows:

- the cultures were washed with EBSS
- 8 dilutions of the solved test item were tested on two 96-well plates (100 µL/well) both plates were pre-incubated for 1 hour in the dark
- after one hour one 96-well plate was irradiated through the lid at 1.65 mW/cm2 (4.95 J/cm2), for 50 min at 25 °C, the other plate was stored for 50 min at 25 °C in the dark.
- after irradiation the test item was removed and both plates were washed twice with EBSS.
- fresh culture medium was added and the cells were incubated overnight at 37 ± 1.5 °C and 7.5 ± 0.5% CO2

4.3 Determination of Neutral Red Uptake
The medium was removed and 0.1 mL serum free medium containing 50 µg Neutral Red / mL was added to each well. The plates were returned to the incubator for another 3 hours to allow uptake of the vital dye into the lysosomes of viable cells. Thereafter, the medium was removed completely and the cells were washed with EBSS. Then 0.15 mL of a solution of 49% (v/v) deionised water, 50% (v/v) ethanol and 1% (v/v) acetic acid were added to each well to extract the dye. After an additional 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.

4.4 Data Recording

The data generated were recorded in the laboratory raw data file. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive control. Arithmetic means ± standard deviation were calculated for every test group.
The ED50 values, the Photo-Irritancy-Factor (PIF), as well as the Mean Phototoxic Effect (MPE), were calculated using the software Phototox (Version 2.0) (distributed by [redacted], Germany, and recommended by the OECD guideline).
The ED50 values (effective dose where only 50% of the cells survived) were determined by curve-fitting software.

4.5 Evaluation of Results
Based on the results obtained, the test item is evaluated as follows:
If **PIF < 2 or MPE < 0.1**: no phototoxic potential predicted.
If **PIF > 2 and < 5 or MPE >0.1 and <0.15** a probable phototoxic potential is predicted.
If **PIF > 5 or MPE > 0.15** a phototoxic potential predicted

II. Results and discussion
The study was performed to assess the phototoxic potential of Flurtamone (AE B107599), technical material. The test was performed using BALB/c 3T3 c31 cells.
The following concentrations of the test item solved in DMSO (further diluted in EBSS, final concentration of DMSO in EBSS was 1% (v/v), dose calculation was adjusted to purity) were tested in the presence and in the absence of irradiation in both experiments: 0.48, 0.95, 1.90, 3.80, 7.60, 15.2, 30.4, 60.8 µg/mL.
As solvent control EBSS containing 1% (v/v) DMSO was used. Chlorpromazine was used as positive control. One test group of cells treated with the test item was irradiated with artificial sunlight for 50 minutes with 1.65 mW/cm2 UVA, resulting in an irradiation dose of 4.95 J/cm2 UVA. Another test group of test item treated cells were kept in the dark for 50 minutes.

Table 1: Summary of Results

	Substance	ED_{50} (+UV) [µg/mL]	ED_{50} (-UV) [µg/mL]	PIF	MPE	% viability of solvent control of irradiated versus non-irradiated plate
RFE	Test Item	-	-	-	0.006	97.5
	Positive control	0.16	8.94	55.60	0.705	93.3
ME	Test Item	-	-	-	-0.005	89.4
	Positive control	0.14	11.05	80.67	0.750	103.9

Cytotoxic effects did not occur after exposure of the test item to the cells, neither in the presence nor in the absence of irradiation with artificial sunlight in the RFE as well as in the ME. Therefore, ED_{50}-values or a PIF could not be calculated. The resulting MPE value was 0.006 and -0.005, respectively. Consequently, the test item is classified as not phototoxic (see chapter 3.9).
The mean of solvent control values of the irradiated group versus the non-irradiated group met the acceptance criteria.
The positive control chlorpromazine induced phototoxicity in the expected range in the presence of irradiation.

III. Conclusion
The test item is not phototoxic.

CA 5.3 Short-term toxicity

The studies below were already submitted at the time of the first Annex I inclusion.

Short-term toxicity studies were performed with flurtamone in rats, mice and dogs with repeated oral exposure for subacute and subchronic periods, also the effects after repeated exposure *via* the dermal administration were investigated in the rat. All studies were performed according to the current OECD guideline and in compliance with the GLP requirements.

No new studies were performed since Annex I inclusion.

CA 5.3.1 Oral 28-day study

In a 28-day study in Sprague-Dawley rats with flurtamone concentrations of 0, 300, 3000, 10000 or 20000 ppm in the diet. The NOAEL was 300 ppm, equivalent to 23.4 and 27.4 mg/kg/day in males and females, respectively.
In a 28-day dog toxicity study, 4 beagle dogs/sex/group were orally administered gelatine capsules containing 0, 40, 200, 500 or 800 mg/kg/day flurtamone. A NOAEL of 200 mg/kg/day was established in this study.

Report:	KCA 5.3.1 /01; [redacted];1988;M-160649-01
Title:	Four-week pilot oral toxicity study in rats with RE-40885-31 (SX-1762)
Report No:	R001490
Document No:	M-160649-01-1
Guidelines:	**USEPA (=EPA): 82-1;not specified**
GLP/GEP:	**yes**

Report:	KCA 5.3.1 /02; [redacted];1988;M-160652-01
Title:	4-week pilot feeding study in mice with Chevron RE-40885 technical (SX-1789)
Report No:	R001491
Document No(s):	Report includes Trial Nos.: 2107-157 M-160652-01-1
Guidelines:	**not specified;not specified**
GLP/GEP:	**no**

Report:	KCA 5.3.1 /03; [redacted];1990;M-201685-01
Title:	Four-week pilot oral toxicity study in dogs with Chevron RE-40885 (SX-1795)
Report No:	C016077
Document No(s):	Report includes Trial Nos.: 2107-159 M-201685-01-1
Guidelines:	**not specified;not specified**
GLP/GEP:	**yes**

CA 5.3.2 Oral 90-day study

In a subchronic dietary rat toxicity study, Sprague-Dawley rats were administered flurtamone at 0, 100, 1000, 7500, 10000 ppm in the diet for 90 days. The NOAEL in this study was 100 ppm, equivalent to 5.6 mg/kg/day.

In a 28-day dietary toxicity study, CD-1 mice were treated with concentrations of 0, 100, 300, 1000, 3500 or 7000 ppm in diet. A NOAEL of 300 ppm (equivalent to approximately 45 mg/kg/day in both sexes) was established.
In a subchronic dietary toxicity study, CD-1 mice (10/sex/dose) were exposed to flurtamone at concentrations of 0, 100, 1000, 3500 or 7000 ppm for 13 weeks. A NOAEL of 1000 ppm was established (estimated to be equivalent to approximately 15 mg/kg/day).
In a 52-week dog study, flurtamone technical was administered to 5 male and 5 female beagle dogs by capsule at dose levels of 5, 200, and 800 mg/kg bw/day. The NOAEL was 5 mg/kg/day.

Report:	KCA 5.3.2 /01; [redacted];1989;M-160641-02
Title:	13-week oral toxicity study in rats with RE-40885 (SX-1789)
Report No:	M-160641-02-1
Document No:	M-160641-02-1
Guidelines:	**USEPA (=EPA): 82-1;Deviation not specified**
GLP/GEP:	**no**

Report:	KCA 5.3.2 /02; [redacted];1989;M-160675-01
Title:	13-Week Pilot Feeding Study in Mice with RE-40885 Technical (SX-1794)
Report No:	R001501
Document No(s):	Report includes Trial Nos.: 2107-158 M-160675-01-1
Guidelines:	**USEPA: 82-1 Subchronic oral toxicity (rodent): 90-day study;not specified**
GLP/GEP:	**yes**

Report:	KCA 5.3.2 /03; [redacted];1991;M-163539-01
Title:	One-Year oral toxicity study in dogs with Chevron RE-40885 technical (SX-1802)
Report No:	R002894
Document No(s):	Report includes Trial Nos.: 2107-160 M-163539-01-1
Guidelines:	**USEPA (=EPA): 81-3;not specified**
GLP/GEP:	**yes**

CA 5.3.3 Other routes

In a 29-day dermal study, Sprague-Dawley rats (six/sex/dose) were exposed to flurtamone in aqueous suspension with 0.25% Carboxy Methyl Cellulose (CMC) to doses of 0, 40, 500 or 1000 mg/kg/day, 5 days per week, over a 29-day period (21 applications). The NOAEL was 1000 mg/kg/day.
In a second dermal toxicity study, Sprague-Dawley rats (five/sex/dose) were administered dermally flurtamone dissolved in polyethylene glycol (PEG 400) at doses of 0, 40, 500 or 1000 mg/kg b.w./day for 21 consecutive days without any treatment-related effect. Thus, also in this study a NOAEL of 1000 mg/kg/day was established.

No further studies were performed.

Report:	KCA 5.3.3 /01; [redacted];1989;M-160644-01
Title:	Four-Week Repeated-dose dermal toxicity Study in Rats with RE-40885 Technical

(SX-1802)
Report No: R001488
Document No: M-160644-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Report: KCA 5.3.3 /02; [redacted];1993;M-203228-01
Title: A 21-day dermal toxicity study in rats with flurtamone technical
Report No: C016883
Document No: M-203228-01-1
Guidelines: **USEPA (=EPA): 82-2;Deviation not specified**
GLP/GEP: **yes**

CA 5.4 Genotoxicity testing

The genotoxic potential of flurtamone was assessed in in-vitro and in-vivo genotoxicity studies according to the current OECD guidelines and according to GLP. These studies were already submitted for the first Annex I inclusion.
Flurtamone was negative for genotoxicity, both with and without metabolic activation, in an Ames study with S. typhimurium. Furthermore, a chromosomal aberration assay in Chinese Hamster ovary cells and a Mouse Lymphoma (L5178Y TK+/-) mutagenicity assay with and without metabolic activation were negative.
Also in an in-vivo test, the micronucleus test in mice with oral doses of 0, 200,1000 and 2000 mg/kg bw no clastogenic effect of flurtamone was seen.
Therefore, flurtamone is not considered as a genotoxic compound for humans.

No further studies were conducted.

CA 5.4.1 In vitro studies

Report: KCA 5.4.1 /01; [redacted];1986;M-160646-01
Title: L5178Y TK+/- mouse lymphoma mutagenicity assay with RE-40885 technical
Report No: R001486
Document No(s): Report includes Trial Nos.:
T5206.701
M-160646-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

Report: KCA 5.4.1 /02; [redacted];1988;M-160666-01
Title: Microbial/mammalian microsome plate incorporation mutagenicity assay with RE-40885 technical (SX-1802)
Report No: R001497
Document No: M-160666-01-1
Guidelines: **USEPA: Guideline 84-2;not specified**
GLP/GEP: **yes**

Report:	KCA 5.4.1 /03; [redacted];1988;M-160655-01
Title:	Chromosome aberrations in Chinese hamster ovary (CHO) cells RE-40885 technical
Report No:	R001492
Document No(s):	Report includes Trial Nos.: T8228.337003 M-160655-01-1
Guidelines:	**USEPA (=EPA): 84-2;not specified**
GLP/GEP:	**yes**

CA 5.4.2 In vivo studies in somatic cells

Report:	KCA 5.4.2 /01; [redacted];1986;M-160678-02; Amended: 1987-02-06
Title:	Acute in vivo cytogenetics assay in male and female rats
Report No:	M-160678-02-1
Document No(s):	Report includes Trial Nos.: TS206.105 M-160678-02-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

Report:	KCA 5.4.2 /02; [redacted];1990;M-160685-01
Title:	Test for chemical induction of unscheduled DNA synthesis in rat primary hepatocyte cultures by autoradiography : RE-40885 Technical
Report No:	R001505
Document No(s):	Report includes Trial Nos.: 0089-5100 M-160685-01-1
Guidelines:	**OECD: 482; USEPA (=EPA): 84-2; Deviation not specified**
GLP/GEP:	**no**

CA 5.4.3 In vivo studies in germ cells

Based on the toxicity and genotoxicity profile, in vivo studies in germ cells were not required.

CA 5.5 Long-term toxicity and carcinogenicity

Studies on long-term toxicity and carcinogenicity were performed in rats and mice, following the current OECD guidelines and in compliance with GLP.
In a two year chronic toxicity and oncogenicity study, 65 male and 65 female Sprague-Dawley Crl: CD®(SD)BR rats were exposed to flurtamone at concentrations of 0, 75, 500, 2500 ppm (equivalent to 0, 2.84, 18.4, 98 mg/kg/day in males and to 0, 3.73, 24.6 and 136 mg/kg/day in females), for 2 years with an interim sacrifice after one year. There was no increased incidence of neoplastic lesions compared to controls. The NOAEL for systemic toxicity was 75 ppm (equivalent to 2.84 and 3.73 mg/kg/day in males and females, respectively) and the NOAEL for oncogenicity was 2500 ppm (equivalent to 98 and 136 mg/kg/day in males and females, respectively).
In a combined chronic/oncogenicity study, 60 Crl: CD-1®(ICR)BR mice/sex, were given diets containing flurtamone at concentrations of 0, 30, 300, 3500 and 7000 ppm (equivalent to

approximately 0, 4.5, 45, 525 or 1050 mg/kg/day in both sexes) for at least 78 weeks. The no effect level for chronic effects was 30 ppm (approximately 4.5 mg/kg/day) and the NOEL for oncogenicity was 300 ppm (approximately 45 mg/kg/day).

These studies were submitted for the first Annex I inclusion. No new studies were performed.

Report:	KCA 5.5 /01; [redacted];1992;M-165798-01
Title:	Combined chronic Oral Toxicity/Oncogenicity Study in Rats with RE-40885 Technical
Report No:	R004014
Document No:	M-165798-01-1
Guidelines:	**USEPA (=EPA): 83-5;Deviation not specified**
GLP/GEP:	**yes**

Report:	KCA 5.5 /02; [redacted];1992;M-165800-02
Title:	Eighteen-months feeding bioassay in mice with Chevron RE-40885 technical (SX-1802)
Report No:	M-165800-02-1
Document No(s):	Report includes Trial No's: 2107-161 M-165800-02-1
Guidelines:	**USEPA (=EPA): 83-1;not specified**
GLP/GEP:	**yes**

CA 5.6 Reproductive toxicity

Flurtamone technical has been tested in a two-generation reproduction study in rats and in developmental toxicity studies in rats and rabbits in compliance with GLP and according to EEC guidelines.
These studies have been submitted for the first Annex I inclusion already and no new studies were performed.

CA 5.6.1 Generational studies

In the 2-generation rat study Flurtamone technical (91.9% purity) was administered to Sprague-Dawley rats (Crl: CD®BR) via the diet at concentrations of 0, 75, 500, 2000, and 5000 ppm. The NOAEL for adult toxicity was 500 ppm with a NOEL at 75 ppm. The NOEL for litter effects was 500 ppm, reproductive parameters were not affected at any doses.

Report:	KCA 5.6.1 /01; [redacted];1992;M-203254-01
Title:	Two-generation (two-litter) reproduction study in rats with RE-40885 technical (SX-1802)
Report No:	C016896
Document No:	M-203254-01-1
Guidelines:	**83-4;Deviation not specified**
GLP/GEP:	**yes**

CA 5.6.2 Developmental toxicity studies

In a rat developmental toxicity study, 25 pregnant Sprague-Dawley Crl: CD(SD) BR rats were treated with doses of 0 (aqueous suspension of 0.25% CMC containing 1% Tween 80), 5, 50, 250, or 1000 mg/kg/day by gavage from day 6 to day 15 of presumed gestation. The maternal NOEL was 50 mg/kg bw/day. The foetal NOEL was 250 mg/kg bw/day.
In a rabbit developmental toxicity study, dose levels of 0 (aqueous suspension of 0.25% CMC containing 1% Tween 80), 20, 200, or 600 mg/kg/day of flurtamone were administered by gavage to groups of 20 pregnant New Zealand rabbits on gestation days 6 to 19. The NOAEL for maternal toxicity was 20 and the developmental NOAEL 200 mg/kg bw/day.

Report:	KCA 5.6.2 /01; [redacted]; 1989; M-160656-01
Title:	Oral teratogenicity and developmental toxicity study in rabbits with Chevron RE-40885 technical
Report No:	R001493
Document No(s):	Report includes Trial Nos.: CEHC88-8, S-3115, M-160656-01-1
Guidelines:	**USEPA (=EPA): 83-3;not specified**
GLP/GEP:	**yes**

Report:	KCA 5.6.2/02; [redacted]; 1989; M-160671-02; Amended: 1998-06-29
Title:	Oral teratogenicity and developmental toxicity study in rats with Chevron RE-40885 technical
Report No:	R001500
Document No:	M-160671-02-1
Guidelines:	**USEPA (=EPA): 83-3;not specified**
GLP/GEP:	**yes**

CA 5.7 Neurotoxicity studies

No special studies on a neurotoxic potential of Flurtamone have been performed. The extensive toxicity data from all apical studies which are available on Flurtamone do not provide evidence of any direct neurotoxic effects. Therefore, based on the available data, Flurtamone does not present a neurotoxic hazard.

CA 5.7.1 Neurotoxicity studies in rodents

Based on the available information, Flurtamone does not present a neurotoxic hazard. Therefore, neurotoxicity studies were not conducted.

CA 5.7.2 Delayed polyneuropathy studies

Flurtamone does not belong to the class of organophosphates from which some have an OPIDP potential. Therefore, an OPIDP study was not necessary and not performed.

CA 5.8 Other toxicological studies

Other toxicological studies with the active ingredient were not performed since the toxicological profile was sufficiently demonstrated.

New studies with the metabolite Trifluoroacetic acid/acetate (TFA) were performed which are summarized in the following.

CA 5.8.1 Toxicity studies of metabolites

Report: KCA 5.8.1 /01;Airaksinen, M. M.; Tammisto, T.;1968;M-247837-01
Title: Toxic actions of the metabolites of halothane: LD50 and some metabolic effects of trifluoroethanol and trifluoroacetic acid in mice and guinea pigs
Report No: C047142
Document No: M-247837-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Report: KCA 5.8.1 /02;Lind, R. C.; Gandolfi, A. J.;1990;M-247891-01
Title: Covalent binding of oxidative biotransformation reactive intermediates to protein influences halothane-associated hepatotoxicity in guinea pigs
Report No: C047204
Document No: M-247891-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

Report: KCA 5.8.1 /03;Fraser, D. M.; Kaminsky, L. S.;1988;M-247892-01
Title: 2,2,2-Trifluoroethanol intestinal and bone marrow toxicity: The role of its metabolism to 2,2,2-trifluoroacetaldehyde and trifluoracetic acid
Report No: C047205
Document No: M-247892-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **no**

TFA studies:

In the following new studies with TFA which were not included in the Annex I are summarized.

Trifluoroacetic acid/acetate (TFA) (M45)

Report:	KCA 5.8.1 /21; [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:	01691JY
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 ± 3	15 ± 8	224 ± 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 ± 4	13 ± 3	16 ± 6	216 ± 10
1000	–	34 ± 4	103 ± 6	12 ± 3	20 ± 4	223 ± 6
5000	–	25 ± 5	104 ± 5	15 ± 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	–	29 ± 4	92 ± 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	–	37 ± 8*	103 ± 2	18 ± 4	23 ± 4	208 ± 27
1250	–	23 ± 3	95 ± 10	13 ± 5	17 ± 4	219 ± 37
2500	–	21 ± 4	95 ± 9	10 ± 3	20 ± 1	248 ± 40
5000	–	19 ± 2	88 ± 9	14 ± 4	18 ± 2	232 ± 27
Positive controls 2NF: 5.0	–	577 ± 29				
NaN_3: 2.0	–		438 ± 90	438 ± 30		
AAC: 50.0	–				75 ± 12	
MMC: 0.2	–					620 ± 8
Experiment 1						
Solvent control	+	30 ± 7	107 ± 16	17 ± 3	20 ± 5	202 ± 30
TFA 1.6	+	36 ± 8	92 ± 12	13 ± 3	17 ± 3	193 ± 16
8	+	36 ± 10	99 ± 19	14± 7	20 ± 1	162 ± 10
40	+	49 ± 7	99 ± 5	19 ± 3	13 ± 3	180 ± 14
200	+	33 ± 8	107 ± 6	16 ± 2	18 ± 4	185 ± 20
1000	+	31 ± 2	88 ± 8	20 ± 2	17 ± 8	189 ± 7
5000	+	36 ± 15	93 ± 8	15 ± 5	22 ± 8	177 ± 45
Positive controls B[a]P: 10.0	+	245 ± 32				
AAN: 5.0	+		977 ± 35	196 ± 24	97 ± 3	
AAN: 20.0	+					492 ± 13

Dose (µg/plate)	S-9 mix (-/+)	*Salmonella typhimurium* strains TA98	TA100	TA1535	TA1537	TA102
		mean ± SD				
Experiment 2						
Solvent control	+	41 ± 5	112 ± 12	17 ± 3	22 ± 2	196 ± 34
TFA 156.25	+	32 ± 10	76 ± 5	12 ± 1	34 ± 7	176 ± 29
312.5	+	29 ± 6	94 ± 5	20 ± 3	23 ± 1	208 ± 3
625	+	38 ± 10	69 ± 5	21 ± 6	18 ± 4	178 ± 29
1250	+	41 ± 6	75 ± 6	10 ± 3	20 ± 4	212 ± 35
2500	+	26 ± 5	93 ± 9	10 ± 3	21 ± 2	216 ± 6
5000	+	34 ± 3	90 ± 4	15 ± 4	20 ± 4	232 ± 18
Positive controls B[a]P: 10.0	+	399 ± 62				
AAN: 5.0	+		1058 ± 107	290 ± 72	624 ± 16	
AAN: 20.0	+					796 ± 246

* Dunnett's test, significant 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: KCA 5.8.1 /22; [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: 2014/84-116173
Document No: M-260699-01-1
Guidelines: **OECD 476; JAKEMS 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	85.10
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		

Dose (µg/mL)	-S9		+S9	
	% rel. total growth	mutant frequency§	% rel. total growth	mutant frequency§
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: KCA 5.8.1 /23; [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: 01692L/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	 Excluding gaps	Mitotic Index (mean)
Experiment 1 (3 hour treatment + 17 hour recovery, +/- S9)					
Solvent	+	200	1	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	4	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 $p \leq 0.001$

Numerical aberrations

No increases in the frequency of cells with numerical aberrations, that exceeded the historical negative control range, were generally observed in cultures treated with TFA in the absence and presence of S9. The only exception to this was observed in Experiment 1 in a single culture at the lowest concentration analysed following 3+17 hour treatment in the presence of S9. In this culture the numerical aberration frequency marginally exceeded the historical control range. In isolation, this increase is not considered to be of any biological relevance.

Table 5.8.1/23-2: Summary of numbers and types of numerical aberrations

Substance Dose (µg/mL)		+/- S9	Cells scored	Numerical aberrations			Total abs	% with num abs
				hyperdiploid	endoreduplicated	polyploid		
Experiment 1 (3 hour treatment + 17 hour recovery, +/-S9)								
Solvent		+	200	0	0	0	0	0
TFA	340	+	203	0	0	3	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	+	197	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	-	102	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: KCA 5.8.1 /24; [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" ([redacted] 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:	KCA 5.8.1 /25; [redacted] ,2001;M-202165-01
Title:	Trifluoroacetate - Exploratory 14-day toxicity study in the rat by dietary administration
Report No:	C016316
Document No(s):	Report includes Trial Nos.: SA01136
	M-202165-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	Trifluoroacetate
Description:	white powder
Lot/Batch no:	129H3458
Purity:	98.7% (Sigma-Aldrich)
2. Positive control:	clofibric acid (positive control for peroxisomal proliferation)
3. Test animals	
Species:	Wistar (HAN) rats
Strain:	RJ: 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. ([redacted], 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

	Trifluoroacetate				Pos.	Trifluoroacetate				Pos.
Dose (ppm)	**0**	**600**	**1200**	**2400**	**Contr.**	**0**	**600**	**1200**	**2400**	**Contr.**
Body weight (g)	males					females				
Day 1	336	335	341	336	336	221	224	223	223	223
Day 7	376	373	383	368	322*	239	240	237	235	222*
Day 14	406	410	421	408	327*	251	249	248	246	227*

Pos. = positive; Contr. = control

* statistically different from control $p \leq 0.01$

Table 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	26	23	22	-1*

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: Haematology summary

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	103	214*	69	60	77	63	67
Urea (mmol/L)	4.71	4.63	4.69	5.09	7.09**	5.12	5.14	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	3.85	7.20	4.38	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	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	--	205	--	--	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**	234	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 ≤ 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 mg/kg bw/day) in males and 2400 ppm (190 mg/kg bw/day) in 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). Trifluoroacetate is a very weak peroxisome proliferator in male rats at doses ≥ 1200 ppm (85 mg/kg bw/day).

Report:	KCA 5.8.1 /26; [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 ([redacted], 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 means (unit)	Dose group (ppm) males					Dose group (ppm) females				
	0	600	1800	5400	16000	0	600	1800	5400	16000
ALAT (IU/L)	38	44	48	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 ≤ 0.05, p ≤ 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	1	1	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	2.1	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 of the histopathological findings encountered were considered to have arisen spontaneously.

III. Conclusion

A NOAEL of 16000 ppm (equivalent to 1315 / 1344 mg/kg bw/day, males/females) was established.

Report:	KCA 5.8.1 /27; [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: 225 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. ([redacted], 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	488	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	253	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 ($p \leq 0.01$)

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 dosage 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 ± 178 (+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 (--)	5.37 ± 0.86 (--)	50 ± 12 (--)	73 ± 12 (--)	38 ± 9 (--)
160	1.8 ± 0.4 (−14%)	5.13 ± 0.56 (−4%)	45 ± 10 (−10%)	82 ± 17 (+12%)	40 ± 10 (+5%)
1600	1.0 ± 0.6** (−52%)	4.19 ± 0.45** (−22%)	53 ± 11 (+6%)	87 ± 16 (+19%)	47 ± 17 (+26%)
16000	0.5 ± 0.2** (−76%)	4.62 ± 0.11** (−14%)	50 ± 12 (±0%)	85 ± 12 (+16%)	45 ± 5 (+18%)

Bili = total bilirubin; Gluc = glucose; ALP = alkaline phosphatase; AST = aspartate amino transferase
ALP = alanine amino transferase
* = statistically significant different from control ($p \leq 0.05$); ** = statistically significant different from control ($p \leq 0.01$)

Several males from all treated and control groups had elevated aspartate aminotransferase and alanine aminotransferase activities. These effects were considered to be treatment-related at 16000 and 1600 ppm in males as they were of high magnitude and/or outside the in-house historical control data. There was no effect on these parameters in females at any dose level.

The other statistically significant differences were considered not to be treatment-related in view of the variation of the individual values and/or their low magnitude.

Urine analysis

When compared to the control groups, higher ketone levels were noted at 16000 and 1600 ppm in both sexes.

No other treatment-related change was noted for the parameters assayed. The few other statistically significant differences were considered to be incidental.

Table 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	1	0	0	0	0	0	0	0
	2	1	0	0	0	0	0	0	0
	3	1	0	0	1	0	0	0	0
	4	0	0	0	0	0	0	0	0
Protein	0	0	0	0	0	9	10	10	10
	1	0	1	0	2	0	0	0	0
	2	9	9	8	8	0	0	0	0
	3	1	0	0	0	0	0	0	0
	4	0	0	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 / Dose (ppm)	Mean (% change when compared to control)					
	Absolute liver weight (g)		Liver to body weight ratio		Liver to brain weight ratio	
	Males					
0	12.15	(--)	2.327	(--)	566.930	(--)
160	11.61	(–4%)	2.258	(–3%)	546.177	(–4%)
1600	13.25*	(+9%)	2.657**	(+14%)	613.081	(+8%)
16000	14.48	(+19%)	3.102**	(+33%)	701.329**	(+24%)
Dose (ppm)	**Females**					
0	5.96	(--)	2.243	(--)	307.108	(--)
160	6.25	(+5%)	2.343	(+4%)	316.173	(+3%)
1600	6.71*	(+13%)	2.520**	(+12%)	334.508	(+9%)
16000	7.36**	(+23%)	2.880**	(+28%)	382.960**	(+24%)

* = statistically significant different from control ($p \leq 0.05$); ** = statistically significant different from control ($p \leq 0.01$)

J. Gross necropsy

Unscheduled death

One male was found dead on study day 15. This animal was noted to have torsion and a dark content within the ileum and jejunum. This intestinal torsion was considered to be the cause of death and was therefore incidental. All other macroscopic findings were related to agonal changes found at the histopathology examination and were considered not to be treatment-related.

Terminal sacrifice

With the exception of the higher incidence of foci (red or white) within the liver observed in males at 16000 ppm, all the other changes were considered to be incidental and not treatment-related

K. Micropathology

Unscheduled death

In addition to agonal changes, degenerative cardiomyopathy was noted. This change is a common spontaneous finding observed in the Wistar rat of this strain and age, it was considered not to be treatment-related. The cause of death was considered to be the intestinal torsion noted at necropsy.

Terminal sacrifice

Treatment-related histopathological changes were observed in the liver.

In all male and most females at 16000 ppm, as well as in a proportion of males at 1600 ppm, a minimal to moderate diffuse centrilobular to panlobular hepatocellular hypertrophy with ground-glass appearance of the hepatocellular cytoplasm was observed. This latter observation is usually induced by peroxisome proliferators. This change was associated with a loss of the periportal hepatocellular vacuolation observed at 16000 ppm in both sexes and at 1600 ppm in males. The effect was dose-related and correlated with the higher mean liver weight noted in these groups.

There was also a higher incidence of hepatocellular necrotic foci in males at 16000 ppm when compared to controls, which was considered to be adverse. This finding was correlated with higher individual values of aspartate aminotransferase and alanine aminotransferase activities observed in clinical chemistry evaluation.

A higher incidence of minimal to slight degenerative cardiomyopathy was noted in males at 16000 ppm. As this change is a common spontaneous finding observed in the Wistar rat of this strain and age, including in untreated control animals, with a similar severity and incidence, it was considered not to be treatment-related.

No effect of treatment was seen in any other organ examined microscopically. Some other histopathological findings were noted in animals of all groups but they were considered to be incidental, as they were within the range of expected changes for rats of this age and strain kept under laboratory conditions.

Table 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		0
Total	4	3	0	0	5	6		0
Hepatocellular necrotic focus (i), focal/multifocal								
Minimal	1	0	1		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	4	1	1	1	1

III. Conclusion

Based on the study results (changes in haematological, clinical chemistry parameters, organ weights and histopathological liver findings) the NOAEL after 90-day dietary exposure was 160 ppm (equivalent to 9.9 / 12.2 mg/bw/day, males/females).

Report: KCA 5.8.1/28; [redacted];2010;M-411209-01
Title: Trifluoroacetic acid: Embryo-fetal oral gavage toxicity study in rats
Report No: 09-435
Document No: M-411209-01-1
Guidelines: **US-EPA OPPTS 870.3700; OECD 414;**
Deviations: none
GLP/GEP: **Yes**

Report:	KCA 5.8.1 /17; [redacted];1999;M-207969-01
Title:	Trifluoroacetic acid: Health effect risk assessment, flurtamone
Report No:	C019618
Document No:	M-207969-01-1
Guidelines:	**Not applicable;**
	Deviation: not applicable
GLP/GEP:	**n.a.**

I. Methods

A health effects assessment for trifluoroacetic acid (TFA) was done by reviewing literature data for pharmacokinetic, acute toxicity, irritation and sensitization, repeated dose toxicity, genotoxicity, carcinogenicity, and reproductive toxicity. The reviewed papers were published between 1966 and 1996 and comprised *in vivo* and *in vitro* study data.

II. Results and discussion

Summary of TFA toxicological profile

Acute Toxicity

TFA has a moderate acute toxicity; it is a skin and eye irritant. All this effects are mainly due to acidic properties and that such effects would not occur at lower concentrations. No clear information is available on the skin sensitizing properties.

Repeated toxicity

Available studies investigated the toxic effects of TFA after administration by gavage or by mixing in the diet.

Gavage administration did not produce observable effects. Dietary administration evidenced in both 14-day and 26-day dietary toxicity study that TFA is a rodent peroxisome proliferator.

Genotoxicity

TFA was negative to Ames test and to DNA repair test.

Long term toxicity

No long term studies are available. Studies carried out with HCFCs which are mainly metabolized to TFA did not show carcinogenic potential.

Reproductive toxicity

TFA had not been tested for teratogenicity of fetotoxicity.

An *in vivo* study with mice has shown that TFA may accumulate in the amniotic fluid. *In vivo* and *in vitro* studies have shown that TFA does not induce testicular toxicity.

III. Conclusion

TFA toxicological profile has been reviewed. On the basis of existing information, it is concluded that no health concern may arise from low exposure to TFA.

CA 5.8.2 Supplementary studies on the active substance

Since the toxicological profile was sufficiently demonstrated, no supplementary studies were necessary.

CA 5.8.3 Endocrine disrupting properties

It should be noted that to date, no clear criteria are available to define endocrine disrupting properties.

Flurtamone has no effects on reproductive indices nor fertility nor reproductive tissues and organs as shown in the multi-generation study and is not a developmental toxicant.
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 flurtamone 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 flurtamone.

CA 5.9 Medical data

The latest status is given as follows.

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Occupational medical surveillance of workers exposed to Flurtamone performed since 2005 annually on a routine basis, not directly related to exposures, did not reveal any unwanted effects in the workers. The examinations included the above laboratory parameters and clinical and technical examinations.
During the production period since 2005 no accidents with Flurtamone occurred in the workers. No further consultations of the Medical Department due to work or contact with Flurtamone were required

CA 5.9.2 Data collected on humans

No such data are available.

CA 5.9.3 Direct observations

Not available.

CA 5.9.4 Epidemiological studies

No such observation or epidemiologic studies are known.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

No human poisoning cases are known.
In animal experiments single high dose application did not cause symptoms.

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 does not seem to be required in regard of the low toxicity. It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious.
Induced vomiting can remove maximum 50% of the ingested substance.
Note: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!
Treatment:
- Gastric lavage does not seem to be required in regard of the low toxicity of the compound
- The application of activated charcoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.
- As there is no antidote, treatment has to be symptomatic and supportive.

CA 5.9.7 Expected effects of poisoning

In regard of the low toxicity no persisting effects are to be expected.