Annex I to the CLH report:

Robust Study Summaries

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

esfenvalerate (ISO); (*S*)-α-cyano-3-phenoxybenzyl-(*S*)-2-(4-chlorophenyl)-3-methylbutyrate

EC Number: **not assigned**

CAS Number: **66230-04-4**

Index Number: **608-058-00-4**

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Chemicals Regulation Division
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CONTENTS

The study summaries provided in Annex I to the CLH report are taken from the relevant sections of the EFSA RAR (June 2014). Where appropriate, additional information from the study reports has been added to facilitate the evaluation for classification.

1 PHYSICAL HAZARDS

Physical hazards are not required to be evaluated in this CLH dossier. A summary table was provided in the main CLH report for information (7. Physicochemical properties). Please refer to the IUCLID technical dossier for summaries of the studies presented in the RAR.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 [Study 1]

Study	IIA 5.1.1/01 Metabolism of fenvalerate (Sumicidin) in rats
Reference	Ohkawa H, Kaneko H, Tsujii H, Miyamoto J (1979)
Date performed	Not reported
Test facility	Pesticides Division, Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº AM-90-0090, published in J Pesticide Science, 4, 143-155, 1979
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	No, study predates the introduction of GLP
Test material	[^{14}C-cyano]-fenvalerate, [^{14}C-cyano]-esfenvalerate, [^{14}C-carbonyl]-esfenvalerate, [^{14}C-benzylic]-esfenvalerate, [^{14}C-chlorophenyl]-chlorophenyl-isovaleric acid (CPIA) Purities and batch numbers were not reported
Study acceptable	Yes

METHODS

Various test substances were administered in one single oral treatment (8.4 mg/kg) or in a repeated treatment (five daily doses of 1.7 mg/kg by oral intubation) to male Sprague Dawley rats. The test substances were the following: [^{14}C-cyano]-fenvalerate, [^{14}C-cyano]-esfenvalerate, [^{14}C-carbonyl]-esfenvalerate, [^{14}C-benzylic]-esfenvalerate, [^{14}C-chlorophenyl]-chlorophenyl-isovaleric acid (CPIA). Urine, faeces and expired CO_2 were collected daily for up to 14 days after single treatment and up to 6 days after the last repeat treatment, and at the end of these periods the animals were sacrificed and the tissues analysed for radioactive content, whole body was autoradiographed 6 and 24 hours after a single dose of [^{14}C-cyano]-esfenvalerate.

RESULTS

Following a single oral dose or five consecutive daily doses of [^{14}C-carbonyl] esfenvalerate and [^{14}C-benzylic] esfenvalerate, radioactivity was almost completely eliminated and excreted in urine and faeces within six days. No $^{14}CO_2$ was expired. Following [^{14}C-cyano] fenvalerate and esfenvalerate, excretion was slower and $^{14}CO_2$ was also expired. The maximum blood concentration (Cmax) of fenvalerate was approximately 0.4 µg/g and was reached (Tmax) at the first timepoint, 1 hr after dosing. By 6 hours after dosing the concentration of fenvalerate in blood had declined to approximately 0.05 µg/g. The CN group of the alcohol moiety was rapidly converted mainly to thiocyanate which retained relatively longer in selective tissues including skin and hair. Fenvalerate and esfenvalerate yielded two faecal ester metabolites which resulted from hydroxylation at the 4' and 2'-phenoxy positions. Other significant metabolites were 3-phenoxybenzoic acid and its hydroxy derivatives (free and conjugated) from the alcohol labelled compound, 3-(4-chlorophenyl)isovaleric acid and its hydroxy derivatives (free, lactones and conjugates) from the acid labelled compound, and thiocyanate and CO_2 from the CN-labelled compounds. There were no apparent differences in the

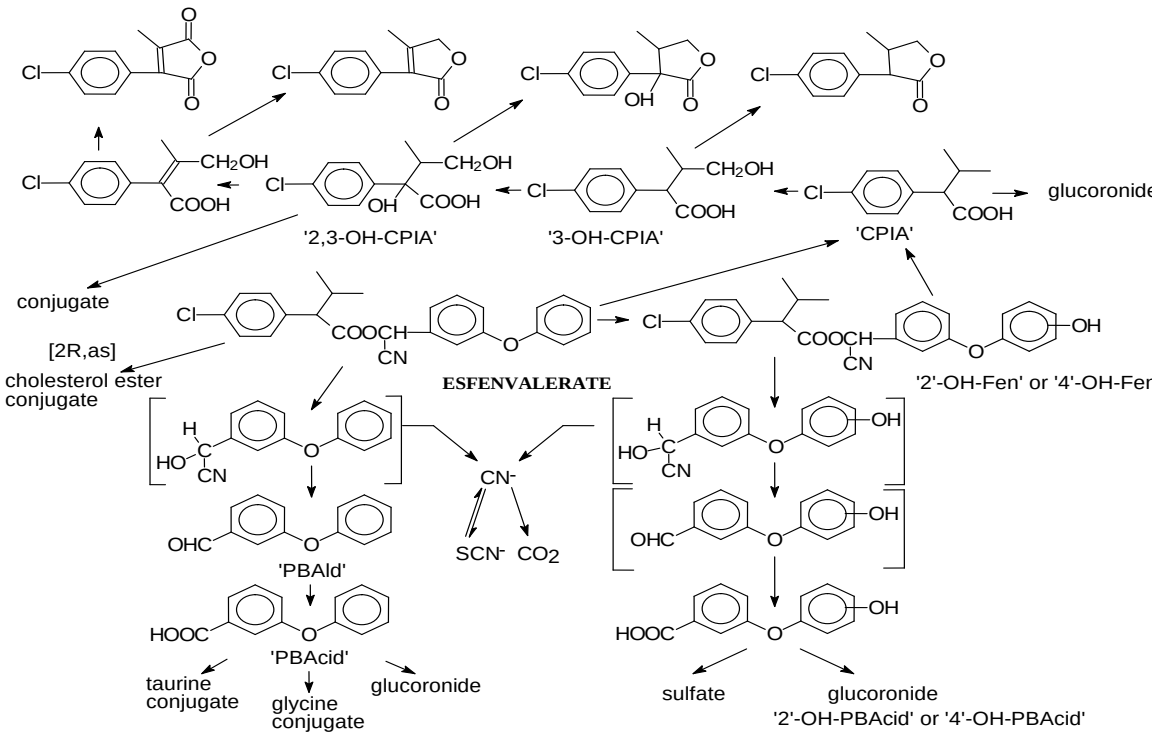
nature and amount of metabolites, and in the patterns of ^{14}C excretion and tissue residues between fenvalerate and esfenvalerate.

Tissue residues following [^{14}C-carbonyl] or [^{14}C-benzylic] esfenvalerate was very low and levels after 14 days were lower than those after six days. Tissue levels following [^{14}C-cyano] fenvalerate or esfenvalerate were higher, and the highest radioactivity was found in skin and hair. Radioactivity six days after the last of the repeat administrations was similar to levels after single administration.

Unmetabolised fenvalerate accounted for 85 - 90% of the radioactivity in the fat and thiocyanate 86% of ^{14}C in the blood. There was no significant difference in excretion rates and tissues residue levels between fenvalerate and esfenvalerate. Similar tissue residues were found following whole body autoradiography.

The metabolic pathways for fenvalerate after oral administration to mammals are given in Figure B.6.1.1-1

Figure B.6.1.1-1 Metabolic pathways of esfenvalerate in mammals



CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

The excretion, tissue residues and metabolite excretion patterns between fenvalerate and esfenvalerate were similar following a single or 5 daily oral doses and it was concluded that both compounds would be metabolised in a similar way. More than 20 metabolites were identified. The significant metabolic reactions were oxidation at the 2- and 4- positions of the acid, and at the 2'- and 4'- positions of the alcohol moiety, cleavage of the ester linkage and conversion of the cyano group to SCN^- and CO_2. The major radioactive products in the faeces were unmetabolised fenvalerate and two ester metabolites ('2'-OH'-Fen' and '4'-OH-Fen'). No unmetabolised fenvalerate was found in the urine. The major urinary metabolites were chlorophenylisovaleric acid ('CPIA'), 3-phenoxybenzoic acid ('PBacid') SCN-, and/or products of further oxidative and conjugation reactions. Tissue residues were low after single or repeat dosing.

2.1.2 [Study 2]

Study	IIA 5.1.1/02 Comparative metabolism of fenvalerate and the [2S - αS] isomer in rats and mice
Reference	Kaneko H, Ohkawa H, Miyamoto J (1981)
Date performed	Not reported
Test facility	Pesticides Division, Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº AM-10-0141, published in J Pesticide Science, 6, 317 - 326, 1981
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	No, study predates the introduction of GLP
Test material	Fenvalerate (carbonyl labelled, benzylic carbon labelled and CN group labelled). Esfenvalerate (clorophenyl labelled, phenylring labelled and CN group labelled). Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

Fenvalerate (carbonyl labelled, benzylic carbon labelled and CN group labelled), and esfenvalerate (clorophenyl labelled, phenylring labelled and CN group labelled) were administered once by gastric intubation to Sprague-Dawley rats and ddy mice at dose levels of 6.7 – 7.8 mg/kg and 3.7 – 4.9 mg/kg for fenvalerate and esfenvalerate respectively. Each of the ^{14}C-fenvalerate preparations were also administered to SD rats at the rate of 30 mg/kg. Another group of male Wistar rats and mice were fed diet containing unlabelled fenvalerate at a concentration of 500 mg/kg for two weeks after which they were given a single oral dose of ^{14}C-fenvalerate at 2.1 and 8.4 mg/kg respectively.

Urine, faeces and expired air of treated animals were collected at intervals up to seven days. At termination tissues were removed and analysed for radiocarbon content; whole body was autoradiographied.

RESULTS

SD rats dosed with 30 mg/kg showed acute toxic symptoms such as tremor and salivation one to four hours after dosing and then recovered. On single oral administration of each of the ^{14}C-acid, alcohol and -CN-labelled preparations of fenvalerate and the [2s, αS]-isomer at 4.2 to 30 mg/kg, the radiocarbon from the acid and alcohol moieties was almost completely eliminated from the body of rats and mice, and the ^{14}C-tissue residues were generally very low. Radiocarbon from the CN-labelled preparations was slowly excreted and higher ^{14}C-tissue residues were found in hair, skin and stomach contents. There were no differences in recovery of radioactivity (67 - 102% of that administered), half-life (1.0 - 2.6 and 0.5 - 0.9 for the CN-labelled and other radiolabel positions, respectively) and ^{14}C-tissue residues between the compounds or sexes. Two weeks pre-feeding with unlabelled fenvalerate resulted in a more complete elimination of radiolabelled fenvalerate and lower tissue levels in rats and mice than those in the non-feeding treatment.

Fenvalerate and the [2S, αS]-isomer were similarly metabolised, by oxidation at the 2'-and 4'-phenoxy positions of the alcohol and at the C-2 and C-3 positions of the acid moiety, cleavage of the ester linkage, conversion of the CN group to SCN^- and CO_2, and conjugation of the resultant carboxylic acids and phenols with glucuronic acid, sulphuric acid and/or amino acids.

No significant differences in the nature or amounts of the metabolites, were seen between the sexes, dose levels or isomers; the following species differences were observed: taurine conjugate of 3-phenoxybenzoic acid was found in mice but not rats; 4'-hydroxylation of the alcohol moiety and sulphate conjugate of 3-(4'-hydroxyphenoxy)benzoic acid occurred to greater extents in rats than in mice; a greater amount of thiocyanate was excreted in mice than in rats.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012.

Elimination of fenvalerate and esfenvalerate in rats and mice was virtually complete (94 – 100%) within 6 to 7 days of dosing with half-lives ranging from 0.5 to 2 days, depending on the position of the label.

Differences between the species were observed in the nature and amounts of metabolites derived from the alcohol moiety. These were as follows:

i) 4'-hydroxylation was greater in rats than in mice.

ii) 3-phenoxybenzoic acid ('PBacid') was taurine conjugated in mice, but not in rats.

iii) sulphate conjugation of 3-(4'-hydroxyphenoxy)benzoic acid ('4'-OH-PBacid') was greater in rats than in mice.

iv) conversion of the cyano group to SCN^- was greater in mice than in rats.

2.1.3 [Study 3]

Study	IIA 5.1.1/03 Comparative Metabolism of Esfenvalerate and Fenvalerate in Rats and Mice – I. Single or 10 consecutive oral administration
Reference	Anonymous (1985a)
Date performed	June – December 1985
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº LLM-50-0007.
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	No
Test material	[^{14}C-chlorophenyl] esfenvalerate; [^{14}C-phenoxybenzyl] esfenvalerate; [^{14}C-chlorophenyl] fenvalerate; [^{14}C-phenoxybenzyl] fenvalerate. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

The dose levels and administration of fenvalerate and esfenvalerate in rats and mice were as follows:

- Single oral treatments to rats and mice of [^{14}C-chlorophenyl] or [^{14}C-phenoxybenzyl] esfenvalerate at 2.5 mg/kg.

- Single oral treatments to rats and mice of [^{14}C-chlorophenyl] or [^{14}C-phenoxybenzyl] fenvalerate at 2.5 and 10 mg/kg.

- Repeat treatment orally for ten consecutive days to mice of [^{14}C-chlorophenyl] esfenvalerate.

- Repeat treatment orally for ten consecutive days to mice only of [^{14}C-chlorophenyl] fenvalerate.

Faeces and urine were collected daily for seven days (from animals receiving the single treatment) or for 16 days (from the day of the first administration from animals receiving 10 treatments). At the end of seven day treatment-free sampling period, the animals were killed and major tissues isolated for analysis.

RESULTS

Excretion: Following single dose oral administration of [^{14}C-chlorophenyl] or [^{14}C-phenoxybenzyl] esfenvalerate and fenvalerate to rats and mice, there was almost complete elimination of radioactivity from the animals within seven days of administration. The distribution of radioactivity excreted in urine and faeces was very similar for esfenvalerate, and fenvalerate. There was no significant

difference between the sexes. Total recoveries in urine and faeces in rats and mice are given in Table B.6.1.3-1. There was rapid elimination of radioactivity from mice at the end of the ten-day treatment period. The proportion recovered from the urine was within the range determined following a single administration and there was no difference in the excretion patterns from esfenvalerate and fenvalerate.

Table B.6.1.3-1 Percentage recovery of applied radioactivity in urine and faeces over seven days following a single oral administration of esfenvalerate and fenvalerate in rats and mice

Species	Position of label	Urine	Faeces	Total
Rat	[^{14}C-chlorophenyl] [^{14}C-phenoxybenzyl]	20 - 39 24 - 35	59 - 79 61 - 71	97 - 101 95 - 101
Mouse	[^{14}C-chlorophenyl] [^{14}C-phenoxybenzyl]	38 - 57 49 - 60	37 - 64 42 - 49	95 - 102 94 - 103

Tissue residues: The tissue residues were very low, although significant levels of radioactivity were detected in fat (see Table B.6.1.3-2). Some residues of fenvalerate were also detected in the adrenal, liver, ovary and spleen. There was little difference between tissue residues following administration of [^{14}C-phenoxybenzyl] esfenvalerate and fenvalerate, but residues were slightly higher following administration of [^{14}C-chlorophenyl] fenvalerate compared with esfenvalerate in some tissues.

Table B.6.1.3-2 Residues in fat (ng equivalents/g wet tissue) seven days after single oral administration

Test substance	Dose level (mg/kg)	Mouse		Rat	
		Male	Female	Male	Female
[^{14}C-chlorophenyl] esfenvalerate	2.5	301	389	178	201
[^{14}C-phenoxybenzyl] esfenvalerate	2.5	384	362	294	294
[^{14}C-chlorophenyl] fenvalerate	2.5	118	235	182	301
[^{14}C-chlorophenyl] fenvalerate	10	1340	1570	1318	1323
[^{14}C-phenoxybenzyl] fenvalerate	10	1160	1579	1289	819

Metabolite identification: The radioactivity in faeces contained a high proportion of unchanged parent material. Urine contained none or only small quantities of parent compound. Major metabolites were '4'-OH-Fen', 2'-OH-Fen', CPIA' and '2,3-OH-CPIA'. There was no overall difference in the metabolism of esfenvalerate, and esfenvalerate with added [2S, αR]-, [2R, αS]- and [2R, αR]-isomers. There was also little difference between esfenvalerate and fenvalerate except that a trace amount of 'CPIA cholesterol ester' was produced in mouse faeces with fenvalerate. There were no significant differences in the behaviour of these substances in mice or rats and no sex-related differences were noted.

Major biotransformation reactions are as follows:

(1) oxidation at the 2- and 3- positions of the acid moiety and at the 2'- and 4'- phenoxy positions of the alcohol moiety, (2) cleavage of the ester linkage and (3) conjugation of the resultant carboxylic acids, alcohols and phenols with glucuronic acid, sulfuric acid, glycine and taurine.

Overall, it can be concluded that esfenvalerate undergoes the same metabolic fate as fenvalerate except that [^{14}C-chlorophenyl] fenvalerate gives slightly higher tissue and produces a trace of 'CPIA cholesterol ester'.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

Esfenvalerate and fenvalerate isomers were excreted readily in both rats and mice following single oral dosing in both species and repeated oral dosing for 10 days in mice. There was almost complete elimination of radioactivity within 7 days after dosing. There was no difference in excretion patterns following dosing with either esfenvalerate or fenvalerate. Tissue residues were generally very low. There was little difference in metabolism between esfenvalerate and fenvalerate, except that a trace amount of 'CPIA cholesterol ester' was found in the faeces of mice dosed with fenvalerate.

2.1.4 [Study 4]

Study	IIA 5.1.1/04 Comparative Metabolism of Esfenvalerate and Fenvalerate in Rats and Mice – II. 28 days Dietary Administration in Mice
Reference	Anonymous (1985b)
Date performed	July – December 1985
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº LLM-50-0008.
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	GLP status not reported
Test material	[^{14}C-chlorophenyl] esfenvalerate; [^{14}C-chlorophenyl] fenvalerate. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

Male and female ddy mice were fed diets containing [^{14}C-chlorophenyl] esfenvalerate (25 ppm) or [^{14}C-chlorophenyl] fenvalerate (25 ppm) diluted in corn oil for 28 days, and a basal diet for subsequent 28 days.

Concentration of test substance in diet were observed; the body weight and total radioactivity in adrenal, brain, fat, kidney, liver, lymph node, ovary, skin and hair, spleen and tests was determined 10, 19, 24 and 28 days after start of administration plus 4, 7, 21 and 28 days after start of untreated diet treatment (equivalent to 32, 35, 49, and 56 days after start of administration).

RESULTS

There were no significant differences between food consumption and body weight gain of the different dose groups of either sex during the course of the study. The concentration of radioactivity in all tissues approached a plateau after 28 days administration and then declined during administration of untreated diet. Total radioactivity after 28 days administration and 28 days of untreated diet following ^{14}C esfenvalerate and ^{14}C fenvalerate at 25 mg/kg, are given in Tables B.6.1.4-1 and B.6.1.4-2. The percentage of the radioactivity accounted for by the metabolite CPIA cholesterol ester is also presented in the tables.

Table B.6.1.4-1 Total radioactivity (µg/g tissue) equivalents and % of 'CPIA cholesterol ester' present after 28 days administration of test substance in the diet.

	Esfenvalerate 25 mg/kg		Fenvalerate 25 mg/kg	
	male	female	male	female
Adrenal	1.3	1.0	5.3 (75%)	5.9 (88%)
Blood	0.25	0.26	0.25 (8%)	0.31 (13%)
Brain	0.02	0.02	0.04	0.04
Fat	6.56	6.50	5.41	6.69
Kidney	0.63	0.32	0.59 (42%)	0.55 (55%)
Liver	0.50	0.54	1.83 (61%)	2.89 (69%)
Lymph	1.1	1.7	3.3 (67%)	3.5 (77%)
Skin	1.26	2.03	1.24	2.22
Spleen	0.19	0.13	0.62 (68%)	0.69 (81%)
Testes	0.04	--	0.23 (70%)	--
Ovary	--	0.40	--	2.11 (81%)

Table B.6.1.4-2 Total radioactivity (µg/g tissue) equivalents and % of 'CPIA cholesterol ester' present after 28 days administration of test substance followed by 28 days administration of untreated diet

	Esfenvalerate 25 mg/kg		Fenvalerate 25 mg/kg	
	male	female	male	female
Adrenal	*	*	6.0 (93%)	5.0 (96%)
Blood	*	*	0.01	0.01
Brain	*	*	0.02	0.02
Fat	1.97	1.19	3.84	1.31
Kidney	0.01	0.00	0.09 (89%)	0.17 (88%)
Liver	0.01	0.01	0.28 (73%)	0.44 (59%)
Lymph	0.1	0.1	1.4 (86%)	2.4 (92%)
Skin	0.06	0.21	0.33	0.35
Spleen	0.00	0.00	0.39 (90%)	0.66 (94%)
Testes	0.01	--	0.23 (74%)	--
Ovary	--	0.0	--	2.0 (95%)

* Below the limit of determination

Total radioactivity in some tissues (adrenals, spleen, ovary) following feeding with [^{14}C chlorophenyl] esfenvalerate was lower than in tissues from animals fed ^{14}C fenvalerate at the same dosage level (25 mg/kg); in other tissues total radioactivity levels were similar. Total radioactivity in tissues following feeding with [^{14}C chlorophenyl] fenvalerate at 100 mg/kg was approximately four times greater that following feeding with [^{14}C chlorophenyl] fenvalerate at 25 mg/kg. There were no differences between sexes.

The depletion of radioactivity from treatment with [^{14}C chlorophenyl] esfenvalerate was faster than with [^{14}C chlorophenyl] fenvalerate for all tissues except fat where depletion rate was slow following all treatments. After administration of [^{14}C chlorophenyl] esfenvalerate the major compounds in liver and kidney where the unchanged a.i. and as metabolites were the 'CPIA' and the hydroxylated derivate of 'CPIA' and both disappeared after administration of untreated diet. These metabolites were also found following administration of ^{14}C fenvalerate together with 'CPIA cholesterol ester' in liver, kidney and some other tissues. 'CPIA cholesterol ester' was also found following fenvalerate after the 28 day administration of untreated diet and accounted for the total radioactivity found in these tissues at the end of the study. 'CPIA cholesterol ester' was not found following administration of [^{14}C-chlorophenyl] esfenvalerate in any tissue at any time. The persistence of ^{14}C in tissues of mice fed ^{14}C-fenvalerate was attributable to the formation of this ester.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

Radioactive residues in the tissues were very low in mice fed esfenvalerate or fenvalerate in the diet for 28 days. Levels approached a plateau after 28 days of feeding and subsequently declined when animals were returned to untreated diet. Total radioactivity in some tissues (adrenals, spleen and

ovaries) was lower following feeding with esfenvalerate than with a similar dose level of fenvalerate. In other tissues, the levels of total radioactivity were similar.

The major metabolites in the liver and kidney of animals fed esfenvalerate or fenvalerate were 'CPIA' and the hydroxylated derivative of 'CPIA'. These disappeared after feeding untreated diets. In addition, 'CPIA-cholesterol ester' was found in mice fed fenvalerate but not in those fed esfenvalerate. This metabolite was also present at the end of the 28 days on untreated diets and accounted for the majority of the radioactivity present at this time.

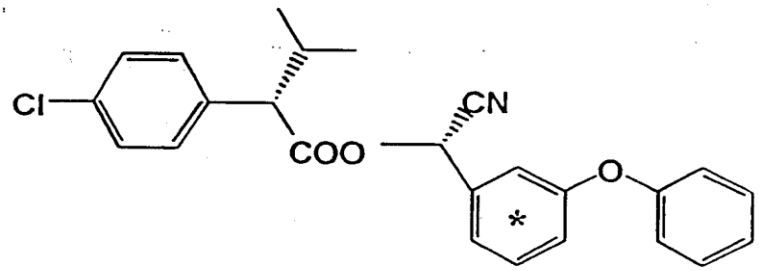
2.1.5 [Study 5]

Study	IIA 5.1.1/05 Biliary excretion of 14C-esfenvalerate in the rat.
Reference	Anonymous (1998)
Date performed	July – December 1985
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº LLM-0042
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	Yes
Test material	[^{14}C-phenoxy] esfenvalerate, purity 98.1, specific activity 8.67 MBq/mg
Study acceptable	Yes

METHODS

Esfenvalerate labelled at the phenoxyphenyl group was 98,1% pure with specific activity of 8,67 MBq/mg, being the C-labelled position shown below. Unlabelled compound was 96,6% pure.

Chemical Structure:



[phenoxyphenyl-^{14}C]S-5602Aα * :^{14}C

Analysis of radiochemical purity of dosing solutions and of metabolites in bile, urine and faeces was performed by thin-layer chromatography (TLC), unlabeled standards on the TLC plates were detected by viewing them under UV light and the radioactive metabolites on the TLC plates were detected by autoradiography. Radioanalysis was performed in organic solvents, bile, urine and alkaline solutions, measuring the radioactivity in terms of disintegration per minute (dpm) by the external standard method. Then the radioactivity was measured by liquid scintillation (LSC). Samples of faecal homogenates and faecal residues were combusted and analysed with a $^{14}CO_2$ absorbent and a scintillator. The limit of quantitation of radioactivity were calculated on the basis of background dpm X 2 (S/N=2). Samples which contained counts less than the detection limit were considered to be background and were not used for calculation.

(1) Biliary Excretion Study: Male Sprague-Dawley (S.D.) rats underwent cannulation of both bile-duct and duodenum and intraduodenal infusion of bile from other rats (bile-supplier). After these operations, the rats were given oral dose of ^{14}C-esfenvalerate by gavage at 0,5 mg/kg/5 ml corn oil (Group A) or at 0,5 mg/kg/5 ml 10% Tween 80 aqueous solution (Group B). After dosing, the treated rats were housed individually in Bollman cages to collect bile, urine and faeces, separately. The cannula of a bile-supplier was connected to the duodenum cannula of the dosed rat through a peristaltic pump, and then bile from the bile-supplier was infused into the duodenum of the dosed rat at an appropriate or natural flow rate. Bile and urine (including cage washes) samples were collected 6, 24 and 48 hours after administration, faeces were collected 24 and 48 hours after dosing. Rats were sacrificed 48 hours after dosing; gastrointestinal tract and carcass were treated separately, however, the radioactivity in the GI tract (with contents) was not measured because the material balance from the other substrates for a given rat was >90%.

(2) General Excretion Study: Male S.D. rats were given a single oral dose of ^{14}C-esfenvalerate by gavage at 0,5 mg/kg/5 ml corn oil (Group C) or at 0,5 mg/kg/5 ml 10% Tween 80 aqueous solution (Group D). Animals were housed individually in glass metabolism cages to collect urine and faeces separately.

Each group A, B, C and D consisted of four test rats, additionally groups A and B had six bile-supplier rats, two of them being "spare" rats planned to be used if the other bile-supplier

produced very little bile. Results of replicate analysis were averaged and a standard deviation (mean ± S.D.).

Findings :

1. **^{14}C-Recovery**: Cumulative ^{14}C-Recoveries in bile, urine, faeces and carcass within 48 hours after single oral administration are presented in Table 1:

Table B.6.1.5-1 (*Table 1*) Cumulative ^{14}C-recoveries in bile, urine, faeces and carcass within 48 hours after single oral administration of ^{14}C-esfenvalerate to male rats

Sample	% of the dosed ^{14}C			
	Group A (biliary excretion study with corn oil)	Group B (biliary excretion study with Tween 80)	Group C (general excretion study with corn oil)	Group D (general excretion study with Tween 80)
Urine	13,4 ± 4,48	20,3 ± 2,79	40,5 ± 3,86	61,6 ± 2,23
Faeces	71,9±7,74	51,7 ± 5,10	55,1 ± 4,48	36,1 ± 1,08
Bile	11,3 ± 5,08	24,2 ± 2,82	---	---
Carcass (without GIT)	5,8 ± 3,06	2,9 ± 0,75	3,8 ± 0,82	2,4 ± 0,51
Total	102,4 ± 4,46	99,1 ± 1,72	99,5 ± 3,13	100.1 ± 0,96

Data are means ± S.D. of four rats,

---: not applicable,

GIT: gastrointestinal tract and contents.

In general, rats of Groups B and D in which the test material was dosed in 10% aqueous Tween 80 vehicle showed higher ^{14}C-urinary and bile excretion rates (about 1,5 times higher), as compared with Groups A and C with corn oil respectively. As a result of comparison of the sum of ^{14}C excreted into the bile and urine of bile-duct cannulated rats (Groups A and B) with the ^{14}C into urine of normal rats (Groups C and D), the ^{14}C in urine of normal rats was greater than the sum of ^{14}C into bile and urine of bile-duct cannulated rats. Therefore, it was clear that the oral absorption rate of esfenvalerate in normal rats was not properly estimated on the basis of the results of the biliary excretion study. The author refers that these differences are often observed in other chemicals (especially high lipophilic compounds), and the reason is considered to be related with restricted movement of rats (in Bollman cage), small food intake due to changed physiological conditions associated with the surgery, change of bile flow, and so on. From these results, it was considered that the exact oral absorption rate could not be estimated using the data obtained from the biliary excretion studies only, and the data obtained from both biliary and general excretion studies should be used to estimate the exact oral absorption rate.

2. **Analysis of metabolites in bile, urine and faeces**: The parent compound itself, esfenvalerate, was not detected in bile or urine, but was detected in faeces. In rat faeces of the biliary excretion

studies, the amounts of esfenvalerate were 95,84% of the faecal ^{14}C in Group A and 80,48% in Group B (see Table 2). These facts indicated that the parent compound found in faeces was excreted without absorption into systemic circulation and degradation of esfenvalerate occurred in the GI tract to some extent. In rat faeces of the general excretion studies, the amounts of esfenvalerate were 38,52% of the dosed ^{14}C (69,85% of the faecal ^{14}C) in Group C and 8,84% (24,48% of the faecal ^{14}C) in Group D (see Table 3).

Table B.6.1.5-2 (*Table* 2) Amount of metabolites (% of faecal ^{14}C) in faeces within 48 hours after single oral administration of ^{14}C-esfenvalerate to male rats, which underwent cannulation of both bile dust and duodenum

	Amount (% of the faecal ^{14}C)	
	Group A (with corn oil)	Group B (with Tween 80)
Esfenvalerate	95,84 ± 1,512	80,48 ± 1,980
Metabolites	3,43 ± 1,345	17,26 ± 2,112
Unextractable	0,73 ± 0,217	2,26 ± 0,195

Data are means ± S.D. of four rats.

Table B.6.1.5-3 (*Table 3*) Amount of metabolites (% of the dosed ^{14}C) in faeces within 48 hours after single oral administration of ^{14}C-esfenvalerate to male rats (general excretion study)

	Amount (% of the dosed ^{14}C)	
	Group C (with corn oil)	Group D (with Tween 80)
Esfenvalerate	38,52 ± 4,704	8,84 ± 1,908
Metabolites	13,14 ± 1,725	24,04 ± 1,592
Unextractable	3,49 ± 1,201	3,23 ± 0,367
Total	55,15 ± 4,481	36,11 ± 1,077

Data are means ± S.D. of four rats.

3. Estimation of oral absorption rate: Taking into consideration that esfenvalerate was not detected in bile or urine, but was detected in faeces for Groups A and B, indicating that the parent compound found in faeces was unabsorbed into systemic circulation through GI tract, the oral absorption rate of esfenvalerate can be estimated by the following equation:

"Absorption rate (%)" = "Oral dosage (100%)" - "Unabsorbed esfenvalerate in faeces (% of dose)" (1)

From the biliary excretion study, however, it was found that esfenvalerate was degraded in the GI tract, the degradation rate being dependent on the vehicle. The degradation rate in the GI tracts was 4,16% and 19,52% (metabolites plus unextractable) of the faecal ^{14}C for corn oil and 10% Tween 80 aqueous solution, respectively. Therefore, equation (1) should be corrected by

degradation of esfenvalerate in the GI tract and modified as follows:

"Absorption rate (%)" = 100% - "CR" x "the rate (%of dose) of esfenvalerate in faeces for Group C or D"

"CR" (Correction Rate) = amount of esfenvalerate + amount of degradation of esfenvalerate in faeces / amount of esfenvalerate in faeces

Since oral absorption observed in rats that have not been surgically modified is likely to be more reliable than those that have, the faecal esfenvalerate values measured in non-cannulated rats (Groups C and D) should be used as a starting point for calculating absorption. In Groups C (corn oil) and D (10% Tween 80), esfenvalerate in faeces was 38,52% and 8,84% of the dosed ^{14}C. The correction rates to be used for degradation were obtained from the bile cannulated rats [100/95,84 for corn oil (Group A) and 100/80,48 for 10% Tween 80 (group B)].

Therefore, the oral absorption rate of esfenvalerate can be estimated as follows:

a) With corn oil vehicle (Groups A and C):

Absorption rate (%) = 100% - (100/95,84) x 38,52% = 59,81%

b) With Tween 80 vehicle (Groups B and D):

Absorption rate (%) = 100% - (100/80,48) x 8,84% = 89,02%

Conclusions :

The oral absorption rate of esfenvalerate with corn oil as a dosing vehicle was about 60% and with 10% Tween 80 aqueous solution was about 90%. The above findings show that the oral absorption rate of esfenvalerate differed greatly between the two dosing solutions. The results from the studies with 10% Tween 80 aqueous solution are considered to be more appropriate for calculation of the AOEL, for the following reasons: the current NOEL for the calculation of the AOEL was based on the rat multigeneration study, which is a feeding study. In the feeding study, rats have been fed on a diet containing esfenvalerate and drinking water. The aqueous Tween 80 vehicle is likely to more closely mimic the absorption pattern seen with dietary or drinking water administration of the test material.

In conclusion, this oral absorption study showed oral absorption to be 90%, based on the results from the aqueous Tween 80 vehicle studies in rats.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012.

The results from excretion studies on bile duct cannulated or non cannulated rats dosed with ^{14}C-esfenvalerate at 0.5 mg/kg in either corn oil or 10% Tween 80 aqueous suspension were used to derive the oral absorption of esfenvalerate. It was shown that unchanged esfenvalerate was not excreted in bile and that unabsorbed esfenvalerate present in faeces was subject to partial degradation in the gastro-intestinal tract. The oral absorption rate could not be derived solely from the bile cannulated rats because the sum of ^{14}C excretion into bile and urine was less than that in the urine of non cannulated rats. This was attributed to the effects of surgery on gut transit time and metabolism of esfenvalerate in the gastrointestinal tract of bile cannulated animals.

The oral absorption of esfenvalerate has been re-evaluated for the Annex 1 renewal (see section B.6.1.9). The revised proposal for oral absorption is 64%, based on the results for non-cannulated rats given esfenvalerate in Tween 80, taking the % dose recovered in the urine (61.6%) added to that recovered from the carcass (2.4%); see Table B.6.1.5-1.

2.1.6 [Study 6]

Study	IIA 5.1.1/06 Comparative Metabolism of Esfenvalerate and Fenvalerate in Rats and Mice – III Placental transfer in pregnant rats.
Reference	Anonymous (1985c)
Date performed	July – December 1985
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº LLM- 0-0009
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	No
Test material	[^{14}C-chlorophenyl] esfenvalerate; [^{14}C-chlorophenyl] fenvalerate. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

One single oral dose or 3 consecutive oral doses of [^{14}C-chlorophenyl] esfenvalerate or [^{14}C-chlorophenyl] fenvalerate were administered to a 13-day pregnant Charles River rats at the rate of 2.5 and 10 mg/kg/day. Before the 3rd oral dose, both unlabelled compounds were given to the animals. Total radioactivity in material blood, foetus, placenta, amniotic fluid and ovary was determined 3, 6, 12, 24 and 48 hours after single dosing, and 3, 6, 12, 24 and 48 hours after the last treatment of the repeated dosing.

RESULTS

The maximum levels of radioactivity were found within 3-6 hours for blood after administration of the single dose and last of the consecutive doses, and for most tissues the maximum levels were found after six hours. Thereafter, radioactivity levels declined rapidly. The repeat administration led to higher levels of radioactivity in the tissue than the single administration.

The total radioactivity in tissues six hours after administration is given in Table B.6.1.6-1.

Table B.6.1.6-1 Total radioactivity in tissues six hours after single/last administration of repeat doses

	Esfenvalerate 2.5 mg/kg/day		Fenvalerate 10.0 mg/kg/day	
Tissue	single	repeat	single	repeat
Maternal blood	324	768	808	3240
Foetus	28	152	79	393
Placenta	100	526	293	1764
Amniotic fluid	21	41	54	173
Ovary	217	760	642	3283

All measurements are given in ng equivalents/g wet tissue

Over the whole experimental period radioactivity in the foetus was less than 0.07% of applied radioactivity indicating that transfer of radioactivity from maternal blood to foetus did not readily occur.

The local radioactivity following fenvalerate was approximately four times that following esfenvalerate which equated with the four fold difference in application rate of the two compounds. There were no substantial differences between esfenvalerate and fenvalerate in the transfer ratio of radioactivity from maternal blood to foetus, placenta or amniotic fluid.

The major metabolites of esfenvalerate and fenvalerate were the same and were 'CPIA', '3-OH-CPIA' in free and active form and parent compound. Levels of 'CPIA' were slightly higher following fenvalerate but otherwise differences in levels of these metabolites between the two active substances were small. The only difference between esfenvalerate and fenvalerate was 'CPIA-cholesterol ester' which was only found following fenvalerate and then only at trace levels in the material blood and placenta and not in the foetus.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

The placental transfer of esfenvalerate or fenvalerate was investigated in pregnant rats following single or repeat dosing during gestation. Less than 0.07% of the applied radioactivity was found in the foetuses indicating that transfer of radioactivity from maternal blood to the foetuses did not readily occur. There was no evidence of accumulation of esfenvalerate in the foetal tissue or amniotic fluid of rats.

2.1.7 [Study 7]

Study	IIA 5.1.1/07 Metabolism of fenvalerate in dogs
Reference	Kaneko H, Izumi T, Matsuo M, Miyamoto J (1984)
Date performed	Not reported
Test facility	Pesticides Division, Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº AM-40-0159, published in J. Pesticide Sci., 9, 269 - 274 (1984)
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	No
Test material	[^{14}C-chlorophenyl] fenvalerate; [^{14}C-phenoxybenzyl] fenvalerate. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

[^{14}C-chlorophenyl] and [^{14}C-phenoxybenzyl] fenvalerate were administered in one single oral dose in gelatin capsules at a rate of 1.7 mg/kg. Urine, faeces and blood were collected daily for three days and metabolites were analysed using thin layer chromatography (TLC) using four solvent systems.

RESULTS

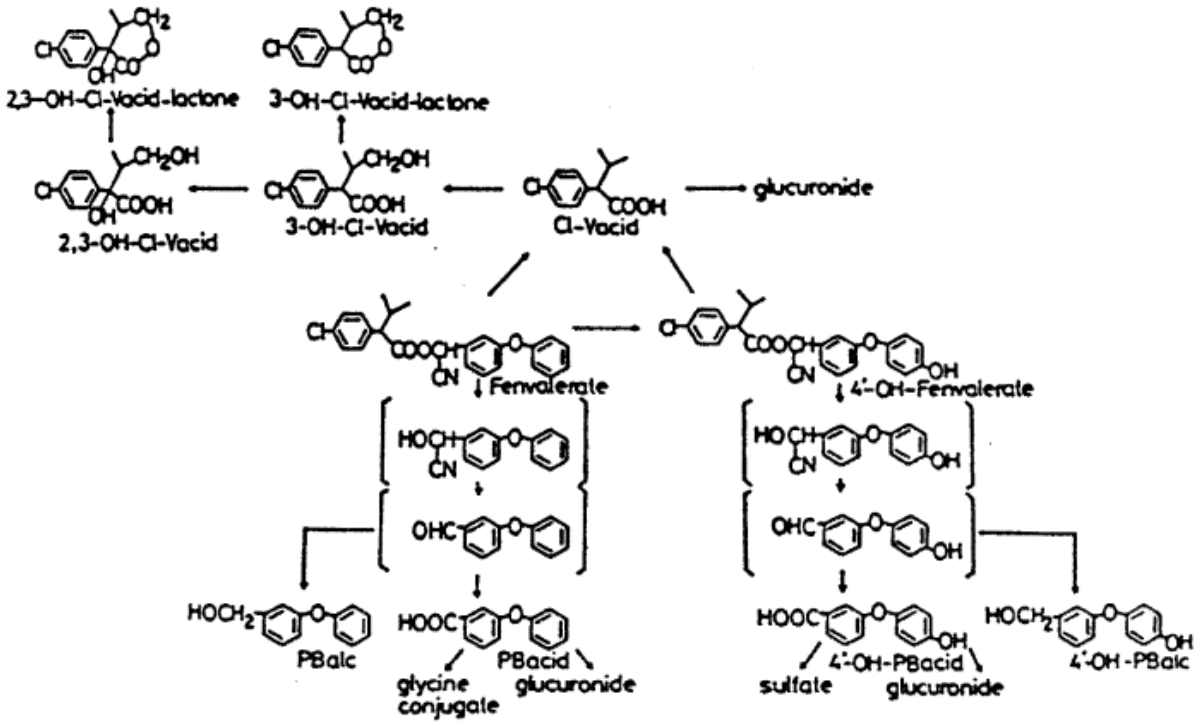
Fenvalerate was rapidly eliminated after administration. The recovery in urine and faeces for the two labelling positions is given in Table B.6.1.7-1. The Tmax for fenvalerate was the first sampling timepoint (approximately 1 hour) and the Cmax was approximately 0.4 µg/mL. The biological half-life for fenvalerate in the blood was about 2 hr and the level of a.i. decreased below the detection limit (0.01 ppm) in 48 hr after dose.

Fenvalerate was metabolised mainly by oxidation at the 4'phenoxy position of the alcohol moiety and at the C-2 and C-3 positions of the acid moiety, cleavage of the ester linkage and conjugation of the resultant carboxylic acids, phenols and alcohols with glucuronic acid, sulphate and/or amino acid. The metabolic pathway for fenvalerate in male dogs is presented in Figure B.6.1.7-1.

Table B.6.1.7-1 % Total radioactivity recovered after three days and mean half-life

Labelling of fenvalerate	Urine	Faeces	DT_{50} (days)
^{14}C-chlorophenyl	31.6	55.5	1.0
^{14}C-phenoxybenzyl	36.8	42.3	0.7

Figure B.6.1.7-1 Proposed metabolic pathways for fenvalerate in male beagle dogs.



CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

Although total recovery of radioactivity was less in dogs than in rodents following a single oral dose of fenvalerate, the disappearance pattern and half-lives in dogs and rats were similar. The following species differences in the metabolism were noted:

i) 2'-hydroxylation of the alcohol moiety did not occur in dogs.

ii) 3-phenoxybenzylalcohol ('PBalc') and 3-(4'- hydroxyphenoxy)benzylalcohol ('4'-OH-Pbalc') were detected in dogs only.

iii) 3-phenoxybenzoylglycine (glycine conjugate of 'PBalc') was the predominant conjugate of the alcohol moiety in dogs, but a minor one in rodents.

iv) glucuronides of the acid moiety and its hydroxy derivatives were greater in dogs.

2.1.8 [Study 8]

Study	IIA 5.1.1/08 Differential metabolism of fenvalerate and granuloma formation - I. Identification of a cholesterol ester

	derived from a specific chiral isomer of fenvalerate.
Reference	Kaneko H, Matsuo M, Miyamoto J. (1986)
Date performed	Not reported
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº AM-60-0160, published in Toxicology and Applied Pharmacology 83, 148 - 156 (1986)
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	GLP status not reported
Test material	[^{14}C-chlorophenyl] fenvalerate; [^{14}C-phenoxybenzyl] fenvalerate. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

[Chlorophenyl-^{14}C] fenvalerate [2S, αS], [2R, αS], [2S, αR] and [2R, αR] was administered once p.o. to four ddy mice and two rats at a level of 2.5 mg/kg. Blood was collected under light diethyl ether anesthesia 6 days after administration and then several tissues were excised to determinate radioactivity by combustion and liquid scintillation counting. Male ddy mice were fed a diet containing 500 ppm of [chlorophenyl-^{14}C] [2S, αS] and [2R, αR] isomers for one and two weeks. Tissues were pooled and extracted after killing at the end of one or two weeks. Further more seven male ddy mice were treated with a single p.o. dose of [chlorophenyl-^{14}C] [2R, αS] at about 70 mg/kg equivalent to 500 ppm. Tissues were excised at appropriate intervals to determine the concentrations of the metabolites.

RESULTS

Following a single oral administration to rats and mice at 2.5 mg/kg [^{14}C - chlorophenyl] [2R, αS] isomer of fenvalerate, led to a higher radioactive residues in tissues than [^{14}C - chlorophenyl] [2S, αS], [2S, αR] and [2R, αR]. Following administration in the diet at 500 mg/kg for two weeks in mice [^{14}C - chlorophenyl] [2R, αS] also led to higher radioactive residues than the [^{14}C - chlorophenyl] [2S, αS] and [2R, αR] isomers especially in the adrenals, liver and mesenteric lymph nodes.

The [2R, αS] isomer produced a lipophilic metabolite in all the examined tissues on the basis of thin-layer chromatography analysis, but not for the other isomers. The amount of lipophilic metabolite differed among tissues, being higher in adrenal, liver, and mesenteric lymph nodes following feeding to mice at 500 ppm of the [2R, αS] isomer for 2 weeks. However, the amount did not increase proportionally with time and apparently reached a plateau within a rather short time. This metabolite was identified as cholesterol [2R]-2-(4-chlorophenyl)isovalerate ([2R]-CPIA-cholesterol ester) on the basis of spectroanalysis and chromatographic behaviour after purification on silica gel, Florisil, thin-layer, and high-pressure liquid chromatography. The presence of the same metabolite also was indicated in rat tissues. The CPIA-cholesterol ester was rapidly formed and found in all the analysed tissues of mice 1 hr after a single p.o. administration of the [2R, αS] isomer.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

[^{14}C-Chlorophenyl] fenvalerate was administered to groups of male rats and male mice as a single oral dose of 2.5 mg/kg as either the [2S,αS]-, [2S,αR]-, [2R,αS]- or [2R,αR]-isomers of fenvalerate. Additional groups of male mice were fed diets containing 500 mg/kg [2R,αS]-, [2S,αS]- or [2R,αR]-isomer for 1 or 2 weeks. 'CPIA cholesterol ester' was found predominantly in mice and the [2R,αS]-isomer of fenvalerate was established as the only source of the 'CPIA cholesterol ester'.

2.1.9 [Study 9]

Study	IIA 5.1.1/09 Stereoselective formation of a cholesterol ester

	conjugate from fenvalerate by mouse microsomal carboxyesterase(s).
Reference	Miyamoto J, Kaneko H, Takamatsu, Y (1986)
Date performed	Not reported
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº LLM-60-0016, published in Journal of Biochemical Toxicology (2): 79-94 (1986).
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	GLP status not reported
Test material	Four [^{14}C-chlorophenyl] chiral isomers of fenvalerate; [^{14}C-chlorophenyl CPIA; 4-[^{14}C]-cholesterol; 4-[^{14}C]-cholesteryl-oleate; [^{14}C]-oleic acid; [^{14}C]-lecithin. Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

The objective of this study was to elucidate the enzymic mechanism involved in the stereoselective formation of the cholesterol ester that can cause granulomatous changes in mammalian tissues. The test substances used were the following:

Four [^{14}C - chlorophenyl] chiral isomers of fenvalerate, each with specific activity 53.2 mCi/mmol.:

i) [2S, αS]

ii) [2S, αR]

iii) [2R, αS]

iv) [2R, αR]

[^{14}C - chlorophenyl CPIA 31.6 mCi/mmol.

4-[^{14}C] - cholesterol, 60 mCi/mmol.

4-[^{14}C] - cholesteryl-oleate, 59.4 mCi/mmol.

[^{14}C] - oleic acid, 56.7 mCi/mmol.

[^{14}C] - lecithin, 55.9 mCi/mmol.

Microsomal fractions of various tissues (brain, kidney, liver, spleen, adrenals, intestines and lymph nodes) were prepared from male ddy mice, male Sprague Dawley rats, male Beagle dogs and male Rhesus monkeys.

Each of the chiral isomers were dissolved in acetone and incubated with 2 ml of the microsomal fractions from the mouse kidney after treatment with detergents for 30 minutes at 37ºC. The incubation mixture was then lyophilised and the metabolites extracted from the residues with chlorine:methanol (2:1 v/v) and analysed by thin layer chromatography (TLC) in two solvent systems. Enzyme activities were assayed by incubation of chiral isomers in acetone with tissue fractions at 37ºC for one or two hours. After incubation the metabolites were extracted with chloroform:methanol (2:1 v/v) and analysed by TLC using two solvent systems.

RESULTS

All the [^{14}C-chlorophenyl] isomers produced the metabolite 'CPIA' to varying extents according to the tissue used. [^{14}C-chlorophenyl] [2R, αS] also produced 'CPIA cholesterol ester' to varying extents according to the tissue and animal species as shown in Table B.6.1.8-1. A trace amount was also produced with the [2R, αR] isomer from the kidney preparation. The kidney, brain and spleen of mice showed relatively higher capacities to form cholesterol-ester compared to other tissues, the enzyme activity was mainly localised in microsomal fractions. The 'CPIA cholesterol ester' did not appear to be produced by three known biosynthetic pathways of endogenous cholesterol esters; acyl-CoA: cholesterol O-acyltransferase (ACAT), lecithin:cholesterol O-acyltransferase (LCAT) and cholesterol

esterase. Carboxyesterase(s) of mouse kidney microsomes solubilised by digitonin hydrolysed only the [2R, αS] isomer of fenvalerate, yielding 'CPIA', whereas they yielded the corresponding cholesterol ester in the presence of artificial liposomes containing cholesterol.

Table B.6.1.8-1 'CPIA cholesterol ester' formed from [2R, αS]- isomer (% of total radioactivity applied)

Tissue	Mouse	Rat	Dog	Monkey
Adrenal	-	0.6	ND	-
Blood	ND	ND	0.1	-
Brain	6.9	1.1	1.6	0.8
Intestine	ND	ND	ND	-
Kidney	9.1	0.3	ND	0.1
Liver	1.1	0.4	0.5	ND
Lung	1.6	0.2	0.1	-
Lymph node	0.4	0.2	ND	-
Ovary	-	ND	-	-
Pancreas	1.2	ND	-	-
Spleen	4.8	1.5	0.1	0.8
Testis	0.9	ND	ND	ND

In conclusion, it appears that the stereoselective formation of the 'CPIA-carboxyesterase ester results from the [2R, αS]-isomer, which subsequently undergoes cleavage by cholesterol to yield the CPIA-cholesterol ester.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

'CPIA cholesterol ester' formation was investigated in excised tissue samples from mice, rats, dogs and monkeys incubated with fenvalerate isomers. Mouse kidney, brain and spleen produced the most 'CPIA-cholesterol ester'. Free 'CPIA' was not a substrate for the formation of 'CPIA-cholesterol ester'. 'CPIA-cholesterol ester' was formed only from the [2R,αS]-isomer in all tissues and species examined, apart from the mouse kidney, which produced only a trace amount from the [2R,αR]-isomer.

2.1.10 [Study 10]

Study	IIA 5.1.1/10 Substrate specificity for formation of cholesterol ester conjugates from fenvalerate analogues and for granuloma formation.
Reference	Kaneko H, Takamatsu Y, Okuno Y, Abiko J, Yoshitake A, Miyamoto J (1988)
Date performed	Not reported
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report nº AT-80-0452, published in Xenobiotica, 18, 11-19 (1988)
Guideline(s)	None cited
Deviations from the guideline	N/A
GLP	GLP status not reported
Test material	Four [^{14}C-chlorophenyl] chiral isomers of fenvalerate; a range of fenvalerate and cycloprate analogues and other pyrethroids.

	Purities and batch numbers were not reported.
Study acceptable	Yes

METHODS

The objectives of this study were to investigate the relationship between the formation of cholesterol esters *in vitro* from fenvalerate analogues and granuloma formation *in vivo*.

The test substances used in the study were the following:

Four [^{14}C - chlorophenyl] chiral isomers of fenvalerate, each with specific activity 53.2 mCi/mmol.:

i) [2S, αS]

ii) [2S, αR]

iii) [2R, αS]

iv) [2R, αR]

Also a range of fenvalerate, cycloprate analogues and other pyrethroids.

For the enzymic part of the study kidneys of male ddy-mice were excised from animals at necropsy. Microsomal fractions were obtained by centrifugation and resuspension in phosphate buffer. Supernatant was obtained and subjected to analysis.

Each of the chiral isomers were dissolved in acetone and incubated with 2 ml of the microsomal fractions or dilysate fractions plus various steroids for one hour at 37ºC. Metabolites were extracted with chloroform methanol (2:1 v/v) and subjected to thin layer chromatography.

For the dietary part of the study, 0 and 3000 mg/kg diet were given to male ddy mice for a month, and the histopathological examination of the liver, spleen, mesenteric and mandibular lymph nodes and adrenals was made.

RESULTS

Among the four isomers of fenvalerate only the [2R, αS]-isomer yielded 'CPIA (chorophenyl-isovaleric acid) cholesterol ester'. In addition, some fenvalerate analogues produced cholesterol ester conjugates, but no other pyrethroids or methoprene produced these conjugates. Some cycloprate analogues gave the corresponding cholesterol ester, the yields of which were dependent on carbon chain length. Two of the fenvalerate analogues, namely '4'-Br-Fen' and 'desphenoxy-Fen' led to granuloma formation in mice following administration in the diet at 3000 mg/kg for one month. These were the same analogues which produced the cholesterol ester conjugates. The desisopropyl-fenvalerate and descyano-fenvalerate produced no granulomas and no cholesterol ester conjugates.

Dehydroisoandrosterone and pregnenolone in the presence of egg lecithin as well as cholesterol gave the corresponding esters of 'CPIA' when incubated with [2R, αS] fenvalerate indicating that other steroids can be used as the carboxyesterase.

CONCLUSION (updated)

This conclusion is taken from Doc MII section 3 submitted in 2012

The substrate specificity of microsomal carboxyesterases responsible for the formation of 'CPIA-cholesterol ester'(cholesteryl [2R]-2-(4-chlorophenyl) isovalerate) from fenvalerate was investigated by incubating mouse kidney microsomes with 14C-cholesterol and the following substrates: fenvalerate isomers, fenvalerate analogues, other pyrethroids, methoprene and cyclopropate analogues. Of the fenvalerate isomers, only the [2R,αS]-isomer produced a cholesterol ester. Some fenvalerate analogues produced cholesterol ester conjugates; the other pyrethroids and methoprene did not. Some cyclopropane analogues yielded the corresponding cholesterol ester.
Cholesterol ester formation *in vitro* from the fenvalerate analogues correlated well with granuloma formation observed *in vivo* previously when the analogues were fed to mice at 3000 ppm for 1 month.

2.2 Re-evaluation of the extent of oral absorption

Summary

At the conclusion of the EU review for initial Annex I inclusion, oral absorption was considered to be 90% from the results of the low dose biliary excretion study in rats (IIA 5.1.1/05, Tomigahara, 1998) and the associated calculations of the percentage oral absorption (Environmental Health Science Laboratory, Discussion of oral absorption of esfenvalerate, Sumitomo Chemical Co., Ltd., Unpublished report No.: LLM-0043). The Applicant proposes a revised value of 64% based on re-evaluation of the data to current regulatory standards. The UK RMS agrees with the Applicant's re-evaluation.

A summary of the original evaluation and the re-evaluation are presented below.

Original evaluation

Two methods were previously used to calculate oral absorption in report LLM-0043.

In the first method absorption was calculated by:

1. Subtracting the percentage esfenvalerate in faeces of non cannulated rats from the total administered (100%)
2. This value is then corrected for loss of unabsorbed esfenvalerate due to metabolism in the gastrointestinal tract

In principal this should be an acceptable method for calculation of the absorption of esfenvalerate, but the problem is how to estimate the loss of unabsorbed esfenvalerate due to metabolism in the gastrointestinal tract. The method used in the reports assumes that the extent of metabolism of esfenvalerate in the gastrointestinal tract is the same in both bile duct cannulated and non cannulated rats. However, the physiological conditions are not the same, because cannulation of the bile duct of rats involves major surgery and, in this study, the cannulated rats were dosed with esfenvalerate only approximately 2 hours after dosing. The effects of surgery are very likely to affect gut transit time and metabolism of esfenvalerate in the gastrointestinal tract so the extent of metabolism of esfenvalerate in the gastrointestinal tract of normal rats is unlikely to be the same as for cannulated animals.

In the second method absorption was calculated by:

1. Adding the percentages of metabolites in faeces and unextractable metabolites in non cannulated rats
2. Correcting this value for possible metabolism in the gastrointestinal tract
3. Adding the corrected value to the percentage of the dose eliminated in the urine of non cannulated rats

This method relies on the same assumption concerning the similarity of the extent of metabolism of esfenvalerate in the gastrointestinal tract of cannulated and non cannulated rats and so it is also considered to be flawed.

When the two methods were combined oral absorption was calculated to be in the range 78.8-89%.

Re-evaluation by Applicant

An oral absorption value of as high as 90% is not sufficiently justified by the experimental data. The Applicant believes that a more robust and scientifically justified value can be derived based on the sum of % dose in urine and in the carcass of normal (non-cannulated) rats given ^{14}C-esfenvalerate in 10% Tween 80 aqueous suspension (considered to mimic more closely the absorption process when rats are fed treated diet compared with animals that are dosed with corn oil). As shown in Table B.6.1.5-1 (IIA 5.1.1/05, Tomigahara, 1998), the mean % dose recovered in the urine of non-

cannulated rats was 61.6% and that recovered from the carcass was 2.4% (total recovery of radioactivity was 100%). Thus, the total absorbed dose is calculated to be 64% of administered dose.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 [Study 1]

Study	IIA 5.2.1/01 Acute oral toxicity of S.1844 in rats
Reference	Anonymous (1985d)
Date performed	May-July 1985
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report nº LLT-50-005
Guideline(s)	OECD 401
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

Esfenvalerate (S-1844; ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity as total isomer was 94.5%) dissolved in corn oil was administered into the stomach by using a plastic syringe attached gastric probe in concentrations of 0, 5, 10, 20, 40, 55, 75, 100, 130 and 180 mg/kg bw, to ten males and ten female Sprague Dawley rat, for each dosage group, and the animals were fasted for 20 hours prior to dosing the test material. The toxic signs and the mortality were observed at 1/6, 1/2, 1, 2, 4, 6 and 8 hours after administration on the first day and daily for 2 weeks there after. Body weight was weighed on days 0, 7 and 14 observation period. All animals which died during the course of observation and all animals which were alive at the end of observation period were necropsied for gross pathological examination. Statistical method: Litchfield and Wilcoxon.

RESULTS

At dose levels up to and including 40 mg/kg there was no mortality in either the male or female groups. There was 10, 40, 50, 90 and 100% mortality with male and female rats dosed 55, 75, 100, 130 and 180 mg/kg, respectively.

Signs of toxicity seen in groups of rats treated with 10 mg/kg esfenvalerate and above, included muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration, dyspnoea, salivation, hyper-excitability and choreoathetotic syndrome. These signs gradually developed one hour after treatment, however, they had disappeared in all animals within three days, with the majority of signs being resolved by 2 days after dosing. Most signs were observed at dose levels associated with mortality (≥55 mg/kg), although muscular fibrillation and occasional signs of tremor, limb paralysis and ataxia were observed at 40 mg/kg. Muscular fibrillation and decrease of spontaneous activity were the only signs observed at 10 mg/kg.

Male rats at the 75 and 100 mg/kg dose level had reduced body weight gain compared to the control group, seven days after treatment. However, body weights in these animals were not different at the end of the study. The only treatment-related gross pathological observation noted in some rats that died during the study was gastric haemorrhaging. Rats that survived to the end of the study showed no significant differences to the control groups.

The acute oral LD_{50} value for esfenvalerate to both male and female rats was 88.5 mg/kg and the NOEL was 5 mg/kg.

CONCLUSION

Esfenvalerate is toxic to rats when administered as a single oral dose.

According to Directive 67/548/EEC esfenvalerate is classified as T; R25 Toxic if swallowed. According to Regulation (EC) 1272/2008 the classification is Acute Tox. 3 (H301 Toxic if swallowed).

3.1.1.2 [Study 2]

Study	IIA 5.2.1/02 Acute oral toxicity of S-1844 in mice
Reference	Anonymous (1986a)
Date performed	June-December 1985
Test facility	Sumitomo Chemical Co. Ltd.
Report reference	Report nº LLT-60-0017
Guideline(s)	OECD 401
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

Esfenvalerate (S-1844; ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity as total isomer was 94.5%) suspended in 0.5% methyl cellulose solution was orally administered into the stomach, in concentrations of 0, 5, 15, 50, 70, 100, 140, 200, 280 and 400 mg/kg bw to ten male and ten female ICR mice, for each dosage group, and the animals were fasted 20 hours prior to dosing the test material.

Animals were observed for signs of toxicity and mortality at 10 and 30 minutes and 1, 2 and 4 hours after treatment on the first day, and daily for a period of 14 days. Body weights were recorded at the beginning, mid-point and end of the study. A control group was treated with methylcellulose solution alone. Food and water were available ad libitum throughout the study, except for the fasting period before dosing. All animals were submitted for gross pathological examination at the end of the study. Statistical method: Litchfield and Wilcoxon.

RESULTS

At dose levels up to and including 100 and 140 mg/kg there was no mortality in female and male mice groups, respectively. There was 10, 30, 60 and 90% mortality with female mice dosed 140, 200, 280 and 400 mg/kg, respectively and 20, 30 and 100% mortality in male mice dosed 200, 280 and 400 mg/kg, respectively.

Signs of toxicity in groups of mice treated with 15 mg/kg esfenvalerate and above, included muscular fibrillation, tremors, decrease of spontaneous activity, ataxia, limb paralysis, irregular respiration and salivation. These signs gradually developed 10 minutes after treatment, however, they had disappeared in all surviving animals within two days. Most signs were observed at dose levels associated with mortality (≥140 mg/kg), although muscular fibrillation and occasional signs of tremor,

limb paralysis and ataxia were observed at 70 and 100 mg/kg. Muscular fibrillation and decrease of spontaneous activity were the only signs observed at 15 mg/kg.

There was no treatment-related effect on body weight. The only gross pathological observations noted in some mice that died during the study was gastric haemorrhaging. Mice that survived to the end of the study showed no significant differences to the control groups.

The acute oral LD_{50} values for esfenvalerate were 320 and 250 mg/kg for male and female mice, respectively; the NOEL was 5 mg/kg.

CONCLUSION

Esfenvalerate is of moderate toxicity to mice when administered as a single oral dose.

3.1.2 Human data

No relevant human studies available.

3.1.3 Other data

Acute neurotoxicity studies were considered potentially relevant and are summarised in section 3.11.1.

3.2 Acute toxicity - dermal route

Not applicable.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 [Study 1]

Study	IIA 5.2.3/01 Acute inhalation toxicity of S-1844 in rats
Reference	Anonymous (1985f)
Date performed	November 1985
Test facility	Sumitomo Chemical Co. Ltd.
Report reference	Report nº LLT-50-0002
Guideline(s)	OECD 403
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

Groups of ten male and ten female Sprague Dawley rats were exposed to atmospheric concentrations of 2.40, 13.8, 205, 395, 550 and 1130 mg/m^3 of esfenvalerate (S-1844; ratio as optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity of total isomer was 94.5%)) diluted in corn oil, during four hours in a test chamber; a group of control animals were exposed to compressed air only and another group exposed to corn oil spray alone. Food and water was available ad libitum except during the exposure period. The mean aerodynamic diameter of the particles ranged from 0.94 to 1.07 µm.

The animals were observed at regular intervals during exposure and for up 14 days after exposure for signs of toxicity and mortality. The body weights of individual animals were recorded at various periods throughout the study. All surviving animals were sacrificed and subjected to a gross post mortem examination at the end of the study. Histopathological examination of the nasal cavity, trachea and lung was carried out on surviving animals from selected exposure groups.

Statistical method: Litchfield, J.T. and Wilcoxon; for the means body weight it was used the Student's T-test.

RESULTS

There was 10, 90 and 100% mortality in male rats exposed to concentrations of 395, 550 and 1130 mg/m^3 of esfenvalerate, respectively. Female rats showed 20, 20 and 100% mortality at the same concentrations of test material, respectively. Deaths occurred within two hours after termination of exposure. No treatment-related signs of toxicity were evident in rats exposed to a concentration of 2.40 mg/m^3. At 13.8 mg/m^3 some rats showed signs of irregular respiration, however, this had disappeared within one hour after termination of exposure.

Further signs of toxicity at higher exposure levels included hyperpnoea, dyspnoea, nasal discharge, urinary incontinence, hypersensitivity to sound, muscular fibrillation, abnormal gait, decrease of spontaneous activity, ataxia, lachrymation and salivation, however, all these symptoms had disappeared within two days of the exposure. Choreoathetotic movement, tremors and aggressive sparring were observed in rats exposed to doses of 395 mg/m^3 or greater. However, all symptoms had completely cleared within five days after exposure. Male rats exposed to 205 mg/m^3 and above had lower body weight gains than those in the control group. Female rats exposed to levels of 395 mg/m^3 and above also showed depression of body weight gain. These body weight variations were transient and the body weights for both sexes had recovered by the end of the study.

The post mortem and histopathological examination revealed no significant treatment-related differences compared to the control group, except for autolysis of the intestinal tract of animals that died during the study.

The acute inhalation 4 h LC_{50} of esfenvalerate to male and female rats was 480 and 570 mg/m^3 (0.48 and 0.57 mg/L), respectively. The NOEL was 2.40 mg/m^3 for both sexes.

CONCLUSION

Esfenvalerate is toxic to rats following a single 4 hour exposure to an atmosphere containing the substance in a corn oil mist.

According to Directive 67/548/EEC esfenvalerate is classified as T; R23 Toxic by inhalation. According to Regulation (EC) 1272/2008 the classification is Acute Tox. 2 (H330 Fatal if inhaled), based on the 4h LC_{50} of 0.48 mg/L for females.

3.3.2 Human data

No relevant studies available.

3.3.3 Other data

No other relevant data available.

3.4 Skin corrosion/irritation

No applicable.

3.5 Serious eye damage/eye irritation

Not applicable.

3.6 Respiratory sensitisation

Not applicable.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 [Study 1]

Study	IIA 5.2.6/01 Skin sensitization of S-1844 in guinea pigs - maximisation test
Reference	Anonymous (1986b)
Date performed	August 1985
Test facility	Sumitomo Chemical Co. Ltd.
Report reference	Report nº LLT-60-0154
Guideline(s)	OECD 406 (Maximisation)
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

The test substance was esfenvalerate (S-1844; ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity as total isomer was 94.5%).

A group of 20 male Hartley guinea pigs were given intradermal injections of 0.05 cm^3 on their shorn scapular sites with (a) Freund's complete adjuvant in water, (b) a 25% solution of esfenvalerate (94.5% a.i.) in corn oil and (c) a 50:50 mixture of Freund's adjuvant in water and the esfenvalerate preparation. The control groups were treated similarly.

One week after the injections, the same patch of skin was re-shorn, pre-treated with a sodium lauryl sulphate solution and exposed to undiluted esfenvalerate (0.4 cm^3) applied with lint and held in place for 48 hours with an occlusive patch. The control groups were treated similarly with 0.5% DNCB in corn oil or corn oil alone (0.4 cm^3).

The test and control animals were challenged topically two weeks after the induction period with esfenvalerate (approximately 0.2 cm^3) applied to the shaved flank area. The test substance was kept in contact with the skin by means of an occlusive dressing for a period of 24 hours. The irritation responses were recorded 24 and 48 hours after removal of the occlusive dressings. The vehicle only control group animals received the same topical challenge.

Body weights were recorded at the initial induction and challenge applications.

RESULTS

In the esfenvalerate treated group, there was slight to moderate erythema in 15 out of 20 guinea pigs after 24 hours (85%). Three animals also exhibited slight oedema. The number of animals with erythema increased to 17 after 48 hours. There was no response in the vehicle only control group animals. There were no treatment-related effects on body weight.

DNCB caused moderate to severe skin sensitisation reactions in all animals.

CONCLUSION

Technical esfenvalerate is a skin sensitiser. According to Directive 67/548/EEC esfenvalerate is classified as Xi; R43 May cause sensitisation by skin contact. According to Regulation (EC) 1272/2008 the classification is Skin Sens. 1B (H317 May cause an allergic skin reaction), based on the observation of a ≥30% response at a >1% intradermal induction dose.

3.7.1.2 [Study 2]

Study	IIA 5.2.6/02 Skin sensitization of S-1844 in guinea pigs – Buehler test
Reference	Anonymous (1986c)
Date performed	November – December 1985
Test facility	Sumitomo Chemical Co. Ltd.
Report reference	Report nº LLT-60-0153
Guideline(s)	OECD 406 (Buehler)
Deviations from the guideline	A smaller number of animals were used, and the number of applications was higher - nine applications instead of three in the induction phase.
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

A 0.5 ml aliquot of esfenvalerate (S-1844; ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity as total isomer was 94.5%i.) was applied without dilution to the shorn flank of a group of ten male Hartley guinea pigs by way of a lint dressing. The substance was held in place for six hours. This procedure was repeated three times a week for three weeks (nine applications). A positive control group was treated similarly with 0.5% solution of DNCB in acetone (0.5 cm^3).

Two weeks after the induction phase, the same patch of skin was re-shorn and exposed to undiluted esfenvalerate. This was held in place for 48 hours with a lint patch. The irritation responses were recorded 24 and 48 hours after removal of the occlusive dressings. The vehicle only control group animals received the same topical challenge.

Body weights were recorded at the initial induction and challenge applications.

RESULTS

There were no signs of erythema or oedema in animals (all scores 0,00) treated with esfenvalerate. DNCB treated animals showed slight to moderate erythema and slight to severe oedema. Body weight changes were normal in all groups despite reduced body weight gain during the induction phase.

CONCLUSION

Technical esfenvalerate is not a sensitiser to guinea pig skin according to the Buehler method.

3.7.1.3 [Study 3]

Study	IIA 5.2.6/03 Guinea pig sensitization of MO70616 - Buehler
Reference	Anonymous (1986d)
Date performed	August – September 1986
Test facility	Anonymous
Report reference	Report nº LLT-61-0116
Guideline(s)	OECD 406 (Buehler)
Deviations from the guideline	The group size for the treated animals was 10, instead of the

	recommended 20.
GLP	Yes
Test material	Esfenvalerate (MO70616). ID no. WRC Tox 730C, name KKA LR no. TCA 01955-1 Lab. TX207. Purity not reported.
Study acceptable	Yes

METHODS

Forty Duncan-Hartley short-haired albino guinea pigs [ten, five males and five females, treated with ethanol as a vehicle control group - group A - ten (five males and five females), treated with 2,4-DNCB as a 0.1% w/v solution in diethyl ether as a positive control group - group B - ten (five males and five females), treated with the undiluted MO 706616 technical as a test group - group C - and ten (five males and five females), treated with the undiluted MO 70616 technical as an irritation control group - group D] - were treated at days 1, 8 and 15 as induction period; on day 29 (challenge treatment) all groups were treated in an identical manner as on the previous three treatment days with the addition of a second test site in the groups A, B, C. The treatment sites were observed for signs of irritation 24 and 48 hours after the removal of the dressings during the induction phase and after the challenge application.

Statistical method: Analysis of variance was used to compare body weight changes.

RESULTS

There were no significant dermal reactions observed during the induction period in the animals treated with esfenvalerate (all scores 0.00). Positive control animals showed no irritation after the first induction, but there was increasing irritation following subsequent inductions. After the challenge applications no dermal reactions were observed (all scores 0.00) in the animals treated with esfenvalerate. 2,4-DNCB did produce evidence of hypersensitivity.

There was no mortality nor significant differences in body weight gain between the groups.

CONCLUSION

Esfenvalerate did not produce evidence of delayed contact hypersensitivity in guinea pigs in this study.

3.7.2 Human data

No relevant data available.

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 [Study 1]

Study	IIA 5.4.1/01 Reverse mutation test of S-1844 in Salmonella typhimurium and Escherichia coli
Reference	Kogiso S (1985a)
Date performed	October – December 1985
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report no. LLT-50-0009
Guideline(s)	OECD 471
Deviations from the guideline	The positive control substances differed from those recommended

	in the guideline. Kanechlor 400 was used instead of Aroclor 1254 in the preparation of S-9 mix.
GLP	Yes
Test material	Esfenvalerate. Lot no. PKG-85109, purity 95.5% all isomers (87.4% as esfenvalerate)
Study acceptable	Yes

METHODS

Esfenvalerate (S-1844), Lot nº PKG 85109; purity 95.5% all isomers (the ratios of fenvalerate Aα, Aβ, Bα and Bβ isomers were 91.5, 5.7, 2.7 and 0.1, respectively), was tested in two experiments in bacterial cultures of the *Salmonella typhimurium* mutants TA100, TA98, TA1535, TA1537 and TA1538, obtained from Dr. B.N. Ames (University of California, Berkley U.S.A.) and of *Escherichia coli*, strain WP2 *uvr* A from National Institute of Genetics, Mishima, Japan. The concentrations of test material used were 0 - 15 - 50 - 150 - 500 - 1500 and 5000 µg/plate for all mutants, for main and repeat test. The esfenvalerate was formulated in dimethylsulfoxide (DMSO), which also was used as vehicle control.

The metabolic activation S-9 mix was prepared from adult male Sprague-Dawley rats; for enzyme induction the animals received a single intraperitoneal injection of Kanechlor 400 - 500 mg/kg; on the 5th day after injection the animals were sacrificed, the livers were removed aseptically and were homogenised and combined with appropriate co-factor solution according to established procedures.

The positive controls used for each condition were as follows: without S-9 mix, methyl-methane sulfonate for TA100; 2-nitrofluorene for TA98 and TA1538; sodium azide for TA1535; 9-aminoacridine for TA1537 and N-ethyl-N'-nitro-N-nitrosoguanidine for WP2 *uvr* A (Esch. coli). With S-9 mix: benzeno (a) pyrene for TA100, TA98, TA1537, TA1538 and 2-amino-anthracene for TA1535 and WP2 *uvr* A (*Esch. coli*).

Evaluation criteria: Revertant colonies induced by the test compound are over twice or more than those induced spontaneously (control). Increase of revertant colonies induced by the test compound has a dose/response relationship. The results must be reproducible.

RESULTS

Cytotoxicity test: The esfenvalerate tested up to the concentrations of 5000 µg/plate revealed no cytotoxic effects on the tester strains in presence or absence of S-9 mix. The test substance was precipitated in the concentrations of 1500 µg/plate and above without S-9 mix and in the highest concentration with S-9 mix.

The test substance did not induce any significant increase in revertant colonies in all the concentrations treated in presence or absence of S-9 mix in five strains of *S. typhimurium* and in one strain of *Esch. coli*. The positive controls induced remarkable number of revertants.

CONCLUSION

Considering the result of this experiment it is concluded that esfenvalerate is not mutagenic in the bacterial systems tested.

3.8.1.2 [Study 2]

Study	IIA 5.4.1/02 *In vitro* chromosomal aberration test of S-1844 in Chinese Hamster Ovary Cells (CHO-K1)
Reference	Kogiso S (1985b)
Date performed	October – December 1985
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report no. LLT-50-0010
Guideline(s)	OECD 473
Deviations from the guideline	Kanechlor 400 was used instead of Aroclor 1254 in the preparation of S-9 mix.
GLP	Yes

Test material	Esfenvalerate. Lot no. PKG-85109, purity 95.5% all isomers (87.4% as esfenvalerate)
Study acceptable	Yes

METHODS

The test substance was esfenvalerate (S-1844), Lot nº PKG 85109; purity 95.5% all isomers (the ratios of fenvalerate Aα, Aβ, Bα and Bβisomers were 91.5, 5.7, 2.7 and 0.1, respectively).

Chinese hamsters ovary cells (CHO-K1) were exposed to the concentrations of 10^{-5}; 2 x 10^{-5}; 5 x 10^{-5} and 10^{-4} M in the absence of S-9 mix and to the concentration of 5 x 10^{-5}; 10^{-4}; 2 x 10^{-4} and 5 x 10^{-4} M in the presence of S-9 mix of esfenvalerate (Lot. nº PKG 85019 of 95.5% of purity of total isomers (87.4% of esfenvalerate). Mitomycin C in concentration of 3 x 10^{-7} M and benzo (a) pyrene in dose of 5 x 10^{-5} M were used as positive controls respectively without and with S-9 mix. The test substance and benzo-a-pyrene were dissolved in dimethylsulfoxide (DMSO), and mitomycin C was formulated in physiological saline. DMSO was also used as vehicle control. For each treatment group, four separated cultures were concurrently prepared and mixed two by two at the slide preparation. The exposure times of the cultures to the test material were 24 or 48 hours in non-activated and of 6 hours in activated cultures. After the exposure, in the test with S-9 mix, the culture medium with the test material was discarded and the cells were cultivated in the fresh medium for further 18 hours.

The S-9 mix was prepared from adult male Sprague-Dawley rats; for enzyme induction the animals received a single intraperitoneal injection of Kanechlor 400 (500 μg/kg); on the 5th day after injection the animals were sacrificed and the liver supernatant fluid was prepared combined with appropriate co-factor solution, according to established procedures.

The cells were arrested in C-metaphase by addition of colcemid, treated with trypsin and collected by centrifugation. One hundred cells from each treatment group were examined for aberrations and additional 400 cells in mitosis were observed from each dose to determine signs of polyploid cells. The percentage of cells with aberrations was compared with the control values by Chi-square test for significance.

RESULTS

For cytotoxicity tests the cells were exposed to concentrations of 10^{-6} to 10^{-3} M of test material without and with S-9 mix. In comparison to negative control, significant growth inhibition of cells was observed at 5 x 10^{-5} M and upper doses (-S-9 mix) and at the dose of 5 x 10^{-4} M (+ S-9 mix).

Esfenvalerate induced no significant increase of chromosomal aberrations in the presence and absence of S-9 mix. Polyploid cells did not increase under any of the conditions tested.

The positive controls, mitomycin C (- S-9 mix) and benzo(a) pyrene (+ S-9 mix) induced significant number of cells with aberrations demonstrating that the test system was working satisfactorily.

CONCLUSION

Esfenvalerate did not induce chromosome aberrations in cultured CHO cells (CHO - k_1) either in the presence and absence of metabolic activation.

3.8.1.3 [Study 3]

Study	IIA 5.4.1/03 Gene mutation test of S-1844 (esfenvalerate) in Chinese hamster cells (V79) in culture
Reference	Kogiso S (1985c)
Date performed	October – December 1985
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report no. LLT-50-0012
Guideline(s)	OECD 476
Deviations from the guideline	The positive control substances differed from those recommended in the guideline. Kanechlor 400 was used instead of Aroclor 1254

	in the preparation of S-9 mix
GLP	Yes
Test material	Esfenvalerate. Lot no. PKG-85109, purity 95.5% all isomers (87.4% as esfenvalerate)
Study acceptable	Yes

METHODS

Chinese Hamster lung cells (V 79) in culture were exposed to esfenvalerate (lot. nº PKG 85109 of 95.5% purity of total isomers (the ratios of fenvalerate Aα, Aβ, Bα and Bβ isomers were 91.5, 5.7, 2.7 and 0.1%, respectively) in the concentrations of 10^{-5}; 3 x 10^{-5}; 10^{-4} ; and 3 x 10^{-4} M without S-9 mix and 3 x 10^{-5}; 10^{-4}; 3 x 10^{-4} and 10^{-3} M with S-9 mix.

N-methyl-N'-nitro-N-nitrosoguanidine and 3-methylchloranthrene were used in the concentrations of 3 x 10^{-6} M and 10^{-5} M respectively without and with S-9 mix.

Test compound and positive controls were dissolved in dimethylsulphoxide (DMSO), and the same substance was used as vehicle control. In this study, the mutagenic frequency at the hypoxantine-guanine-phosphorybosyl transferase (HGPRT) locus of Chinese hamster cells in culture (V 79) was detected in the presence or absence of S-9 mix, after exposition to the test substance. Three dishes were used for each treatment group, six and nine days after the treatment with test material. The mutation frequency and plating efficiency were examined. Previous to the mutagenic frequency cytotoxicity test was performed by treating the cells at doses from 10^{-6} to 10^{-3} M in the presence and absence of metabolic activation.

The activation S-9 mix was prepared from adult male Sprague-Dawley rats; for enzyme induction the animals received a single intraperitoneal injection of Kanechlor 400 (500 mg/kg). Five days later the animals were sacrificed and the liver supernatant fluid was prepared combined with appropriate co-factor solution, according to established procedures.

Evaluation criteria: the test compound was considered to be positive when the increase of the mutation frequency was statistically significant and dose dependent. The statistical analyses were performed according to the method of Kastenbaum and Bowman.

RESULTS

Cytotoxicity test with and without S-9 mix: In preliminary cytotoxicity test the survival of the cells was 22% and 20% of the control groups respectively at the concentration of 10^{-3} M (+ S-9 mix) and 3 x 10^{-4} M (- S-9 mix). These concentrations were considered as top doses in this study.

The plating efficiency with or without S-9 mix in all treated groups and in the control group was over 80% at the both expression times (6 and 9 days).

The mutation frequency without S-9 mix was 4.6 per 10^{-6} clonable cells and 3.4 per 10^{-6} clonable cells in the vehicle control group respectively at 6 and 9 days expression times. In the treatment groups the frequency of mutations were varying between 5.1 per 10^{-6} and 8.2 per 10^{-6} clonable cells at the 6 days expression time, and between 2.1 per 10^{-6} and 6.3 per 10^{-6} clonable cells at the 9 days expression time.

The mutation frequency with S-9 mix was 5.8 per 10^{-6} and 4.5 per 10^{-6} clonable cells in the vehicle control group respectively at 6 and 9 days expression times. In the treatment groups, the frequency of mutations were varying between 2.4 per 10^{-6} and 7.6 per 10^{-6} clonable cells at 6 days expression time, and between 1.5 per 10^{-6} and 4.6 per 10^{-6} clonable cells at 9 day expression time.

There were no significant increases of the mutant frequencies in the esfenvalerate treated groups in presence or absence of metabolic activation in comparison to the vehicle controls. The positive controls N-methyl-N'-nitro-N-nitrosoguanidine and 3-methylcholanthrene showed remarkable increase in mutation frequencies without or with S-9 mix.

CONCLUSION

From the above results, it is concluded that esfenvalerate does not have mutagenic potential in V79 Chinese hamster cells in culture.

3.8.1.4 [Study 4]

Study	IIA 5.4.1/04 Unscheduled DNA Synthesis (UDS) assay of S-1844 in the HeLa cells
Reference	Kogiso S (1986)
Date performed	October 1985 – February 1986
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report no. LLT-60-0022
Guideline(s)	OECD 482
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate. Lot no. PKG-85109, purity 95.5% all isomers (87.4% as esfenvalerate)
Study acceptable	Yes

METHODS

The test substance was esfenvalerate (S-1844), Lot nº PKG 85109; purity 95.5% all isomers (the ratios of fenvalerate Aα, Aβ, Bα and Bβ isomers were 91.5, 5.7, 2.7 and 0.1, respectively).

Cultures of HeLa cells (purchased from Dainippon Seiyaku Co., Ltd., Osake - Japan) were exposed to esfenvalerate (Lot. nº PKG 85109, 95,5% of purity of total isomers – 87.4% a.i. -) in concentrations of 3×10^{-6} - 10^{-5} - 3×10^{-5} - 10^{-4} - 3×10^{-4} M (without S-9 mix) and of 10^{-5} - 3×10^{-5} - 10^{-4} - 3×10^{-4} - 10^{-3} M (with S-9 mix). As positive controls were used 4-nitrosoquinoline-1-oxide (4 NQO) and 3-methylchlolanthrene respectively with and without S-9 mix, in concentrations of 10^{-5} M in both conditions. The test material and positive controls were dissolved in DMSO (dimethylsulphoxide). DMSO was also used as vehicle control. The dose range was based on the preliminary cytoxicity test with concentrations ranging from 10^{-6} to 10^{-3} M of test material, with and without S-9 mix. The cells were treated with 50 μCi of [3H] thymidine (final concentration of 10 μCi/mL) and chemical solution at appropriate concentrations. After exposure for three hours, slides were prepared for autoradiography. The study was performed in duplicate.

The S-9 mix was prepared from adult male Sprague-Dawley rats, which received, for enzyme induction, a single intraperitoneal injection of Kanechlor 400 (500 mg/kg). On the 5th day after injection the animals were sacrificed and the liver supernatant fluid was prepared combined with appropriate co-factor solution according to the established procedures.

There is no mention of evaluation criteria in the Report. The histograms of population densities of net grain counts were analysed statistically by the Mann-Whitney U test.

RESULTS

Cytotoxicity test:

The HeLa cells were exposed to concentrations of esfenvalerate ranging from 10^{-6} to 10^{-3} M with and without S-9 mix. The maximum growth inhibition of 51.1% was observed at the highest dose level of 10^{-3} M (- S-9 mix) and of 60.9% (+ S-9 mix), compared to the vehicle control. At the maximum dose level precipitation of test material was occurred in the test without S-9 mix. The top doses for the test were considered 3×10^{-4} M (- S-9 mix) and 10^{-3} M (+ S-9 mix).

UDS Assay:

Test with S-9 mix: The number of net grains in nuclei of cells treated with test material was comparable to that of vehicle control group. The results indicate that S-1844 (esfenvalerate) does not induce UDS in presence of the metabolic activation. On the other hand, the positive control, 3-

methylcholanthrene induced significant increase in number of grains per cell suggesting significant induction of UDS.

Test without S-9 mix: The number of grains in nuclei for cells treated with esfenvalerate was similar to that of the vehicle control group. The results indicate that the test material does not induce UDS without S-9 mix. In contrast, the positive control (4NQO) showed a significant increase of the number of cells with more than 30 grains per nucleus indicating that an intensive degree of UDS was induced.

CONCLUSION

It is concluded that esfenvalerate does not induce UDS in the cultures of HeLa cells with or without metabolic activation, under the conditions of the test.

3.8.2 *In vivo* data

3.8.2.1 [Study 1]

Study	IIA 5.4.2/01 Micronucleus test of S-1844 in mouse bone marrow cells
Reference	Anonymous (1985g)
Date performed	October – December 1985
Test facility	Sumitomo Chemical Co. Ltd., Japan
Report reference	Report no. LLT-50-0011
Guideline(s)	OECD 474
Deviations from the guideline	Justification for using a single sex (males) is not provided. This should not be considered to be a significant deficiency as there is no clear evidence of gender differences in esfenvalerate toxicity
GLP	Yes
Test material	Esfenvalerate. Lot no. PKG-85109, purity 95.5% all isomers (87.4% as esfenvalerate).
Study acceptable	Yes

METHODS

Six male mice per group of ICR strain, eight week old, were treated with esfenvalerate (Lot. nº PKG 85109 of 95.5% purity as all isomers (ratios of fenvalerate Aα, Aβ, Bα, Bβ isomers were 91.5, 5.7, 2.7 and 0.1%, respectively). The test was divided in two studies. In the dose/response study the animals received a single intraperitoneal dose/group of 40, 80 and 150 mg/kg of test material dissolved in corn oil, and micronuclei were examined 24 hours after dosing. In the time course study, the animals were given a single I.P. dose of 150 mg/kg of test material in the same vehicle, and micronuclei were examined 24, 48 and 72 hours after treatment. In both studies the vehicle control group received via I.P. corn oil only. Mitomycin C at a dose of 2 mg/kg dissolved in saline and was injected by the intraperitoneal route, in both studies and served as positive control.

The selection of doses were made from a preliminary study in mice with doses of 100, 200, 500 and 1000 mg/kg via I.P. which provoked dose dependent mortality (0/6; 2/6; 3/6 and 6/6 animals respectively from 100 to 1000 mg/kg). Based on these results, 150 mg/kg was selected as highest dose for this study.

Evaluation: The incidence of micronucleated cells was scored in 1000 polychromatic erythrocytes (PCE) per mouse, (6000/group) and the ratio of PCEs to whole erythrocytes (PCE + normochromatic erythrocytes) was examined. Statistical analyses for the incidence of micronucleated cells was performed according to Kastenbaum & Bowman and T test for ratio of PCE to whole erythrocytes.

RESULTS

In dose response study the ratio of PCE to whole erythrocytes, which was evaluated as the sign of toxicity, was comparable in treated groups and vehicle control group. In the time course study the

ratio of PCE to whole erythrocytes showed significant decrease in the highest dose after 48 hours treatment but 72 hours after treatment the ratio of PCEs was recovered to the control level. Both in the dose response study and time course study, no significant increases of micronucleated PCEs were observed in any esfenvalerate treated groups as compared with the vehicle controls. On the other hand, the positive control, mitomycin C showed significantly increased number of micronuclei in PCE, indicating that the test system was working satisfactorily.

CONCLUSION

From the above findings, it was concluded that esfenvalerate does not induce any micronuclei in bone marrow erythrocytes under conditions used.

3.8.3 Human data

No relevant data available.

3.8.4 Other data

No other relevant data available.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 [Study 1]

Study	IIA 5.5.2/01 Combined chronic toxicity/oncogenicity (feeding) study in Wistar rats
Reference	Anonymous (2011a)
Date performed	June 2008 – July 2010
Test facility	Anonymous
Report reference	LLT-0226 (Lab. report B99213)
Guideline(s)	OECD 453
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Batch no. 60610G, purity 87.3% as Aα
Study acceptable	Yes

METHODS

HanRcc:WIST(SPF) rats, about 6 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.5.1–1: Study design

Test group	Dietary concentration of esfenvalerate TG (ppm)	Number of animals			
		Satellite group killed at 52 weeks		Main study group killed at 104 weeks	
		Males	Females	Males	Females
1	0	20	20	50	50
2	15	20	20	50	50

3	50	20	20	50	50
4	150	20	20	50	50
5	400	20	20	50	50

The test and control diets were prepared weekly during the first two weeks and every two weeks thereafter, using Kliba Nafag 3433 rodent maintenance diet. Esfenvalerate TG was warmed at 50±2°C in a water bath; acetone was added for each dose concentration and mixed with microgranulated feed separately for each dose concentration. The added acetone was shown to have evaporated after this step. An appropriate amount of water was added to aid pelleting. The pellets were dried with air for approximately 48 hours before storage. The achieved concentrations of the test substance in samples of test diet used on the study, taken at 3 month intervals, were generally within 20% of the nominal, demonstrating satisfactory concentration and homogeneity; stability of the test substance in the diet for the period of use was also demonstrated by analysis.

The mean test substance intake (mg/kg bw/day) per treatment group and sex was calculated weekly using the cage mean bodyweight data and food consumption per cage (expressed as g/rat/day). The group mean weekly intake values were then averaged over the duration of the study.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. An FOB and locomotor activity (observed over a 60 min time period) measurements were conducted at week 48 for all satellite animals. Bodyweights and food consumption were determined weekly for the first 14 weeks, and then at 4-week intervals thereafter. Ophthalmoscopy examinations were conducted on 20 main study males and females per group during the acclimatisation period, and the 20 control and high dose males and females were re-examined during week 52.

Blood (from the retroorbital plexus after overnight fasting) and urine samples were taken from satellite animals at weeks 13, 26 and 52. A standard range of haematology, blood clinical chemistry and urinary parameters were measured. At similar intervals, overnight urine samples were collected and a standard range of parameters were assessed. Blood samples were similarly taken (but without overnight fast) from all main study animals at 78 and 104 weeks, for erythrocyte, total leukocyte count and differential leukocyte count, only.

Scheduled necropsies were conducted on all animals, after either 52 weeks (satellite groups) or 104 weeks (main study) exposure. The weights of major organs were recorded for all animals at 52 weeks and 10 animals/group/sex at 104 weeks. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. Microscopic examination was conducted on all tissues sampled from all animals of the control and high dose groups, and on organs with macroscopic changes from other groups.

RESULTS

Received doses were calculated in terms of mg esenvalerate TG/kg body weight. Mean values are shown below:

Table B.6.5.1–2 Mean dose received (mg/kg/day)

Dietary concentration of esfenvalerate TG (ppm)	15	50	150	400
Main study animals				
Males	0.7	2.3	6.9	18.5
Females	0.8	2.7	8.0	21.5
Satellite animals				
Males	0.8	2.7	7.9	21.6
Females	0.9	3.1	8.9	24.7

Survival rates, summarised in Table B.6.5.1-3, were not influenced by esfenvalerate TG treatment. Survival of main study high dose females was slightly reduced in comparison with controls, but the difference was not statistically significant and was considered unrelated to treatment.

Table B.6.5.1–3 % survival at termination for the main study and satellite groups

Group	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Main study, week 104	78	56*	76	78	66	64	66	62	72	52
Satellite, week 52	100	95	100	100	95	95	95	100	100	100

* significantly different from control, p<0.05

There were no treatment related clinical signs of toxicity and the incidence, onset and location of palpable nodules/masses was similar for all dose groups. The only noteworthy FOB difference was a significant reduction in hindlimb grip strength at 400 ppm in both genders (see Table B.6.5.1-4). However, forelimb grip was not affected by treatment. Because forelimb grip was not affected, and there were no related histopathological findings in skeletal muscle, sciatic nerve, and lumbar spinal cord, the hindlimb grip strength findings are of uncertain toxicological significance.

Table B.6.5.1–4 FOB at week 48, group mean grip strength observations (as kg)

Activity	Dietary concentration of esfenvalerate Tg (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Forelimb grip strength (kg)	1.32	1.41	1.23	1.25	1.40	1.16	1.02	1.22	1.05	1.09
Hindlimb grip strength (kg)	1.09	1.10	1.10	1.08	0.98*	0.93	1.03	0.99	0.91	0.68**

* significantly different from control, p<0.05 ** significantly different from control, p<0.05

In the locomotor activity assessment, there was an apparently dose-related decrease in total activity among treated males over the 60 min observation period (see Table B.6.5.1-5); however, statistical significant was not achieved. Also, there were significantly lower activity measurements among males at 15 and 400 ppm for the 30-40 min time period. In the absence of consistent statistically significant differences at other time periods within the 60 min assessment, these locomotor observations could not be conclusively attributed to treatment. It is noted that the neurological climical signs observed from 150 ppm in the 90 day rat studies (IIA 5.3.2/01 and IIA 5.3.2/02) were not seen in this study, which provides support for concluding that the evidence that treatment-related neurological changes occurred in this study is weak.

Table B.6.5.1–5 Group mean locomotor activity, week 48: distance covered (cm) observations

Activity	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Distance covered 30-40 min	101	48*	70	63	34*	82	63	77	102	109
Distance covered, total 0-60 min	577	539	523	474	425	780	660	676	795	794

* significantly different from control, p<0.05

An adverse effect on bodyweight gain was observed in males, as shown in Table B6.5.1-6. For the main study animals at 400 ppm, group mean bodyweight were significantly lower throughout the study, starting from the first week of treatment, with mean bodyweights being 9.7% lower at termination. At 150 ppm, male bodyweights were slightly reduced throughout the study but a dose response relationship was not present (bodyweight gains at 15 ppm were similar to those at 150 ppm) and therefore this difference was not considered to be related to treatment. Among females, bodyweight gain was significantly reduced at 400 ppm for main study animals during the first week of treatment, but thereafter bodyweights were similar to controls. A similar pattern of bodyweight differences was seen in the satellite groups.

Table B.6.5.1–6 Group mean bodyweights (g) and bodyweight gain (as %), main study, selected data

Day	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Bodywt. d 1	159	159	154*	154	157	127	128	129	126	130
Bodywt. d 8	203	204	199	196**	192**	149	151	151	149	150
Bodywt.. d 351	604	601	582	578	559**	320	329	334	323	314
Bodywt. d 715	713	690	693	668	644**	401	403	428	413	386
Gain d 1-8	28	29	29	28	22**	17	18	17	18	15**
Gain d 1-351	280	280	279	277	257*	153	158	159	157	141
Gain d 1-715	348	335	349	336	317	215	215	227	228	196

* significantly different from control, p<0.05 ** significantly different from control, p<0.01

Food consumption at 400 ppm was significantly reduced during the first 2 weeks of treatment for main study males and during the first week of treatment for females, possibly due to palatability problems (see Table B.6.5.1-7). There were no effects on food consumption for the remainder of the study.

Table B.6.5.1–7 Group mean food consumption (g/animal/day), selected data

Day	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
1-8	18.5	19.1	18.8	18.0	15.5**	13.6	14.1	14.1	13.8	12.5**
8-15	20.6	20.9	20.3	20.4	19.6**	14.6	14.8	14.7	14.4	14.2
22-29	19.1	19.5	20.4**	20.9**	20.4**	14.0	14.1	14.4	14.4	14.3
344-351	22.1	22.4	22.6	21.1	20.5*	15.1	15.4	14.7	15.0	15.1
680-687	20.7	18.9*	19.3	20.3	21.0	15.1	14.1	15.0	15.9	14.7

* significantly different from control, p<0.05 ** significantly different from control, p<0.01

There were no treatment-related ophthalmoscopy, haematology, clinical chemistry or urinalysis findings.

There were no organ weight differences or macroscopic necropsy findings that were considered to be treatment-related.

An analysis of the non-neoplastic microscopic pathology findings for the satellite and main study animals revealed statistically significant increases in the high dose group in relation to only occasional tissues, as shown in Table B.6.5.1–8. The incidence of hyaline inclusions at level 3 (but not at level 1 or 2) in the nasal cavity was significantly increased in satellite and main study females at 400 ppm. These inclusions occur in the sustentacular cells of the olfactory and respiratory epithelium. The pathogenetic mechanism and possible toxicological relevance of these inclusions are unknown, but

are considered to be indicative of a slight local irritation or defensive response (Boorman et al. 1990; Greaves, 1990). As the incidence in main study females was within the laboratory historical control range, the control incidence is relatively high compared with the historical control range indicating high natural variation for this parameter, and the incidence of 400 ppm in females is comparable with these of males in the control and 400 ppm group, the presence of these hyaline inclusions in the level 3 nasal cavity can be considered as incidental.

The number of main study males at 400 ppm with spinal cord radiculoneuropathy was significantly increased. However, this is a normal background lesion with a great range of natural variation (evidenced historical control incidence range of 0 - 96%) and the incidences at 400 ppm were within the historical control range. In addition, there was no relationship between radiculoneuropathy and the presence of clinical signs or histopathological changes in the peripheral nerve, central nervous system or skeletal muscle. Overall, it is clear that the radiculoneuropathy observed in this study was not a severe lesion and was an age-related incidental finding.

The number of males with inflammation of the stomach was significantly increased at 150 and 400 ppm; although the historical control range was slightly exceeded, this is a normal background lesion and a clear dose-response relationship was not evident so these differences can also be regarded as incidental. Furthermore, the increases in males can be contrasted with a zero incidence in these two female groups but an increased incidence in the female control group. In addition, most of the animals with inflammation were dead animals (3/4 at 150 ppm), (6/6 at 400 ppm), and they had ulceration of forestomach or squamous hyperplasia. Therefore, inflammation of the stomach must be a concurrent lesion related to ulceration or squamous hyperplasia observed in dead animals.

Occasional isolated statistically significant increases in the incidence of non-neoplastic lesions were seen in the lower dose groups, but in the absence of dose response relationships these differences should not be regarded as treatment related. To confirm, there were no non-neoplastic microscopic findings that were considered to be treatment related adverse effects.

Boorman, GA, Eustis SL, Elwell MR, Montgomery Jr CA, MacKenzie WF (1990) Pathology of the Fischer Rat. Reference and Atlas. Academic Press, San Diego, California (1990)

Greaves P, Faccini JM, Courtney CL (1992) Proliferative Lesions of Soft Tissues and Skeletal Muscle in Rats. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, D.C.

Table B.6.5.1–8 Selected non-neoplastic microscopic pathology findings: satellite and main study animals

Tissue & finding	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Satellite animals (killed week 52)										
Nasal cavity level 3: hyaline inclusions	2/20 [10%]	0/1 [0%]	-	-	5/20 [25%]	3/20 [15%]	0/1 [0%]	-	-	10*/20 [50%]
Main study animals (killed week 104)										
Spinal cord, thoracic: radiculoneuropathy	2/50 [4%]	3/22 [12%]	0/12 [0%]	0/11 [0%]	10*/50 [20%]	7/50 [14%]	1/17 [59%]	1/19 [15%]	0/14 [0%]	3/50 [6%]
Spinal cord, lumbar: radiculoneuropathy	12/50 [24%]	10/22 [45%]	0/12 [0%]	3/10 [30%]	22*/50 [44%]	22/50 [44%]	5/17 [29%]	9/19 [47%]	2*/14 [14%]	15/50 [30%]
Lab. historical control for spinal cord radiculoneuropathy, area unspecified	156/629 [25%, range 0-90%]					130/627 [21%, 0-96%]				

Stomach: inflammation	0/50 [0%]	1/24 [4%]	1/13 [8%]	4† /13 [31%]	6*/50 [12%]	3/50 [6%]	2/17 [12%]	3/19 [16%]	0/14 [0%]	0/49 [0%]
Lab. historical control	13/528 [2%, range 0-8%]					7/524 [1%, 0-7%]				
Nasal cavity level 3: hyaline inclusions	25/50 [50%]	4†/22 [18%]	3/12 [25%]	4/11 [36%]	26/50 [52%]	19/50 [38%]	11/17 [65%]	10/19 [53%]	3/14 [21%]	29*/50 [58%]
Lab. historical control	62/429 [14%, range 0-40%]					93/379 [25%, range 0-68%]				

* significantly different from control, p<0.05 † significantly different from control, p<0.01 - tissue not subjected to microscopic examination

Historical control data is from 5-8 chronic (104 week) toxicity studies conducted at Harlan Laboratories in Wistar rats, completed between July 2005 and February 2009

Concerning neoplastic pathology changes, there was no evidence of a treatment-related increase in the incidence of tumours. The overall incidence of animals with benign and/or malignant tumours was similar in all groups, as shown in Table B.6.5.1-9. The individual tumours types with an incidence in any treatment group that was statistically significantly higher than the concurrent control group is shown in Table B.6.5.1-10; although the incidence of occasional tumour types in the lower dose groups was above the laboratory historical control range, no dose-related patterns were present and therefore it can be concluded that none of the observed tumours were caused by esfenvalerate TG treatment. The percentage animals in the 15, 50 and 150 ppm groups with tumours of the pituitary gland (males and females), thymus (females) and mammary gland (females) was noticeably higher than both the control group and 400 ppm group. This is the due to the fact that for 15, 50 and 150 ppm groups only the animals with gross lesions or those found dead were subject to histopathological examination, and should not be interpreted as evidence of a monotonic dose-response relationship; clearly, there is no coherent dose-response relationship over the range 15 to 400 ppm and it can be concluded that there is no evidence of carcinogenic activity in the pituitary, thymus or mammary gland.

Table B.6.5.1–9 Number [%] of animals with tumours

Finding	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	**15**	**50**	**150**	**400**	**0**	**15**	**50**	**150**	**400**
Satellite groups										
No. of animals examined	20	a	a	a	20	20	a	a	a	20
No. with tumours	0	1	1	0	1 [5]	4 [20]	2	2	1	3 [15]
No. with benign tumours	0	1	1	0	1 [5]	4 [20]	2	2	1	3 [15]
No. with malignant tumours	0	0	0	0	0	0	0	0	0	0
Main study groups										
No. of animals examined	50	a	a	a	50	50	a	a	a	50
No. with tumours	30 [60]	33	26	27	36 [72]	42 [84]	42	41	42	39 [78]
No. with benign tumours	25 [50]	26	20	25	28 [56]	35 [70]	37	38	40	37 [74]
No. with malignant tumours	10 [20]	11	7	5	11 [22]	12 [24]	11	10	13	10 [20]
Satellite and main study groups combined										
No. of animals examined	70	55	58	55	70	70	58	58	58	70
No. with tumours	30 [43]	34 [62]	27 [47]	27 [49]	37 [53]	46 [66]	44 [76]	43 [74]	43 [74]	42 [60]
No. with benign tumours	25 [36]	27 [49]	21 [36]	25 [45]	29 [41]	39 [56]	39 [67]	40 [69]	41 [71]	40 [57]
No. with malignant tumours	10 [14]	11 [20]	7 [12]	5 [9]	11 [16]	12 [17]	11 [19]	10 [17]	13 [22]	10 [14]

a = the exact number of animals examined in the satellite and main study groups 2-4 is not available in the study report

Table B.6.5.1–10 Tumour types showing statistically signficant differences, incidence (no. of affected animals/no. examined) in main study animals, decedents and survivors combined

Tissue & tumour type	Dietary concentration of esfenvalerate TG (ppm)									
	Males					Females				
	0	15	50	150	400	0	15	50	150	400
Testes: Leydig cell tumour	2/50 [4%]	1/27 [4%]	0/17 [0%]	4*/15 [27%]	4/50 [8%]					
Lab. historical control	17/628 [2.7%, range 0-5%]									
Pituitary gland: adenoma pars anterior	8/50 [16%]	17†/27 [63%]	10†/18 [56%]	10†/16 [62%]	13/50 [26%]	20/50 [40%]	23*/35 [66%]	25†/31 [81%]	25†/34 [73%]	21/50 [42%]
Lab. historical control	210/626 [33.5%, range 28.0-38.9%]					349/624 [55.9%, range 42.0-71.3%]				
Parathyroid glands: adenoma	0/38 [0%]	0/17 [0%]	1/12 [8%]	2*/11 [18%]	1/42 [2%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]
Lab. historical control	8/532 [1.5%, range 0-5.1%]					1/550 [0.2%, range 0-1.2%]				
Thymus; thymoma lymphatic type, benign	0/47 [0%]	1/22 [4%]	2*/14 [14%]	1/15 [7%]	1/45 [2%]	0/48 [0%]	6†/25 [24%]	5†/23 [22%]	5†/17 [29%]	3/49 [6%]
Lab. historical control	9/600 [1.5%, range 0-4.4%]					22/615 [3.6%, range 0-16%]				
Haemolymphoreticular system: malignant lymphoma	2/50 [4%]	3/24 [12%]	1/12 [8%]	1/11 [9%]	2/50 [4%]	0/50 [0%]	3*/17 [18%]	0/19 [0%]	0/14 [0%]	0/50 [0%]
Lab. historical control	9/480 [1.9%, range 0-3.7%]					4/480 [0.8%, range 0-2.0%]				
Mammary gland: fibroadenoma	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	10/50 [20%]	14†/27 [52%]	19†/29 [66%]	13†/23 [57%]	13/49 [27%]
Lab. historical control	0/479 [0%, range 0-0%]					180/626 [28.8%, range 22.0-36.0%]				
Mammary gland: adenocarcinoma	0 [0%]	0 [0%]	0 [0%]	0 [0%]	0 [0%]	5/50 [10%]	3/27 [11%]	6/29 [21%]	7*/23 [30%]	5/49 [10%]
Lab. historical control	1/479 [0.2%, range 0-2%]					37/626 [5.9%., range 2.0-12.0%]				

* significantly different from control, p<0.05 † significantly different from control, p<0.01

Historical control data is from 6-8 chronic (104 week) toxicity studies conducted at Harlan Laboratories in Wistar rats, completed between July 2005 and February 2009

CONCLUSION

The different effects observed in the 2-year rat study were discussed at the expert review meeting. The body weight changes at the high dose (less than 10% versus control group) were not considered adverse. The reduction in hind limb grip strength at the high dose was concluded to be adverse. The incidences of radiculoneuropathy showed high variability between sexes and also within the historical control data (e.g. the incidence in control females is the same than the incidence in high dose males). There is no clear dose response relationship. A statistically significant increased incidence is observed in high dose males, above the mean of the historical control data. Taking into account the lack of histopathological examinations at the low and intermediate doses, the experts agreed that this was an adverse effect at the high dose.

Increased incidences of stomach inflammation were observed in the males of the two high dose groups (whereas no incidence was observed in the females of the same dose groups). Due to the low number of animals examined at 150 ppm, the experts agreed that these equivocal results could not be dismissed when considering the statistically significant increase at the high dose of 400 ppm.

The experts agreed to derive a NOAEL of 50 ppm (equivalent to 2.3 mg/kg bw per day) for non-neoplastic findings.

With regard to the neoplastic findings, the increased incidence of Leydig cell tumours at the highest two doses (above the historical control range) was considered treatment related. The relevance to humans could not be excluded on the basis of the available data.

For the other types of tumour, which exceeded in some instances the historical control data, no dose response relationship was observed. The experts agreed that the results for the intermediate doses were less clear, due to the low number of animals examined.

The agreed NOAEL for the neoplastic findings is 50 ppm (2.3 mg/kg bw/day) based on the increased incidences of Leydig cell tumours.

The experts agreed to propose classification as Carc. Cat. 2.

Additional histopathology report submitted to the RMS after the RAR

Study	Esfenvalerate TG: Additional Histotechnique and Pathology Work on Testes of Intermediate Groups for Harlan Study Number B99213
Reference	Anonymous (2015)
Date performed	November 2014 – March 2015
Test facility	Anonymous
Report reference	LLT-0255 (Lab. report D96351)
Guideline(s)	OECD 453
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Batch no. 60610G, purity 87.3% as Aα

METHODS

The purpose of this study was to conduct histopathological evaluation of the testes tissues collected in the combined chronic toxicity / oncogenicity (feeding) rat study with the test item, Harlan study number B99213, from animals of the intermediate dose groups (groups 2, 3 and 4).

The testes from the assigned animals were processed for light microscopy examination using the same procedures as those described in the main study report. The statistical methods used for data evaluation were as described in the histopathology phase report.

RESULTS

The evaluation of additional testes sections from animals of intermediate groups 2 to 4 that were not evaluated during the main study did not reveal new preneoplastic or neoplastic lesions. Therefore, the original data regarding the incidences of Leydig cell hyperplasia and Leydig cell tumours did not change. The total incidences of lesions detected in the testes from all animals are presented in the Table below:

Table 1: Incidence of findings recorded in testes from all main study animals, decedents and survivors combined

	Dietary concentration of esfenvalerate TG (ppm)				
	0	**15**	**50**	**150**	**400**
No. of animals examined	**50**	**50**	**50**	**50**	**50**
Congestion	0	1	0	0	2
Edema, interstitial	4	8	6	4	2

Edema, tubular	1	0	0	0	1
Tubular degeneration	7	16	16	14	9
Multinuclear giant cells	0	0	1	0	1
Arteritis/periarteritis	2	2	6	5	1
Rete testis hyperplasia	0	1	0	0	0
Leydig cell hyperplasia	**2**	**1**	**0**	**1**	**0**
Leydig cell tumour	**2**	**1**	**0**	**4**	**4**
Histiocytic sarcoma	0	1	0	0	0

Statistical analysis (PETO Test) revealed a positive trend for Leydig cell tumor (P = 0.0262). The Leydig cell tumour is not a rare tumour entity, and therefore this was not considered a significant value. This is supported by P-values obtained in the Fisher's exact test that were not statistically significant (P = 0.3389). Furthermore, the incidence of Leydig cell tumours was within the range of historical control data (see Table 2).

Table 2: Historical control data on Leydig cell tumours in RccHanTM: WIST rats compiled by RCC/Harlan (Harlan 2010)

Male	Total n	Total %	Mean %	STDEV %	MIN %	MAX %
Number of rats examined	1740					
Leydig cell tumour, benign	52	3.0	2.8	3.1	0	10.0

Historical control data from 23 studies conducted between 1981-2009

DISCUSSION

Although the Peto trend test revealed a positive trend at P = 0.0262, the finding of Leydig cell tumours is deemed to be incidental. Leydig cell tumours are generally not considered to be rare tumour entities. Nolte et al. (2011)1 reported even higher mean incidences (13.7%). In this study, the incidence of Leydig cell tumours in the control group was 4%. Historical control data revealed up to 10% of the animals affected by Leydig cell tumours in control groups from 104-week studies of this rat strain. In addition, it has been published that mean incidences of spontaneous Leydig cell tumours in RccHanTM: WIST rats are higher than those reported from Sprague Dawley rats (Weber et al., 2011)2. Again this was confirmed by Nolte et al. (2011). They reported a mean incidence of 4.2% in Sprague-Dawley rats, whereby incidences in '…Wistar rats were highly variable, primarily caused by different sources of animals. Mean incidences per breeder varied from 2.8 to 39.9%...'. Furthermore, pre-neoplastic Leydig cell hyperplasia was not observed at increasing incidences.

CONCLUSION

It was concluded that there was no treatment related increase in Leydig cell tumours in the esfenvalerate 2-year rat study (including the additional work performed in this study D96351).

[1] T. Nolte, S. Rittinghausen, R. Kellner, E. Karbe, B. Kittel, M. Rinke, U. Deschl: RITARegistry of Industrial Toxicology Animal data: the application of historical control data for Leydig cell tumours in rats. Exp. Toxicol. Pathol. 63: 645-656 (2011)

[2] K. Weber, T. Razinger, J.F. Hardisty, P. Mann, K.C. Martel, E.A. Frische, K. Blumbach, S. Hillen, S. Song, T. Anzai, H.J. Chevalier: Differences in rats models used in routine toxicity studies. Int. J. Toxicol. 30: 162-173 (2011)

3.9.1.2 [Study 2]

Study	IIA 5.5.3/01 Oncogenicity study with DPX-YB656-84 eighteen month feeding study in mice
Reference	Anonymous (1997)
Date performed	December 1993 – April 1997
Test facility	Anonymous
Report reference	Report No. LLT-0180
Guideline(s)	OECD 451
Deviations from the guideline	None
GLP	Yes.
Test material	DPX-YB656-84 (esfenvalerate), batch no. not reported, purity (84.8% S,S isomer; 98.8% total fenvalerate isomers)
Study acceptable	Yes

METHODS

Three groups of 80 males and three groups of 80 female Crl: CD mice were fed diets containing 0, 35 and 150 ppm of DPX-YB656-84 for 18 months. Additional groups of 80 male and 80 female mice were fed diets containing 350 ppm of DPX-YB656-84. Mice fed 350 ppm of the test substance developed excessive morbidity and mortality due to self-trauma induced by the pharmacological effects of the test substance on dermal sensory nerves and were sacrificed by design on test days 57 and 58.

Data collected from mice received 350 ppm in the diet were not used in the evaluation of the oncogenic potential of the test substance.

RESULTS

DPX-YB656-84 was not oncogenic under the conditions of this study. All of the differences observed between control and 150 ppm exposed animals in the endpoints evaluated can be attributed to an interplay of self-trauma (self-mutilation) secondary to the sensory stimulation resulting from dermal contact with ground chow containing the test substance and probable systemic toxicity (slightly lower mean body weight and moderately lower mean body weight gain) in this group.

Survival was significantly decreased in males (46% compared to 70% in controls) and females (41% compared to 71% in controls) in the 150 ppm group, largely attributable to the number of mice sacrificed “in extremis” because of self-trauma. Survival of animals fed diets containing 35 ppm of the test substance was comparable to controls.

Slightly lower mean body weight and moderately lower body weight gain were observed in 150 ppm males and females. Relative to controls, mean body weights of males and females were depressed 7% and 9%, respectively, by the end of the study. In males the differences were more pronounced during the first half of the study. By the end of the study, mean body weight gains were depressed 19% in males and 22% in females. These differences were most striking during the first few months of the study. The observed depression in mean body weight and mean body weight gain was interpreted to be due to the interplay of increased incidence and severity of dermal self-trauma present in these animals and mild systemic toxicity.

Overall, there was no significant effect on food consumption attributable to DPX-YB656-84 in male or female mice at the 35 or 150 ppm exposure levels. Males and females fed diets containing 150 ppm, had moderately (24% - 47%) lower food efficiency values during the 0 - 56 day interval. Food efficiency values of treated groups were generally comparable to controls, however, for the last 15 months of the study. The lower food efficiency observed during the first few months of the study was interpreted to be the result of the additive effects of self-trauma and systemic toxicity.

Additional information provided by Sumitomo Chemical Agro Europe (dated 12 March 2013) states that evidence of skin scratching and excoriation was present at both 35 ppm and 150 ppm. The

information states that the most common sites of scratching and excoriation were ears, head, face and neck with fewer lesions on the back, sides and base of tail. This is consistent with a higher probability of exposure of the head and neck region to treated diet whilst feeding.

CONCLUSION

Under the conditions of this study, DPX-YB656-84 was not oncogenic in mice of either sex. The no observed effect level (NOEL) was 150 ppm for males and females, related to oncogenicity. The test substance-related increased incidences of gross and microscopic findings in skin, ears, and eyes of males and females in the 35 and/or 150 ppm groups were due to self-trauma induced by the pharmacological effects of DPX-YB656-84 and were considered not to be a target organ toxicity.

Comment by UK RMS

The above study conclusion was copied from an Addendum to the original DAR.

The UK RMS notes that in an Addendum to the original DAR the summary of the reproduction study in rats with esfenvalerate using pelleted diet (IIA5.6.1/02, Anonymous 1999b) concludes that skin itching/tingling and consequent skin trauma occurring at low doses in dietary studies conducted using powered diet is due to dermal contact with the test substance in the diet rather than a systemic effect. This conclusion is supported by the absence of skin trauma in the esfenvalerate dietary rat carcinogenicity study (IIA 5.5.2/01, Anonymous 2011a), a study that used pelleted diet which would result in dermal contact with the test substance being minimised. Skin paraesthesia is an established local effect of dermal contact with pyrethroids in humans (Wilks, 2000; Ray and Forshaw, 2000) and is regarded as a minor, 'nuisance', effect unrelated to the pyrethroid systemic neurotoxicity that can occur at higher systemic dose levels. Because the self-trauma effects seen at 35 ppm in this mouse carcinogenicity were not associated with bodyweight changes, and were likely to be related to dermal contact with the test substance and therefore not relevant to deriving a systemic ADI, the UK RMS concludes that a study NOAEL of 35 ppm (intake of 4.3 mg/kg/day in males and 5.7 mg/kg/day in females). This conclusion is consistent with that of the esfenvalerate JMPR monograph of 2002 (see http://www.inchem.org/documents/jmpr/jmpmono/2002pr04.htm).

Wilks, M.F. (2000). Pyrethroid-Induced Paresthesia – A Central or Local Toxic Effect? Clinical Toxicology, 38(2), 103-105

Ray, D.E. and Forshaw, P.J. (2000). Pyrethroid Insecticides: Poisoning Syndromes, Synergies, and Therapy. Clinical Toxicology, 38(2), 95-101

3.9.2 Human data

No relevant data available.

3.9.3 Other data (e.g. studies on mechanism of action)

3.9.3.1 [Study 1]

Study	IIA 5.5.4/01 Hormonal investigation for rat testicular tumourigenicity using fenvalerate and esfenvalerate
Reference	Anonymous (1999a)
Date performed	April 1998 – January 1999
Test facility	Sumitomo Chemical Co., Japan
Report reference	Report no. LLT-0190
Guideline(s)	None
Deviations from the guideline	N/A

GLP	No
Test material	Fenvalerate, esfenvalerate, purity and batch numbers not available
Study acceptable	Yes

Although this study was taken account of when esfenvalerate was considered for first inclusion in Annex I, a study appraisal by the Portugal RMS could not be located. The following study summary is copied from the esfenvalerate JMPR monograph of 2002 (see http://www.inchem.org/documents/jmpr/jmpmono/2002pr04.htm).

METHODS

The potential for fenvalerate and esfenvalerate to affect the male endocrine system, primarily by testicular tumorigenesis, was investigated in groups of male SLC:Wistar rats fed diets containing fenvalerate (purity, 92.9%) at a concentration of 0, 50, 150, 500 or 1500 ppm or esfenvalerate (purity, 86.0%) at a concentration of 375 ppm for 26 weeks. This study included a component in which female rats were housed in close proximity (but without contact) to subgroups of males given the lowest and highest concentrations of fenvalerate and of those given esfenvalerate. Blood samples were taken at 4-week intervals for analysis of serum luteinizing hormone and testosterone concentrations.

RESULTS

No rats died during the study. Lower body weights and decreased food consumption relative to controls were observed in the groups given 375 ppm of esfenvalerate and/or 1500 ppm of fenvalerate. The serum concentrations of luteinizing hormone and testosterone were not affected to any biologically significant extent; those statistically significant changes that were observed did not show a dose–response relationship or temporal consistency. Marginal increases in relative (but not absolute) organ weights were observed during the phase of the study in which the males were housed separately from the females, as follows: testes (+5%), pituitary (+15%) and liver (+9%) at 1500 ppm of fenvalerate; and epididymides (+4%), seminal vesicles (+9%) and liver (+4%) at 375 ppm of esfenvalerate. There were no gross or histopathological changes that were considered to be related to treatment. Housing males and females in close proximity without physical contact had no effect different from those seen when males were housed alone

CONCLUSION

Esfenvalerate and fenvalerate exposure for 26 weeks has no effect on serum luteinizing hormone and testosterone concentrations.

3.9.3.2 [Study 2]

Study	IIA 5.3.2/01 Subchronic feeding study of MO 70616 in the rat
Reference	Anonymous (1984)
Date performed	June – September 1984
Test facility	Anonymous
Report reference	Report no. LLT-51-0013
Guideline(s)	OECD 408
Deviations from the guideline	None.
GLP	Yes (declaration made by Applicant June 2013)
Test material	MO 70616 (esfenvalerate). Batch WRC Tox. Sample No. 730B, purity not reported.
Study acceptable	Yes

METHODS

MO 70616 (esfenvalerate) was dissolved in acetone and admixed in the diet to groups of 30 male and 30 female Sprague Dawley derived rats/dose at levels of 0, 50, 150, 300 and 500 ppm for up to 13 weeks. The acetone was allowed to evaporate off.

Following seven weeks exposure up to 10 rats/sex/group were randomly selected and evaluated at an interim necropsy. After 13 weeks, up to five animals/sex/group were used for electron microscopy evaluations and the remaining animals sacrificed for post mortem examination. Ophthalmoscopy was only used prior to initiation of the study in order to reject unsuitable animals from the randomization pool. Blood and urine were collected at interim and terminal evaluation, selected organs were weighted at each scheduled necropsy. Gross observations were made on all rats, and tissues and organs designated in the Protocol were evaluated microscopically.

Statistical significance was evaluated according to the method of Dunnett, a multiple comparison method for determining significant difference between a control group and one or more treatments. Body weights were compared using analysis of covariance. Purity and stability of test substance were not determined.

RESULTS

It seems that seven of 30 high dose group female died or became moribund during the study, but exact number of deaths was not reported. Rats receiving 300 ppm (group 4) and above exhibited clinical signs such as jerky leg movements and unsteady gait. The severity of these effects was dose related and the high dose group (500 ppm) showed body tremors and became hypersensitive to sounds; some had convulsions and/or death. The signs were usually observed from within the first few weeks of dosing to termination in the high dose group.

Body weight and food consumption decreased significantly in the high dose group and males of group 4 (300 ppm). This effect appeared also in other treatment groups early in the study but with time, body weight/food consumption differences between control group and treated group values lessened. There were no consistent haematological changes related to treatment: decreased urine volume and concomitant increase in urine specific gravity were noted in animals having reduced food intake. Decreased absolute mean heart weight and increased relative mean brain weight in group 5 (high dose group) appeared to be also related to decreased body weight.

The only gross necropsy observation considered to be treatment related was the scab covered areas located at the base of the tail for a few rats fed with 300 and 500 ppm leading to chronic dermatitis. Gross and microscopic evaluation did not reveal any lethal morphologic alteration in the tissues of rats which died during the study.

Microscopic examination revealed after seven and 13 weeks (group 4 and 5) slight to moderate hypertrophy of the parenchymal cells in the parotid salivary gland and with lower incidence in the pituitary glands.

CONCLUSION

Although one male rat treated with 150 ppm of test substance had jerky leg movements diagnosed during week 11 of the study, this effect did not appear remarkable at subsequent examinations. So the NOEL was considered by Applicant to be essentially the 150 ppm level, equivalent to approximately 7.5 mg/kg/day. However, the Portugal RMS had some doubts about this conclusion and identified the NOEL as 50 ppm, equivalent to approximately 2.5 mg/kg/day.

3.9.3.3 [Study 3]

Study	IIA 5.3.2/02 13-Week Dietary Admix Study of MO 70616 Technical in Rats.
Reference	Anonymous (1987)
Date performed	September – December 1986
Test facility	Anonymous
Report reference	Report no. LLT-71-0086
Guideline(s)	OECD 408
Deviations from the guideline	Tissues were not subjected to histopathological examination
GLP	Yes
Test material	MO 70616 Technical (esfenvalerate). Batch WRC Tox. Sample

	No. 730C, purity not reported.
Study acceptable	Yes

METHODS

This subchronic feeding study was performed in order to evaluate the effect level from a prior subchronic study (LLT-51-0013) where doubts had arisen. MO 70616 Technical (esfenvalerate) was dissolved in acetone and admixed in the diet to five groups of 25 male and 25 female Sprague Dawley derived rats at levels of 0, 75, 100, 125 and 300 ppm for either seven (10 rats/sex/group) or 13 (15 rats/sex/group) weeks.

Physical examinations including ophthalmology of all rats were conducted at pre-test and prior to sacrifice. Tissues from the interim rats and terminal rats were preserved but not prepared, processed and examined microscopically, since histopathological evaluation of tissues for the previous study (LLT-51-0013) revealed no treatment related morphological changes below 300 ppm. Statistical significance was evaluated according to the method of Dunnett. Purity of test substance was not determined.

RESULTS

Treatment related clinical observations were limited to neurological signs in some high dose rats, beginning week 10 of the study and characterised by hyperactivity and/or abnormal limb movements (jerky leg movements characterised by prolonged posterior extension, flexion, and/or elevation of one or both hindlimbs). This last observation had an overall lack of severity and persistence.

Decreased total weight gain was observed in high dose male rats during the first two weeks of the study, but not thereafter, and in female rats of group 4 and 5 (125 and 300 ppm diet) throughout the study. There were no significant differences in food consumption between the groups.

Urinalysis, haematology, clinical chemistry and gross necropsy did not reveal treatment related findings. Among organ weight there were some consistent effects due to test substance as higher absolute kidney weight in high dose female rats and higher relative kidney weight for high dose male and female rats. Relative liver weights of both group 4 and group 5 male rats were significantly elevated compared to control males. These differences were not interpreted by the Applicant signs of hepatic toxicity because the differences were slight and could be attributed to differences in liver glycogen and/or fat levels or enzymatic induction.

CONCLUSION

Based on neurological clinical signs or kidney weights, the NOEL of MO 70616 is 125 ppm, equivalent to approximately 6.25 mg/kg/day. The Portugal RMS considers this level as NOAEL.

3.9.3.4 [Study 4]

Study	IIA 5.3.7/01 Repeat dose dermal toxicity 21-day study in rats
Reference	Anonymous (2000b)
Date performed	April – May 2000
Test facility	Anonymous
Report reference	Report LLT-0207 (Lab. Project ID DuPont-4228)
Guideline(s)	OECD 410
Deviations from the guideline	No
GLP	Yes
Test material	Esfenvalerate technical, Lot # YB656-84 (purity 97.3-98.5%, as sum of all isomers)
Study acceptable	Yes

METHODS

Sprague-Dawley rats, about 55 days old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.4–1: Study design

Test group	Esfenvalerate dose level (mg/kg/day) Exposure period 6 h/day	Number of animals	
		Males	Females
1	0	10	10
2	25	10	10
3	125	10	10
4	500	10	10
5	1000	10	10

The test substance was prepared for dosing by heating in a 60-70°C water bath to liquefy the sample (conditions under which it is known to be stable). After the test substance was liquefied, the temperature of the water bath was lowered to approximately 40-50°C and was maintained at this temperature during dosing procedures.

The rats were acclimatised to the bandaging for one week prior to the commencement of dosing. On the day prior to treatment all rats were shaved free of hair from their backs and trunks. Plastic collars were applied around their necks to inhibit the animals from interfering with the dosing procedures. The appropriate quantity of test substance was applied undiluted to an area covering, where possible, 10% of the total body area. The control group received deionised water at the same dosage volume as used for the high dose group. The application site was then covered by a porous gauze dressing followed by successive layers of stretch gauze and self adhesive bandages. The bandages were removed at the end of each daily 6 h exposure period and the test substance was washed of with warm tap water. After examination, the application site was rewrapped with gauze and self adhesive bandages. Animals were treated for 21 consecutive days.

General clinical observations and signs of dermal irritation at the application site were recorded once daily. A comprehensive functional observation battery (FOB) and motor activity measurements was conduced on all animals prior to exposure and during week 3. Bodyweights were measured twice weekly, and food consumption was monitored throughout the study. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

On day 21, and after an overnight fast, blood samples were taken from the retroorbital sinus of all animals and a standard range of haematology and clinical chemistry parameters were measured.

A necropsy was conducted on all animals the end of the 21 day treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. All organs from the control and high dose animals, together with eyes, treated and untreated skin and gross abnormalities from the other groups, were subjected to microscopic (light) examination.

RESULTS

There were no treatment related deaths.

Treatment-related clinical signs were observed at 125 mg/kg/day and above. During the 1st week, abnormal hind limb gait was observed in all animals in the 500 and 1000 mg/kg/day groups, and in 50% of males and all females in the 125 mg/kg/day group. This observation was consistent with oral toxicity studies conducted with esfenvalerate. Vocalisation was reported for most females at 500 and 1000 mg/kg/day, predominantly during the first 3 days of dosing. Additionally, at 1000 mg/kg/day, most females exhibited hyperactivity at the start of the study and hyperreactivity at other times. Vocalisation, hyperactivity and hyperreactivity may be secondary to the skin sensory stimulation previously reported in both humans and animals, rather than due to direct systemic toxicity.

The application site irritation assessments did not detect any treatment-related local irritation. However, the incidence of probably self-inflicted superficial wounds to the shoulder and forelimb

areas was higher in the esfenvalerate-treated groups, likely to be a response to transient local itching and/or tingling sensations which are a known effect of pyrethroids.

The FOB did not reveal any treatment-related effects. However, the motor activity assessment at week 3 showed increased activity in comparison with baseline and control activity levels, measured as duration of movements and number of movements during the 60 min observation period, among females at 500 and 1000 mg/kg/day (see Tables B.6.3.4-2 and B.6.3.4-3). The increased activity may be secondary to skin sensory stimulation since motor activity was not increased in females in either the 90-day (IIA 5.7.4/01) or acute (IIA 5.7.1/01) neurotoxicity studies in rats. Activity, measured as duration of movements, was also increased at 125 mg/kg/day, but this is not considered to be treatment related because the change from the baseline measurement was marginal.

Table B.6.3.4–2 Motor activity assessment: total duration of movements (sec) in 60 min observation period, for females

Observation	Dose of Esfenvalerate (mg/kg/day)				
	0	25	125	500	1000
Pre-dosing (baseline) mean	810	804	1140	1007	1104*
Pre-dosing (baseline) range	261-2066	429-1738	525-2194	379-1679	676-2043
Week 3 mean	699	786	1210*	1288*	1296*
Week 3 range	164-1082	222-1503	232-2541	600-2518	691-1734
% change at week 3 from control	-	↑12%	↑73%	↑84%	↑85%
% change at week 3 from baseline	↓14%	↓2%	↑6%	↑28%	↑17%

* significantly different from control, $p<0.05$

Table B.6.3.4–3 Motor activity assessment: total number of movements in 60 min observation period, for females

Observation	Dose of Esfenvalerate (mg/kg/day)				
	0	25	125	500	1000
Pre-dosing (baseline) mean	405	435	581	500	462
Pre-dosing (baseline) range	150-673	215-714	341-954	225-892	280-731
Week 3 mean	431	418	636	595*	596*
Week 3 range	144-736	142-851	203-983	432-845	407-881
% change at week 3 from control	-	↓4%	↑9%	↑38%	↑38%
% change at week 3 from baseline	↑6%	↓4%	↑9%	↑19%	↑29%

* significantly different from control, $p<0.05$

There were no treatment-related adverse effects on bodyweight or food consumption.

The ophthalmoscopy examination revealed increased incidences of corneal opacities at 125 mg/kg/day and above, probably due to self-inflicted trauma related to increased scratching rather that to systemic toxicity.

There were no treatment related haematology or clinical chemistry findings.

There were no organ weight differences, macroscopic or microscopic pathology findings that could be attributed to treatment.

CONCLUSION

Dermal exposure to esfenvalerate in the rat at a dose levels 125 mg/kg/day and above for 21 days elicited systemic toxicity, observed as abnormal hind limb gait in both genders during the 1st week of treatment. Additionally, increased incidences of corneal opacities were reported at 125 mg/kg/day and above, probably due to self-inflicted trauma related to increased scratching rather than to direct

systemic toxicity. At higher exposure levels, vocalisation, hyperactivity and hyperreactivity were present as a response to esfenvalerate treatment. There were no overt signs of skin irritation at the application sites. No effects were seen at the lowest exposure level tested, and so the study NOAEL is 25 mg/kg/day.

3.9.3.5 [Study 5]

Study	IIA 5.6.1/01 Reproductive and Fertility effects with DPX-YB656-84 (esfenvalerate) Multigeneration reproduction study in rats
Reference	Anonymous (1994a)
Date performed	June 1993 – April 1994
Test facility	Anonymous
Report reference	No. LLT-41-0169
Guideline(s)	OECD 416
Deviations from the guideline	None
GLP	Yes.
Test material	Esfenvalerate, batch no. DPX-YB 656-84, purity 98.8% as total isomers, 84.8% as SS isomers
Study acceptable	Yes

METHODS

Groups of 30 male and 30 female CDBR rats, with a body weight 250-275 g (males) and 175-200 g (females), were fed diets that contained 0, 75, 100 or 350 ppm DPX-YB 656-84 (esfenvalerate). After 73 days on test, the P1 rats were bred within concentration groups to produce F1 litters. At weaning 30 F1 rats/sex/concentration were randomly selected to produce the F2 generation. Due to progressive clinical signs observed (ataxia, barrel rolls, 'popcorn' seizures, etc) in the high concentration (350 ppm) rats at weaning, the concentration was dropped from 350 to 150 ppm DPX-YB656-84 (esfenvalerate).

An exception to the proceeding feeding schedule occurred in parental F1 generation males and females exposed to 150 ppm (high concentration group) of test compound. Within five weeks or less of exposure to 150 ppm of test compound, all parental F1 generation male rats had either been sacrificed in extremis or found dead with extensive dermal ulceration involving the neck, head/or face. Parental F1 generation females (21/30) exposed to 150 ppm of test compound were sacrificed in extremis because of extensive neck, head/or face ulceration. The surviving 9 females rats from the parental F1 generation 150 ppm group were sacrificed by design after six weeks of exposure to the test compound. All 150 ppm parental F1 rats were submitted for necropsy and gross observations were recorded. Microscopic examination of preserved tissues was not performed since the rats had not lived enough to reach sexual maturity or propagate.

The overall calculated mean daily intake of DPX-YB656-84 was 0, 4.21, 5.55 and 18.8 mg/kg/day for P1 male rats and was 0, 5, 5.6, 7.18 and 25.1 mg/kg/day for P1 female rats fed diet containing 0, 75, 100 or 350 ppm DPX-YB656-84 respectively, during the premating phase.

The calculated overall mean daily intake of DPX-YB656-84 was 0, 5.98 and 7.84 mg/kg/day for F1 male rats and was 0, 7.31 and 10.4 mg/kg/day for F1 females fed diets containing 0, 75 or 100 ppm DPX-YB656-84, respectively, during the premating phase.

The calculated mean daily intake of DPX-YB656-84 for F1 male rats fed diets containing 350/150 ppm DPX-YB6546-84 for the initial 28 days of the premating phase was 18.93 mg/kg/day and for F1 female rats was 19.25 mg/kg/day for rats fed 350/150 ppm DPX-YB656-84 for 92 days. It is notable that immediately after weaning, the mean daily intake of DPX-YB656-84 was considerably higher than later in the premating phase; for example during the first week after weaning mean daily intake of DPX-YB656-84 was 0, 11.8, 15.3 and 22.2 mg/kg/day for F1 male rats and was 0, 11.0, 16.3 and

23.4 mg/kg/day for F1 female rats fed diets containing 0, 75, 100 or 350 ppm DPX-YB656-84, respectively.

DPX-YB656-84 was dissolved in acetone (5 mL/concentration), added to Purina Certified Rodent Chow, and thoroughly mixed for three minutes in a high-speed mixer. Control diets were also mixed for the same period of time. All diets were prepared weekly and refrigerated until used.

Statistical methods: Body weights, body weight gains, food consumption, food efficiency, organ weights and gestation length were analyzed by a one-way analysis of variance. When the test for differences among test groups means was significant ($p \leq 0.05$), pairwise comparisons between test and control groups were made with Dunnett`s test. Incidence of clinical observations was evaluated by the Cochran-Armitage test for trend[3] . When appropriate, evaluation of the incidences of clinical observations between the control and low dose group were analyzed by the Fisher`s Exact Test[4]. Measures of reproduction and lactation performance were evaluated with either the Cochran-Armitage test for trend (mating, fertility and gestation indices, and litter survival) or Jonckheere`s test[5] (pup numbers, survival, viability index, and lactation index). Pup weights were analyzed using analysis of covariance, followed by a linear contrast of the least square means[6]. The covariants were litter size and sex ratio. All other significance was judged at $p \leq 0.05$.

RESULTS

The following effects, observed in P1 rats fed 350 ppm or in F1 rats fed 350/150 DPX-YB656-84, were considered to be compound related when compared with the respective control group.

Adult evaluations:

-Statistically significant decrease in the weekly mean body weights, body weight gains, and food consumption of P1 and F1 male and female rats during premating.

-Statistically significant decrease in the overall food efficiency of P1 female rats during premating.

-Statistically significant decrease in the mean body weight of P1 females during gestation and lactation.

-Statistically significant decrease in mean body weight gain on lactation days 0-7; statistically significant increase in mean body weight gain on lactation day 12-21 in P1 females.

-Statistically significant reduction in the mean final body weights of P1 male and female rats.

-Statistically significant increases in the incidence of dermal lesions observed during clinical observations.

-Incidence of grossly observed skin ulcerations and corresponding incidences of microscopically observed skin ulcerations, inflammation, and acanthosis/hyperkeratosis of the skin of P1 males and F1 male and female rats.

-Statistically significant increase in the incidence of clinical signs related to neurotoxicity were observed in P1 and F1 males and females.

-Increased mortality in F1 male and female rats.

Offspring evaluations:

[3] Evidence of copulation = copulatory plug, found dead pregnant, or delivery of litter.

[4] Including those found dead pregnant during gestation

[5] Determined for each litter. Mean and standard deviation for each dose level were calculated.

[6] Excluding litters sacrificed due to death of dam during lactation.

-Statistically significant decrease in pup survival, and the pup weights of the F1 generation pups.

-Statistically significant increase in the incidence of clinical observations (abnormal gait/mobility, eyes not open, no fur, small whole body, sores, tremors, and weakness).

-Increased mortality in the F1 generation pups

The following effects, observed in P1 or F1 rats fed 100ppm DPX-YB656-84, were considered to be compound related when compared with the respective control group:

Adult evaluations:

-Statistically significant decrease in food consumption of P1 females.

-Statistically significant decrease in the weekly mean body weights, mean final body weights, body weight gain, and food consumption of F1males.

-Statistically significant decrease in the mean body weight of F1 females during premating and gestation.

-Biologically significant increases in the incidence of dermal lesions in F1 males, as well as incidences of grossly observed skin ulcerations and corresponding incidences of microscopically observed skin ulcerations, inflammation, and acanthosis/hyperkeratosis of the skin of F1 males and one F1 female.

Offspring evaluations:

-Statistically significant decreases in the day 21 pup weights of the F1 generation pups.

-Statistically significant decreases in the litter size, and pups weights of the F2 generation pups; an increased incidence of subcutaneous haemorrhage.

The following effects, observed in P1 or F1 rats fed 75 ppm DPX-YB656-84, were considered to be compound related when compared with the respective control group:

Adult evaluations:

-Statistically significant reduction in the mean final body weights of F1 females during premating and gestation.

-Statistically significant reduction in the mean final body weights of F1 male rats.

-Incidences of grossly observed skin ulcerations and corresponding incidences of microscopically observed skin ulcerations, inflammation, or acanthosis/hyperkeratosis of the skin of one P1 male, one P1 female and three F1 male rats.

DISCUSSION AND CONCLUSION

Dose-dependant general toxicity was observed in this study. Previous studies have demonstrated that synthetic pyrethroids, such as DPX-YB656-84, elicit functional and behavioural changes in rodents; those changes include tremors, seizures, abnormal gait, ataxia, hypersensitivity, and skin paraesthesia. The clinical observations have been attributed to a pharmacological effect, in which the compound interacts with sodium channels in the nerve membrane to prolong their opening and transiently increase sodium permeability of the nerve membrane. Those previously described functional behavioural changes are consistent with the toxicity observed predominantly in the high concentration animals of the current study. At weaning of the F1 generation, the observation of tremors, seizures, abnormal gait, ataxia, hypersensitivity, and barrel rolls were strongly and progressively affecting a majority of the animals in the high concentrations group. Therefore the concentration was decreased from 350 to 150 ppm DPX-YB656-84. Dropping the concentrations did cause these observations to

subside. Despite the decrease in concentration, the observation of skin sores and scabs, attributable to skin sensation and paraesthesia, did not decrease, but became progressively worse.

Skin sensations have been reported by occupationally exposed individuals. The sensations are characterized by itching, burning, and tingling of the skin following topical exposure. Observations of alopecia, sores, and scabs were first significantly noted at the weaning of the F1 generation. Prior to weaning, pups are exposed dermally to the chow in the breeding pans and at weaning, rats are generally small enough to fit inside the feed jars, and they appear to spend substantial amounts of time in the feed jars eating.

This behaviour would cause the animals to be exposed to DPX-YB656-84 for substantial amounts of time early in its adulthood. Although it is thought that the cause of the skin sensation is predominantly due to topical exposure, causal systemic exposure can not be ruled out as being in part responsible for the effect. It is not possible to determine the full impact of this early dermal exposure on the effects observed in this study.

It is notable that in the F1 generation observations of alopecia, sores, and scabs in the head and neck region were also noted in the rats fed 100 or 75 ppm DPX.YB656-84. Because these clinical observations jeopardized the life of the rats, vitamin E oil was topically administered daily to the head and neck region of all rats, in an attempt to alleviate the compound-induced skin sensations. Vitamin E oil has been effective as a preventive and ameliorative agent in humans exposed to DPX-YB656-84. The vitamin E oil treatment did not appear to be altogether effective in this study, as it became necessary to terminate the high concentration group due to the severity of the sores and scabs. In fact, the vitamin E oil treatment may have caused slight sores and scabs in the control group. The sores and scabs observed in the F1 generation were classified as slight, moderate, or severe. Although a high number of sores and scabs were observed in all groups, including the control group, it should be stressed that the sores and scabs of the control and low dose groups were primarily classified as slight, and sores of this classification were not life-threatening as were the sores classified as moderate or severe (see Table B.6.6.1–1). Although the treatment with vitamin E oil was not completely effective, it is not considered to have negatively impacted the outcome of the study.

Reproductive toxicity, manifest as decreases in litter size, pup survival, and pup weights, was observed in rats fed 100 ppm and greater. No reproductive toxicity was observed in the rats fed 75 ppm DPX-YB656-84 and there were no compound related effects on mating indices, fertility indices, gestation length, or reproductive organs, regardless concentration.

Table B.6.6.1–1 Summary of dermal effects seen during clinical observations in F1 rats during premating

Concentration ppm	**0**	**75**	**100**	**350/150**
number in group	30	30	30	30
male--alopecia	7	13	21*	27*
total--scabs	16	19	20*	24*
scabs--slight	16	17	16	7
scabs--moderate	0	2	4	12*
scabs--severe	0	0	0	5*
total--sores	12	18	23*	27*
sores--slight	10	14	14	0
sores--moderate	2	2	5	14*
sores--severe	0	2	4*	13*
females--alopecia	10	9	10	28*

total -scabs	6	7	11	28*
scabs--slight	6	7	11	16*
scabs--moderate	0	0	0	12*
scabs--severe	0	0	0	0
total--sores	4	6	6	30*
sores--slight	4	5	5	8
sores--moderate	0	1	1	16*
sores- severe	0	0	0	6*

*Statistically significant trend at $p \leq 0.05$ by Cochran-Armitage test

The observed general toxicity included: decreased body weight, body weight gain, and food consumption; increased incidences in clinical observations indicative of neurotoxicity (e.g. abnormal gait/mobility, ataxia, barrel rolls, hypersensitivity, and 'popcorn' seizures) and dermal lesions observed as alopecia, sores, scabs, and the corresponding gross pathological and microscopic findings of skin ulcerations, inflammation, and acanthosis/hyperkeratosis and finally mortality. Reproductive toxicity was manifested as decreases in litter size, pups survival, and pup weights.

There were no compound-related effects on mating indices, fertility indices, gestation length, or the reproductive organs, regardless of treatment.

Although general compound-related toxicity was observed in rats fed 75 ppm, these effects consisted of very mild decreases in body weight and a few observations of skin lesions. No adverse reproductive effects were observed in rats fed 75 ppm DPX-YB656-84. Therefore, under the conditions of this study, although the overall no-observed-adverse-effect-level (NOAEL) for adult rats and their offspring was not obtained, the reproductive NOAEL for adult rats and their offspring was 75 ppm DPX-YB656-84.

3.9.3.6 [Study 6]

Study	IIA 5.6.1/02 Reproduction study in rats with Esfenvalerate (in pellets diet)
Reference	Anonymous (1999b)
Date performed	Not reported
Test facility	Anonymous
Report reference	No. LLT-0192
Guideline(s)	OECD 415
Deviations from the guideline	None
GLP	Yes.
Test material	Esfenvalerate. Lot no. 60610G, purity 86.0% esfenvalerate (97.3% total fenvalerate isomers)
Study acceptable	Yes

METHODS

In a previous reproduction study of esfenvalerate (Biegel 1994, IIA 5.6.1/01) decreased body weight of F1 generation was noted even at the lowest dose level of 75 ppm, possibly due to skin paraesthesia induced by esfenvalerate mixed in pulverized diet. The present one generation reproduction toxicity study was conducted to determine the no observed effect level (NOEL) for F1 generation when skin paraesthesia is prevented. The dietary mixture of esfenvalerate was pelleted so that dermal exposure to esfenvalerate was minimal.

Dose groups consisted of 4 groups including 3 treatment groups and one control group receiving basal diet only, as shown in Table B.6.6.1-2.

Table B.6.6.1–2 Dose levels

Test substance	Dosage (ppm)
Control*	0
Esfenvalerate	20
Esfenvalerate	40
Esfenvalerate	100

*receiving basal diet (pelleted form) which did not contain the test substance

Twenty four rats/sex of parental (P) generation animals were allocated to each group at the start of treatment. Dosage levels of esfenvalerate were 20, 40 and 100 ppm, and all parental (P) animals received the diet for 70 days prior to mating and throughout mating, gestation and lactation periods until termination. As for the F1 generation, treatment period was for 203-205 days for F1a, and for 105 days for F1b after weaning (following the possible exposure to the test substance *in utero* and through nursing during lactation in the same manner as for P generation).

Dosing formulation (pelleted form) was confirmed to be stable for at least 16 days at room temperature, therefore, the sample was changed every two weeks.

Reason for dosage selection: In a previous dietary reproduction toxicity study (dose levels; 0, 75, 100 and 350/150 ppm), esfenvalerate was administered by mixture of the test substance in pulverized diet. At 75 ppm and higher doses, skin lesions such as sore and scab were observed both in P and F1 generations and body weight in F1 generation was lower than control. At 100 ppm and higher doses, reduced litter size in F1 parental animals, lower viability of F2 pups, and decreased body weights of F1 and F2 pups were noted. In the highest dose group (350/150 ppm), all F1 animals were found dead or euthanized *in extremis* approximately 6 weeks after weaning. Based on these results, the highest dose in the present study was selected to be 100 ppm which was expected to induce some apparent systemic toxicity.

Route of administration and justification: The test article was administered orally as a diet admixture, which is recognized as an efficacious method of absorption. Pelleted diet (*ad libitum*) was used in this present study, because the test substance mixed with diet in pelleted form was expected to be similar in kinetics to the mixture in pulverized form diet, which was used in the previous study. Moreover, the pelleted form was expected not to provoke the skin lesions observed during the previous reproduction study.

Clinical observations, bodyweight, food consumption were recorded throughout the study for P generation and F1 generation post weaning. The P generation mating periods were each 14 days maximum starting from days 71 and 181 of treatment. Parameters assessed in the offspring during lactation were litter size, sex, presence of gross abnormalities, clinical signs and weekly bodyweights.

RESULTS

P generation

General parental toxicity:

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

Bodyweights of both males and females at 100 ppm were slightly reduced on occasions, which were considered to be a treatment-related effects although statistical significance was not achieved when compared with controls.

It should be noted that bodyweight gains of both males and females at 100 ppm were significantly lower than control, which were considered to be a treatment-related effects.

Table B.6.6.1-3 Group mean bodyweights (g) P generation, selected values

Day	Dietary concentration of Esfenvalerate (ppm)							
	Males				Females			
	0	20	40	100	0	20	40	100
Study day 1	376	376	376	376	237	237	237	237
36	521	514	517	507	296	301	295	289
71	601	589	588	579	330	332	328	324
127	661	660	644	655				
197	715	716	703	712				
GD (1st mate) 0					328	318	325	320
14					386	374	380	380
PND (1st mate) 0					362	356	357	357
14					385	375	378	375
77					375	363	376	377
GD (2nd mate) 0					378	358	357	371
14					431	405	403	419
PND (2nd mate)0					423	386	395	400
14					421	399	426	403

GD = gestational day PND = post natal day

Food consumption at 40 ppm in males and 100 ppm in males and females was reduced during the first few days of treatment only; these changes were considered to be attributable to taste aversion which is often observed in an initial phase of the treatment period in dietary studies, and considered to be of no toxicological significance.

The achieved intake of the test substance is shown in Table B.6.6.1-4 below:

Table B.6.6.1-4 Mean and range of the values of chemical intake (mg/kg/day): P generation

P generation	Interval		20 ppm	40 ppm	100 ppm
Male	Treatment period	Weeks 0 - 25 of treatment	0.98 (0.78-1.40)	1.89 (1.49-2.72)	4.74 (3.78-6.97)
Female	**F1a litter**				
	Premating	Weeks 0-10 of treatments	1.38 (1.13-1.60)	2.73 (2.14-3.10)	6.82 (4.94-8.23)
	Gestation	Days 0-21 of gestation	1.08 (0.80-1.37)	2.16 (1.66-2.47).	5.54 (4.13-6.67)
	Lactation	Days 0-14 of lactation	2.14 (1.22-2.98)	4.01 (2.06-5.66)	9.75 (4.56-14.53)
	F1b litter				
	Premating	Week 0-7 of treatment	1.20 (0.97-1.79)	2.24 (1.94-2.83)	5.85 (5.08-6.89)
	Gestation	Days 0-21 of gestation	0.98 (0.82-1.10)	2.07 (1.72-2.25)	4.92 (3.91-5.49)
	Lactation	Days 0-14 of treatment	1.88 (1.06-2.66)	4.05 (2.64-5.66)	8.59 (4.83-13.11)

Reproductive performance:

The mating data are presented in Table B.6.6.1-5

Table B.6.6.1-5 Mating data for P generation

Parameter	Dietary concentration of Esfenvalerate (ppm)							
	P generations males				P generation females			
	0	20	40	100	0	20	40	100
1st mating, producing the F_{1a} generation pups								
No. cohabiting	24	24	24	24	24	24	24	24
No. copulating	24	21	22	23	24	21	22	23
Copulation index (%)	100	87.5	91.7	95.8	100	87.5	91.7	95.8
No. of pregnancies resulting	19	16	18	20	19	16	18	20
Fertility index (%)	79.2	76.2	81.8	87.0	79.2	76.2	81.8	87.0
2nd mating, producing the F_{1b} generation pups								
No. cohabiting	19	15	18	19	19	15	18	19
No. copulating	18	15	15	17	18	15	15	17
Copulation index (%)	94.7	100	83.3	89.5	94.7	100	83.3	89.5
No. of pregnancies resulting	13	10	4	8	13	10	4	8
Fertility index (%)	72.2	66.7	26.7**	47.1	72.2	66.7	26.7**	47.1

Copulation index = (no. of animals with confirmed copulation/no. of animals in cohabitation) x 100

Fertility index = (no. of pregnant females/no. of animals with confirmed copulation) x 100

** significantly different from control, $p<0.01$

In production of F1a pups, the copulation and fertility indices of P generation were comparable to the control and as no statistically significant differences were noted, they were not considered to be affected by treatment.

In production of F1b pups, although the copulation indices of P generation of all groups were comparable to those for F1a, the fertility indices of P generation were lower than expected in all groups including the control group. Statistical significance was attained in the 40 ppm group and the number of dams for F1b pups was reduced and insufficient for evaluation, probably due to the advanced age of the parents as described in "discussion and conclusion". These changes were not considered to be related to treatment because there was no dose response.

Parturition and lactation:

In production of F1a pups, no statistically significant differences were noted in gestation index, duration of pregnancy, number of females with abnormal parturition, number of offspring (total, live or dead) at birth and sex ratio in the treatment groups and therefore, these were not affected by the test substance. In the 100 ppm group, one pregnant female failed to deliver, and necropsy of the animal revealed that the litter was resorbed.

In production of F1b pups, as mentioned above, the fertility index of P generation was generally low, resulting in the decreased number of females which delivered live offspring. The number of females that delivered live offspring was 11, 8, 3 and 8 in the control, 20, 40 and 100 ppm groups, respectively, and was considered to be too small for statistical evaluation. These changes were not considered to be treatment-related, because there was no clear dose-dependency. One female each in the control and in the 20 ppm groups was found dead on day 23 of gestation prior to or during parturition. Part of the litter in that female from the control group was delivered, but the other was not. The whole litter in that 20 ppm female was dead. In addition, one animal each in the control and the 40 ppm groups failed to deliver, and necropsy of these animals revealed that the litter in that control animal included one resorption and one live fetus, and the litter in the 40 ppm animal was resorbed. Furthermore, abnormal parturition was observed in one female in the 20 ppm group. These findings were not considered to be treatment-related, because similar changes were not observed in the 100 ppm group. No treatment-related differences were noted in gestation index, duration of pregnancy, number of females with abnormal parturition, number of offspring (total, live or dead) at birth and sex ratio and therefore, these were not affected by the test substance.

In both F1a and F1b pups delivered, none of the pups was observed with an external anomaly.

Necropsy observations:

There were no treatment-related findings.

F1a generation

Clinical signs and mortality:

In the lactation period of male and female F1 pups, there were no treatment-related deaths in any groups. No abnormal signs were noted in any treatment groups, but in the control group, one male pup showed wound and/or scab, and another male exhibited skin detachment of tail.

After weaning, there were no treatment-related deaths or clinical signs. Wound and/or scab were found in 2, 5, 3 and 6 males in the control, 20, 40 and 100 ppm groups respectively. The incidences in the treatment groups were comparable to those of the control group and therefore, the findings were not considered to be treatment-related. No skin lesions were observed in females in any groups.

Viability:

The values of viability indices on day 0-4 after birth in all treatment groups were similar, and there was no apparent tendency in terms of dose-dependency between the 20 ppm group (96.5%) and the 100 ppm group (95.5%) even though these groups differed by 5 times in concentration. The indices in the treatment groups (95.5-96.5%) were within the historical control range (93.4-99.7%; mean:

98.1%), while in the control value (100.0%) in this study was beyond the range. In the previous reproduction toxicity study, the viability indices on day 0-4 after birth in both F1 and F2 pups at doses high as 100 ppm were similar to or higher than those of the control. Furthermore, there was no treatment-related effect on perinatal mortality and viability index on day 4-21 after birth. The numbers of pups found dead at delivery were 6 (2.6%), 3 (1.5%), 4 (1.7%) and 1 (0.4%) in the control, 20, 40 and 100 ppm groups, respectively. More pups were found dead in the control group than in the treatment groups. Based on these results, the changes in the 40 and 100 ppm groups with statistical significance were considered to be unrelated to treatment.

Body weights:

The pup bodyweight data are presented in Table B.6.6.1-6

Table B.6.6.1-6 Group mean bodyweights (g)- F_{1a} generation offspring

Day post partum	Dietary concentration of Esfenvalerate (ppm)							
	Males				Females			
	0	20	40	100	0	20	40	100
F_{1a} generation offspring								
0	7.0	6.8	6.7	6.4**	6.6	6.4	6.4	5.9**
4	10.8	10.6	10.3	9.5*	10.1	10.1	9.8	8.9*
7	16.7	17.0	16.4	15.3	15.9	16.2	15.7	14.5
14	33.9	34.7	34.2	32.6	32.8	33.6	32.8	31.1
21	57.6	57.8	57.6	55.2	55.3	55.5	55.3	52.0
56	357	347	355	343	227	224	229	209**
105	596	565	597	580	320	320	331	300
224	773	716	777	760	403	398	417	373

* significantly different from control, $p<0.05$ ** significantly different from control, $p<0.01$

Low values of pup body weight in males and females were noted on days 0 and 4 after birth in the 100 ppm group, and these changes were considered to be treatment-related. The pup weights in the 20 and 40 ppm groups during the lactation period were comparable to those of the control group.

After weaning, body weight and body weight gain in females selected as F1a adults were decreased in the 100 ppm group, and were considered to be treatment-related. Body weight and body weight gain of males were not affected.

Food consumption:

For males, there were no treatment-related differences.

For females, decreased food consumption was observed on days 22, 28 and 196 after birth in the 100 ppm group. Although the reductions were only 1 or 2 grams lower than those of the control and transiently noted, it was considered that food consumption in this group was affected by treatment, since these changes were seen only in the highest dose group. No remarkable changes in food consumption were noted in the 20 and 40 ppm groups.

Achieved intake of test substance:

The overall means and ranges of chemical daily intake during the study in the 20, 40 and 100 ppm groups are presented in the Table B.6.6.1–7.

Table B.6.6.1–7 Mean and range of the values of chemical intake (mg/kg/day): F1a generation

F1a generation	Interval		20 ppm	40 ppm	100 ppm
Male	Premating	Weeks 0-29 of treatment	1.23 (0.76-2.94)	2.45 (1.44-5.91)	6.30 (3.71-16.35)

Female	Premating	Weeks 0-29 of treatment	1.41 (0.94-2.97)	2.71 (1.76-6.02)	7.17 (4.65-15.40)

*Necropsy observation*s:

There were no treatment-related findings.

F1b generation

As described before, the number of F1b animals (resulted from reduced fertility of the parental animals) is insufficient for a statistical evaluation.

DISCUSSION AND CONCLUSION

In a previous reproduction toxicity study, as esfenvalerate was administered to the animals as a mixture with pulverized diet, the skin of the animals was possibly exposed to the particles of the test substance, and then, skin paraesthesia, which is commonly known to be induced by pyrethroids, occurred in the treatment groups, resulting in wound and/or scab after scratching. Since it was suggested that skin paraesthesia that occurred in the previous study possibly caused decreased body weight in F1 generation at the lowest dose level of 75 ppm, the NOEL could not be determined. To determine the NOEL for F1 generation rats under the condition of avoiding skin paraesthesia, the pulverized diet admixed with esfenvalerate was formulated in pelleted form in order to minimize the dermal exposure to the test substance in the present study.

There were no dose-dependent increases of the number of animals which exhibited wound and/or scab throughout the present study. The results demonstrated that skin paraesthesia was successfully avoided even at 100 ppm in P and F1 generations by using pelleted diet. Therefore, the appropriate toxicological evaluation of esfenvalerate orally treated in diet was achieved with minimal dermal exposure of animals.

Under the study conditions described above, the actual NOEL was successfully determined in the present study. Body weight and food consumption of males and/or females in P generation were affected at the highest dosage of 100 ppm, but no effect on reproduction was detected even in the 100 ppm group when F1a litter was produced. On the other hand, the endpoints of reproductive performance in production of F1b litter were lower than expected in all groups. Statistically significantly lower fertility index was noted in the 40 ppm group, but not in the highest dosage group of 100 ppm. Although these low fertility values were unusual, these changes were not considered to be treatment-related, due to lack of clear dose dependency. In the present study, the age of the males and females at mating for production of F1b pups was over 35 weeks. In the male rat, the peak reproductive period is reported as between days 100-270 of age (approximately 14-38 weeks of age). Meanwhile, in the female rat, it is known that beginning at 6 months of age, the litter sizes of multiparous rats decrease progressively, and that from the maternal age of 12 months the pregnancy rate is significantly decreased, and from the age of 9 months the litter values are significantly lowered and the resorption rates are increased. These facts indicate that the male and female rats at age of 35 weeks were in some marginal period for the normal reproduction. Therefore, it was strongly suggested that the lower values of the reproductive endpoints at the production of F1b pups were caused by the advanced age of the rats used in the production of F1b pups. Accordingly, it was concluded that esfenvalerate never affected the fertility in the rat up to the dosage level of 100 ppm.

In F1a generations, only slight changes of body weight and food consumption at 100 ppm were considered to be treatment-related and the 20 and 40 ppm groups were not affected by treatment. There was no evidence that the other endpoints including viability were affected by treatment.

In F1b generation, although the numbers of litters and pups were insufficient for statistical evaluation, the results of F1b offspring were considered to be the same as those of F1a.

In conclusion, no effects on P and F1 generations were observed up to 40 ppm, and no reproductive effects were noted up to 100 ppm. Thus, the NOEL for the F1 generation was considered to be 40 ppm (2.45 and 2.71 mg/kg/day for males and females respectively). In addition, it was considered that

the NOEL for P generation animals was 40 ppm (1.89 and 2.73 mg/kg/day, respectively, and the reproductive NOEL was 100 ppm (4.74 and 5.54 mg/kg/day for males and females, respectively). It was demonstrated in this study that skin paraesthesia observed in the previous study was not a systemic effect of the test substance.

3.9.3.7 [Study 7]

Study	IIA 5.10.1/04 Esfenvalerate (DPX-YB656) Technical: 10-Day Hershberger Bioassay for Detecting Androgenic Activity
Reference	Anonymous (2011b)
Date performed	July - September 2011
Test facility	Anonymous
Report reference	LLT-0228 (Lab. ID DuPont-33441)
Guideline(s)	U.S. EPA, OPPTS 890.1400, OECD 441
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Lot DPX-YB656-146, purity 85.7% as Aα
Study acceptable	Yes

METHODS

The androgenic and anti-androgenic effects of esfenvalerate were evaluated using the Hershberger Assay.

Groups of 6 castrated young adult male CD(SD) strain rats were given oral (gavage) doses of 0, 3, 6 or 9 mg/kg bw/day esfenvalerate once daily for 10 consecutive days in an androgenic assay and an anti-androgenic assay. In the anti-androgenic assay the animals were co-administered testosterone propionate (TP) at 0.4 mg/kg bw/day by subcutaneous injection. Positive control compounds were TP (0.4 mg/kg bw/day), an androgen which increases reproductive/endocrine organ weights and flutamide (3 mg/kg bw/day), an anti-androgen which decreases reproductive/endocrine organ weights. The vehicle was corn oil for esfenvalerate, corn oil with 1% ethanol for TP and 0.1% Tween® 80 in 0.5% methylcellulose for FT.

Clinical signs, bodyweights and food consumption were recorded. At termination, 24 hours after the last dose, the ventral prostate, seminal vesicles (including fluid coagulating glands), levator ani-bulbocavernosus muscles (LABC), paired Cowper's glands, the glans penis and the liver were examined and weighed.

RESULTS

There were no treatment-related mortalities.

Neurological clinical signs, including ataxia, abnormal gait, paw shaking and hyperreactivity were observed after dosing in esfenvalerate groups at 9 mg/kg bw/day from day 6 (androgenic study) or day 3 (anti-androgenic study). These signs extended to the 6 mg/kg bw/day group in the latter study. There were no remarkable clinical signs in the positive control groups.

Decreased bodyweight and /or weight gain were observed in esfenvalerate groups at 6 and 9 mg/kg bw/day in the androgenic study but the differences from control were not statistically significant. There were no treatment related effects on bodyweight or body weight gain in the anti-androgenic study or in the positive control groups of both studies.

No gross pathological findings were reported. In both the androgenic and anti-androgenic studies there were no changes in the endocrine/reproductive organ weights in the esfenvalerate treated groups, when compared to the respective vehicle control groups (see Tables B6.8.3-3 to B6.8.3-6). Increased relative liver weight was observed in the esfenvalerate 9 mg/kg bw/day group in the anti-androgenic study. In the positive control (TP) group for the androgenic study, significantly increased

endocrine and reproductive organ weights were observed which were consistent with the known androgenic effects of TP. Conversely, significantly decreased endocrine and reproductive organ weights were observed in the positive control (FT) group for the anti-androgenic study, consistent with the known anti-androgenic properties of FT.

Table B6.8.3-3: Absolute organ weights androgenic study – group mean values

Group/dose level (mg/kg bw/day)	Absolute organ weight (g) (mean ± SD)						
	Final bw	Cowper gland	Glans penis	LABC	Seminal vesicles	Ventral prostate	Liver
Control, corn oil	329.2 ± 17.4	0.0079 ± 0.0026	0.0523 ± 0.0063	0.2102 ± 0.0401	0.1071 ± 0.0219	0.0137 ± 0.0035	13.02 ± 1.62
Esfenvalerate/3	318.9 ± 19.1	0.0066 ± 0.0013	0.0483 ± 0.0068	0.1881 ± 0.0342	0.0903 ± 0.0246	0.0124 ± 0.0023	12.26 ± 1.53
Esfenvalerate/6	315.8 ± 20.5	0.0077 ± 0.0015	0.0522 ± 0.0066	0.2176 ± 0.0272	0.1094 ± 0.0291	0.0159 ± 0.0056	12.57 ± 1.38
Esfenvalerate/9	306.9 ± 23.0	0.0067 ± 0.0026	0.0504 ± 0.0084	0.1900 ± 0.0321	0.0912 ± 0.0239	0.0164 ± 0.0035	12.71 ± 2.18
TP/0.4	342 .6 ± 23.3	0.0460*** ± 0.0080	0.0867* ± 0.0070	0.6117* ± 0.0311	0.9823* ± 0.1204	0.1912* ± 0.0246	14.16 ± 1.91

* $p < 0.05$ *** $p < 0.001$

Table B6.8.3-4: Organ weights relative to final bodyweight androgenic study– group mean values

Group/dose level (mg/kg bw/day)	Relative organ weight (%) (mean ± SD)						
	Final bw	Cowper gland	Glans penis	LABC	Seminal vesicles	Ventral prostate	Liver
Control, corn oil	329.2 ± 17.4	0.0024 ± 0.0009	0.0160 ± 0.0024	0.0642 ± 0.0140	0.0328 ± 0.0083	0.0042 ± 0.0013	3.942 ± 0.302
Esfenvalerate/3	318.9 ± 19.1	0.0021 ± 0.0005	0.0152 ± 0.0027	0.0590 ± 0.0107	0.0284 ± 0.0081	0.0039 ± 0.0007	3.836 ± 0.324
Esfenvalerate/6	315.8 ± 20.5	0.0024 ± 0.0004	0.0167 ± 0.0029	0.0692 ± 0.0099	0.0349 ± 0.0098	0.0050 ± 0.0017	3.970 ± 0.196
Esfenvalerate/9	306.9 ± 23.0	0.0022 ± 0.0008	0.0166 ± 0.0033	0.0624 ± 0.0125	0.0303 ± 0.0103	0.0054 ± 0.0015	4.118 ± 0.429
TP/0.4	342 .6 ± 23.3	0.0135* ± 0.0025	0.0254* ± 0.0028	0.1790* ± 0.0099	0.2893* ± 0.0507	0.0564* ± 0.0108	4.122 ± 0.303

* $p < 0.05$ *** $p < 0.001$

Table B6.8.3-5: Absolute organ weights anti-androgenic study – group mean values

Group / dose level (mg/kg bw/day)	Absolute organ weight (g) (mean ± SD)						
	Final bw	Cowper gland	Glans penis	LABC	Seminal vesicles	Ventral prostate	Liver
Control, corn oil + TP	352.9 ± 16.7	0.00460 ± 0.0072	0.0911 ± 0.0050	0.6932 ± 0.0567	0.9500 ± 0.1425	0.1994 ± 0.0304	14.50 ± 1.40
Esfenvalerate/3 +TP	354.0 ± 18.7	0.0409 ± 0.0035	0.0897 ± 0.0131	0.6544 ± 0.0402	0.9468 ± 0.0579	0.1756 ± 0.0298	15.21 ± 1.21
Esfenvalerate/6 +TP	343.6 ± 21.6	0.0426 ± 0.0064	0.0971 ± 0.0102	0.6192 ± 0.0274	0.9737 ± 0.1349	0.1675 ± 0.0399	14.53 ± 1.90
Esfenvalerate/9 + TP	356.4 ± 25.3	0.0408 ± 0.0129	0.0919 ± 0.0070	0.6274 ± 0.0616	0. 7990 ± 0.1062	0.1666 ± 0.0817	16.41 ± 2.34
FT/3 + TP/0.4	345.2	0.0184***^{NP}	0.0692*	0.3534*	0.2952*	0.0594*^{NP}	14.64

	± 25.6	± 0.0044	± 0.0085	± 0.1087	± 0.0736	± 0.0121	± 1.44

* $p < 0.05$ *** $p < 0.001$

Table B6.8.3-6: Organ weights relative to final bodyweight anti-androgenic study– group mean values

Group / dose level (mg/kg bw/day)	Relative organ weight (%) (mean ± SD)						
	Final bw	Cowper gland	Glans penis	LABC	Seminal vesicles	Ventral prostate	Liver
Control, corn oil + TP	352.9 ± 16.7	0.0131 ± 0.0022	0.0258 ± 0.0015	0.1969 ± 0.0191	0.2696 ± 0.0420	0.0566 ± 0.0084	4.102 ± 0.226
Esfenvalerate/3 +TP	354.0 ± 18.7	0.0116 ± 0.0008	0.0253 ± 0.0029	0.1855 ± 0.0175	0.2678 ± 0.0178	0.0499 ± 0.0097	4.301 ± 0.324
Esfenvalerate/6 +TP	343.6 ± 21.6	0.0125 ± 0.0024	0.0284 ± 0.0035	0.1812 ± 0.0187	0.2851 ± 0.0487	0.0485 ± 0.0105	4.215 ± 0.318
Esfenvalerate/9 + TP	356.4 ± 25.3	0.0116 ± 0.0041	0.0259 ± 0.0033	0.1770 ± 0.0234	0.2259 ± 0.0408	0.0466 ± 0.0223	4.586* ± 0.395
FT/3 + TP/0.4	345.2 ± 25.6	0.0054* ± 0.0014	0.0202* ± 0.0033	0.1017* ± 0.0294	0.0855* ± 0.0202	0.0171* ± 0.0029	4.240 ± 0.249

* $p < 0.05$ *** $p < 0.001$

The performance criteria for acceptable coefficient of variation (CV) were achieved except for the CV of the ventral prostate weight (49%) in the esfenvalerate high dose group in the anti-androgenic study, which exceeded the CV criterion of 40%. However, as this was an isolated finding the study was still considered valid.

CONCLUSION

Esfenvalerate administration to castrated male rats did not cause any treatment related changes in reproductive/endocrine organ weight in the Hershberger Assay. Therefore, it is concluded that esfenvalerate did not exhibit androgenic or anti-androgenic properties under the conditions of this study.

At 6 and 9 mg/kg bw/day, esfenvalerate caused neurological clinical signs, including ataxia, abnormal gait, paw shaking and hyperreactivity. The study NOAEL for neurotoxicity was 3 mg/kg bw/day.

3.9.3.8 [Study 8]

Study	IIA 5.10.1/05 Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats following Oral Administration of Esfenvalerate (DPX-YB656) Technical
Reference	Anonymous (2012a)
Date performed	October - December 2011
Test facility	Anonymous
Report reference	LLT-0230 (Lab. ID DuPont-32088)
Guideline(s)	U.S. EPA, OPPTS 890.1500
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Lot DPX-YB656-146, purity 85.7% as Aα
Study acceptable	Yes

METHODS

The potential effects of esfenvalerate on pubertal development and thyroid function were evaluated using the male pubertal assay.

Groups of 15 juvenile/peripubertal male CD(SD) strain rats were given oral (gavage) doses of 0 (corn oil vehicle only), 3.0 or 9.0 mg/kg bw/day esfenvalerate once daily from post natal day 23 (PND 23) until termination on PND 53 or 54.

Clinical signs and bodyweights were recorded daily. The age of complete balanopreputial separation was assessed from PND 30. At termination all rats were killed and blood was collected for analysis of serum hormone levels (thyroxine (T_4), thyroid stimulating hormone (TSH) and testosterone) and serum chemistry. Weights of endocrine and reproductive organs, liver and kidneys were recorded. Histopathological examination of the testis, epididymis, thyroid and kidney was performed.

RESULTS

There were no treatment-related mortalities.

Neurological signs including tremor, impaired use of or splayed forelimbs/hindlimbs, were observed after dosing in the high dose esfenvalerate group (9 mg/kg bw/day) from PND 23-42, although tremor was confined to the period PND 23 -31. Additional signs observed sporadically included clear material around the mouth at 9.0 mg/kg bw/day and red material.

There were no treatment related effects on bodyweight or bodyweight gain. The coefficient of variation (CV) value (9.32%) for the control group at termination slightly exceeded the laboratory's performance criteria maximum value of 7.47%. However, the mean bodyweights were within the acceptable range and this deviation did not affect the validity of the study.

There were no treatment related effects on balanopreputial separation. The mean ages of attainment of complete balanopreputial separation were 44.3 and 45.3 days in the 3.0 and 9.0 mg/kg bw/day groups, respectively, compared to 44.2 days in the control group. Mean body weights at the age of attainment of balanopreputial separation were 212.6 g and 206.4 g in the same respective groups compared to 211.9 g in the control group. There were also no treatment related changes in males which showed 3 or more days of incomplete separation. The CV value for body weight at the age of attainment of complete balanopreputial separation in the control group (9.71%) was greater than the recommended maximum acceptable value in the performance criteria (7.57%). This was likely attributed to 3 males with incomplete separation for greater than 3 days. In addition, the mean body weight at the age of attainment of complete balanopreputial separation in this group was within the acceptable range; therefore, this deviation did not impact the validity of the study.

There were no treatment related effects on mean serum levels of T_4, TSH or testosterone (Table B6.8.3-7). A slightly lower mean serum testosterone level at 9.0 mg/kg bw/day was not statistically significantly different from the control group mean. This difference was attributed to normal biological variation in the absence of any changes in reproductive organ weights or morphology. The variability in this parameter was reflected in the CV values of 45%, 66% and 73% in the 0, 3.0 and 9.0 mg/kg bw/day groups, respectively, and the top of the acceptable CV range value of 89.7% in the laboratory's performance criteria. Furthermore, the mean serum testosterone value at 3.0 mg/kg bw/day was higher than the control value.

Table B6.8.3-7: Serum hormone levels (ng/mL)– group mean values ± SD

Hormone parameter	Group / dose level (mg/kg bw/day)		
	Control / 0	Esfenvalerate / 3.0	Esfenvalerate / 9.0
Testosterone	2.366 ± 1.0610	2.745 ± 1.8129	1.488 ± 1.0935
Total T_4	5.45 ± 0.755	5.65 ± 0.984	5.17 ± 0.866
TSH	8.28 ± 3.106	8.23 ± 3.406	9.43 ± 7.939

None statistically significant

There were no treatment related effects on serum chemistry parameters. Occasional isolated statistically significant group differences were considered biologically insignificant and were consistent with normal biological variability.

No treatment related gross pathological findings were reported.

There were no treatment related effects on endocrine or reproductive organ weights (Table B6.8.3-8). No statistically significant differences from the control group were noted for any of the absolute or relative organ weights or organ weights adjusted for PND 21 bodyweight as a covariate. Therefore, only the absolute organ weight values are presented in the table. A slightly lower liver weight at 9.0 mg/kg bw/day was considered to be biologically insignificant and consistent with normal biological variability. Slight differences in thyroid, kidney and pituitary weight parameters in the control group compared with the acceptable performance criteria were considered not to have affected the validity of the study. The mean organ weights were within the laboratory's historical control range.

Table B6.8.3-8: Absolute organ weights– group mean values ± SD

Organ weights	Group / dose level (mg/kg bw/day)		
	Control / 0	**Esfenvalerate / 3.0**	**Esfenvalerate / 9.0**
Adrenal glands (mg)	39.7 ± 6.87	38.9 ± 5.52	37.5 ± 5.29
Dorsolateral prostate (mg)	108.1 ± 18.77	109.8 ± 16.17	103.3 ± 23.88
Epididymis, left (mg)	198.5 ± 27.37	195.6 ± 26.95	194.4 ± 20.19
Epididymis, right (mg)	206.3 ± 24.84	200.6 ± 21.41	200.8 ± 20.60
Kidneys (g)	1.88 ± 0.152	1.89 ± 0.140	1.79 ± 0.150
LABC (mg)	496.8 ± 104.24	501.3 ± 83.53	470.3 ± 88.36
Liver (g)	12.66 ± 1.376	12.76 ± 1.383	11.56 ± 1.701
Pituitary (mg)	10.7 ± 1.79	10.9 ± 1.84	10.4 ± 1.48
SV/CG/W/O fluid (mg)	400.8 ± 65.33	410.2 ± 29.84	375.8 ± 49.36
SV/CG/ACC fluid (mg)	659.6 ± 97.79	690.6 ± 100.17	617.9 ± 120.21
Testis, left (mg)	1504.8 ± 189.79	1471.0 ± 196.14	1428.5 ± 77.25
Testis, right (mg)	1570.5 ± 325.53	1461.0 ± 119.86	1454.2 ± 78.59
Thyroids (mg)	10.55 ± 1.890	10.63 ± 3.218	10.19 ± 1.377
Ventral prostate (mg)	228.5 ± 40.08	247.1 ± 43.46	217.8 ± 64.16

None significantly different from control LABC: levator ani plus bulbocavernosus muscles
SV/CG/W/O fluid: seminal vesicle weight including coagulating gland without fluid contents
SV/CG/ACC fluid: seminal vesicle weight including coagulating gland with fluid contents

No treatment related histopathological findings were reported. All findings were considered to be incidental and consistent with the age of the animals.

CONCLUSION

Esfenvalerate administration to juvenile male rats did not cause any treatment related changes in pubertal development, reproductive/endocrine organs or hormone levels in this male pubertal assay conducted to the US EPA guideline.

At the highest dose level tested, 9 mg/kg/day, esfenvalerate elicited neurological clinical signs, including tremor, impaired use of or splayed forelimbs/hindlimbs. The study NOAEL for neurotoxicity was 3 mg/kg bw/day.

3.9.3.9 [Study 9]

Study	IIA 5.10.1/01 Esfenvalerate (DPX-YB656) Technical: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903))
Reference	Anonymous (2012b).
Date performed	December 2011
Test facility	CeeTox, Inc., Kalamazoo, MI 49008, USA
Report reference	LLT-0232 (Laboratory study no. 9144V-100359ERTA)
Guideline(s)	US EPA OPPTS 890.1300, OECD 455 (2009)
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Lot DPX-YB656-146, purity 85.7% as Aα
Study acceptable	Yes

METHODS

Esfenvalerate was tested for its ability to act as an agonist of the human estrogen receptor alpha (hERα) using the hERα-HeLa-9903 cell line in accordance with the US EPA and OECD guidelines. Two independent runs of the assay were conducted.

The vehicle was acetonitrile for esfenvalerate and DMSO for positive control and reference compounds. The stably transfected hERα-HeLa-9903 cell line was used in this study. The cell line was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, 7-6-8 Asagi Saito, Ibaraki-shi, Osaka 567-0085, Japan. The cell line was certified to be free of mycoplasma. The stability of the cell line was monitored by the use of the reference chemicals 17β-estradiol, 17α-estradiol, corticosterone and 17α-methyltestosterone.

Test concentrations of esfenvalerate ranged from $10^{-10.6}$ to $10^{-3.6}$M for both the first and second runs. The highest concentration was the maximum non precipitating or non cytotoxic (< 20% reduction in cell viability) concentration. Stock solutions were analysed separately for stability and concentration verification. The positive control (PC) 17β-estradiol was tested at 1 nM. Reference chemicals were tested in the range 10^{-15} to 10^{-4} M.

The test procedure was as follows. After the 3-hour (minimum) post-seeding incubation, the plates were removed from the incubator and the media was aspirated. 75 µL of fresh media, followed by 75 µL of the 2x concentrated test substance or positive control stock solutions were added to wells containing ~1 X 10^4 cells/well for a final volume of 150 µL/well to yield the final serial concentrations. The plates were incubated in a 5% CO_2 incubator at 37±1°C. The concentrations of acetonitrile and DMSO in the medium were held constant at 0.1% v/v. All concentrations were tested in replicates of 6/plate. In addition, for each concentration, 2 replicates/plate were prepared that incorporated the hERα antagonist ICI 182,780. Replicates incorporating a hERα antagonist allow for the identification of non-specific (i.e., non-hERα-mediated) induction of the luciferase gene as true hERα-mediated induction is inhibited by addition of an antagonist whereas non-specific induction is not. Cell viability was assessed by the propidium iodide (PI) uptake method which yields a fluorescent signal that decreases with a decrease in cell viability. The limit of solubility was determined by visual inspection. hERα-mediated transactivation of luciferase gene expression was evaluated according to a proprietary method.

A complete concentration response curve for each of 4 reference compounds (17β-estradiol, 17α-estradiol, corticosterone and 17α-methyltestosterone) was run each time the transcriptional activation assay was performed.

The results were expressed as the relative transcriptional activity (RTA) compared with the positive control. The mean value from the vehicle control (with or without ICI 182,780) wells or positive control wells was subtracted from each well to normalise the data. The normalised value for each well was divided by the mean value of the normalised PC wells (with the normalised mean of the PC wells

being defined as 100% relative transcriptional activity). The final value for each well was the RTA for that well compared to the mean normalised PC response.

The data were then interpreted according to the following steps:

1. Where appropriate, $logPC_{50}$, $logPC_{10}$, $logEC_{50}$ and Hill slope values were calculated.

2. For the test substance, the maximum response relative to the positive control (RPCMax) was determined. In each individual run of the transcriptional activation assay, if RPCmax was less than 10%, the test substance was considered to have given a negative response for hERα agonism.

3. For each individual run of the transcriptional activation assay, the acceptability of the data was evaluated using the following criteria:

a. The mean normalized luciferase signal of the PC (1 nM 17β-estradiol) should be at least 4-fold that of the mean VC on each plate.
b. The results of the 4 reference chemicals should be within the acceptable ranges.

4. If the acceptability criteria outlined above were met, that run of the transcriptional activation assay was considered to be valid.

5. The test substance was considered negative if RPCMax <10% in at least 2 runs of the transcriptional activation assay. The test substance was considered positive if RPCMax ≥10% in at least 2 runs of the transcriptional activation assay.

RESULTS

Cell viability and precipitation

The cell viability of all esfenvalerate concentrations were > 82% of the vehicle control viability, and were considered acceptable. The concentrations of reference chemicals were also acceptable, except for the highest concentration of corticosterone (10^{-4} M) in the first run (> 20% reduction in cell viability relative to vehicle control) which was excluded from the analysis.

There was no evidence of precipitation with esfenvalerate or the reference chemicals.

Transcriptional activation assay

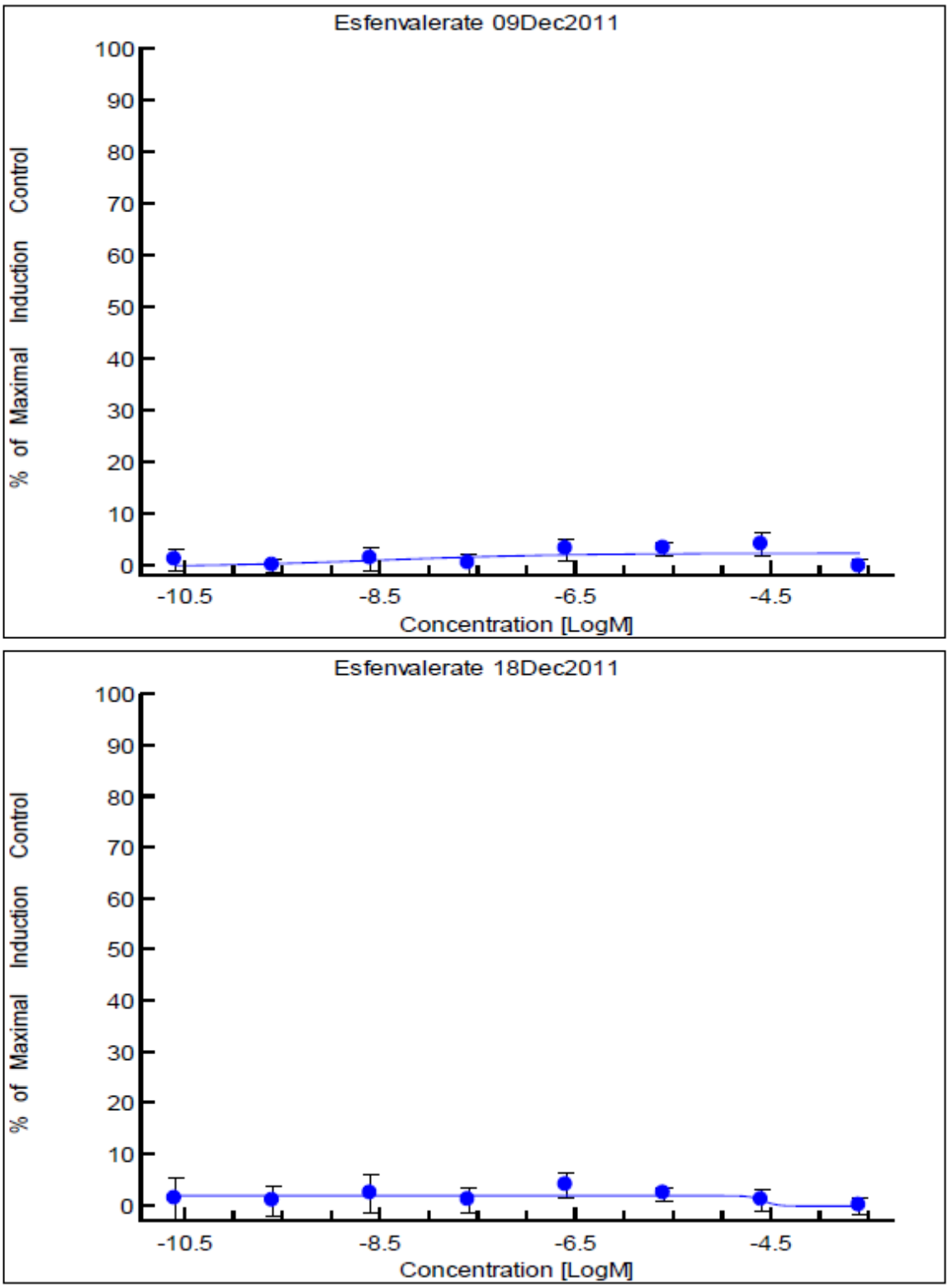
Esfenvalerate did not increase luciferase activity at any of the concentrations tested as the RPCMax mean values were < 10% (4.1 ± 2.3% and 4.0 ± 2.5% in the first and second independent runs of the assay, respectively).

The positive control (1 nM 17β-estradiol) responded as expected with a greater than 4-fold increase in mean luciferase activity compared with the vehicle control. The $logPC_{50}$, $logPC_{10}$, $logEC_{50}$ and Hill slope values for the 4 reference chemicals were within the accepted ranges with the exception of a minor deviation for 17α-methyltestosterone where the $logPC_{10}$ value was marginally higher than the acceptable range and the $logPC_{50}$ value could not be calculated. This minor deviation was considered not to have compromised the validity of the assay.

None of the compounds induced a non-specific (i.e., non-hERα-mediated) induction of the luciferase gene when tested in the presence of the hERα antagonist ICI 182,780.

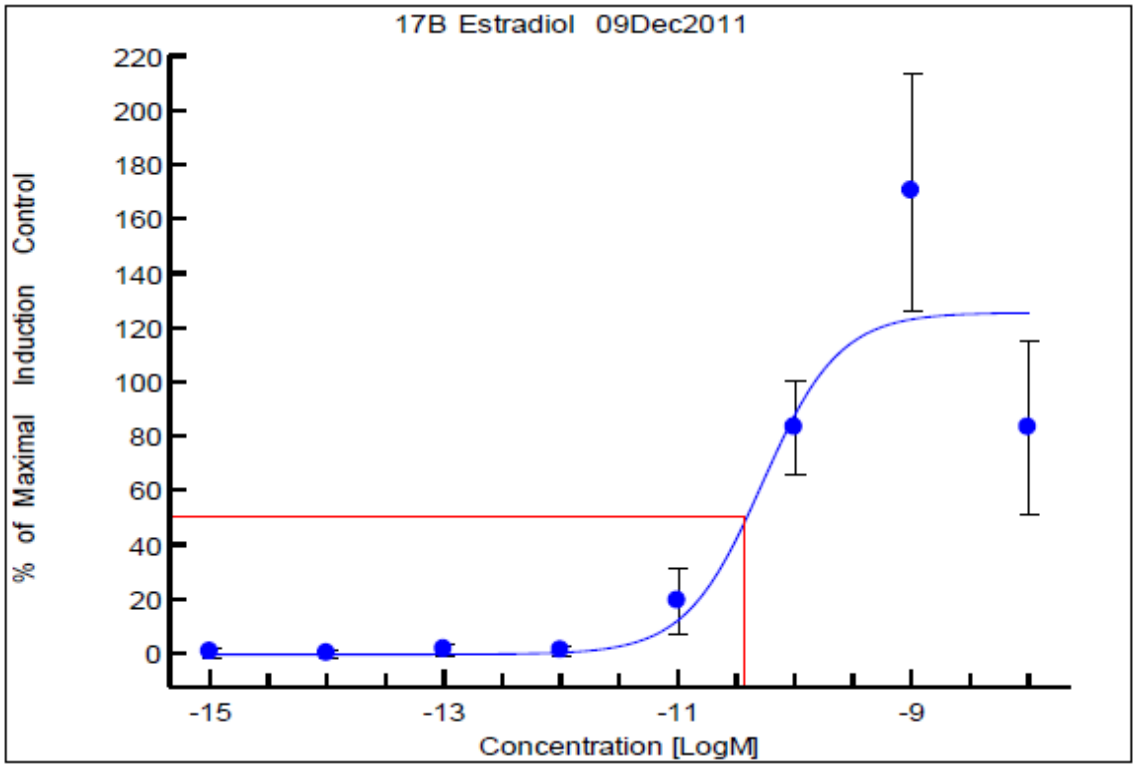
The graphs (Figures B6.8.3-1 to B6.8.3-5) from the study reports are reproduced below because they are more informative than presenting the data tables.

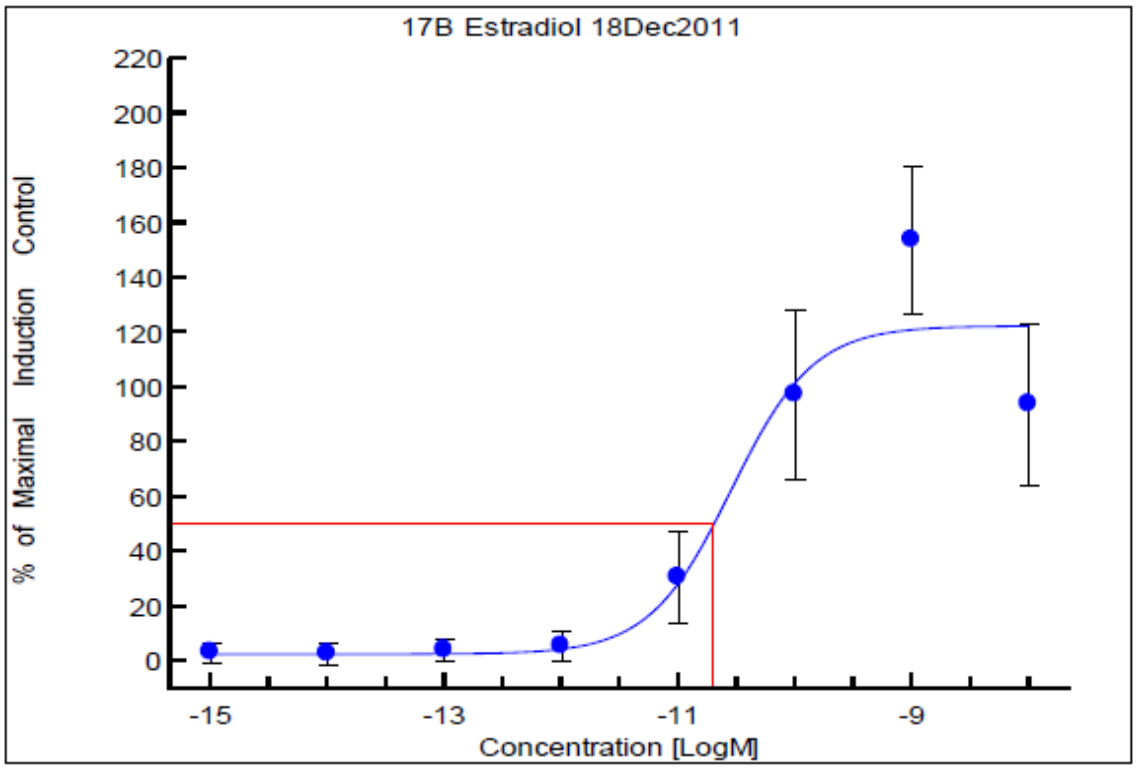
Figure B6.8.3-1: Esfenvalerate - relative transcriptional activity



The two separate graphs represent the data (Means ± standard deviation) from the two different independent runs of the assay in the absence of antagonist (n = 6/concentration).

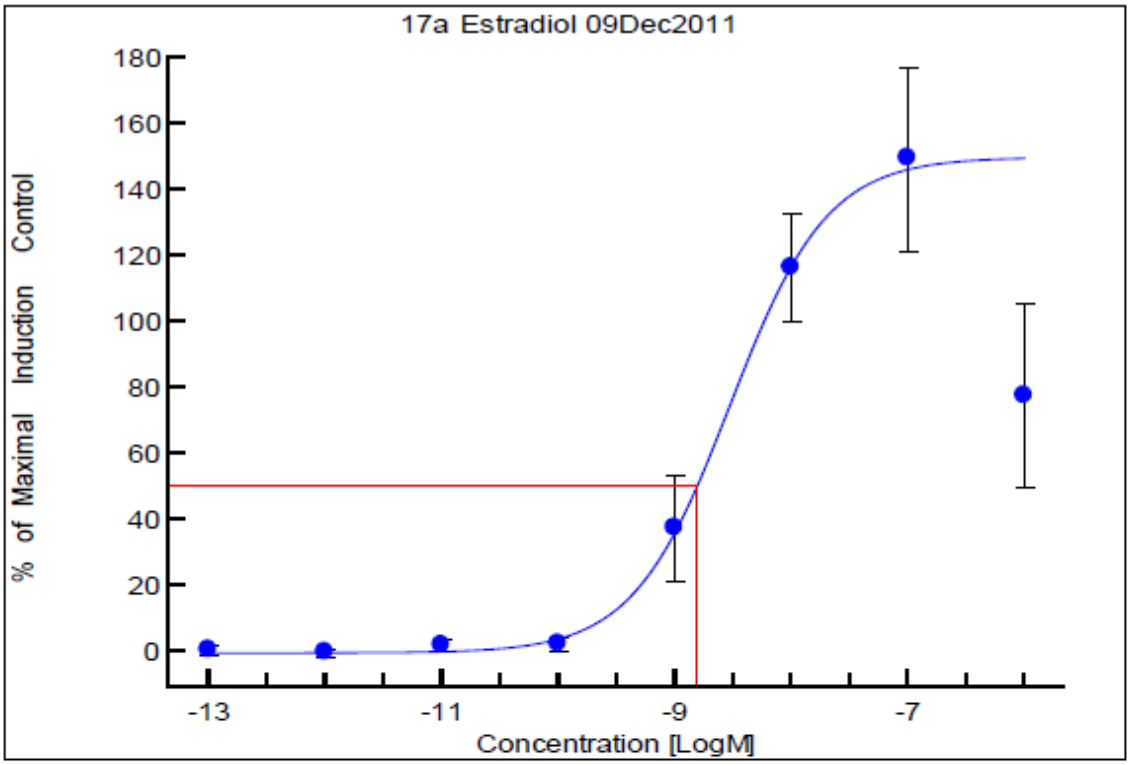
Figure B6.8.3-2: 17β-estradiol - relative transcriptional activity



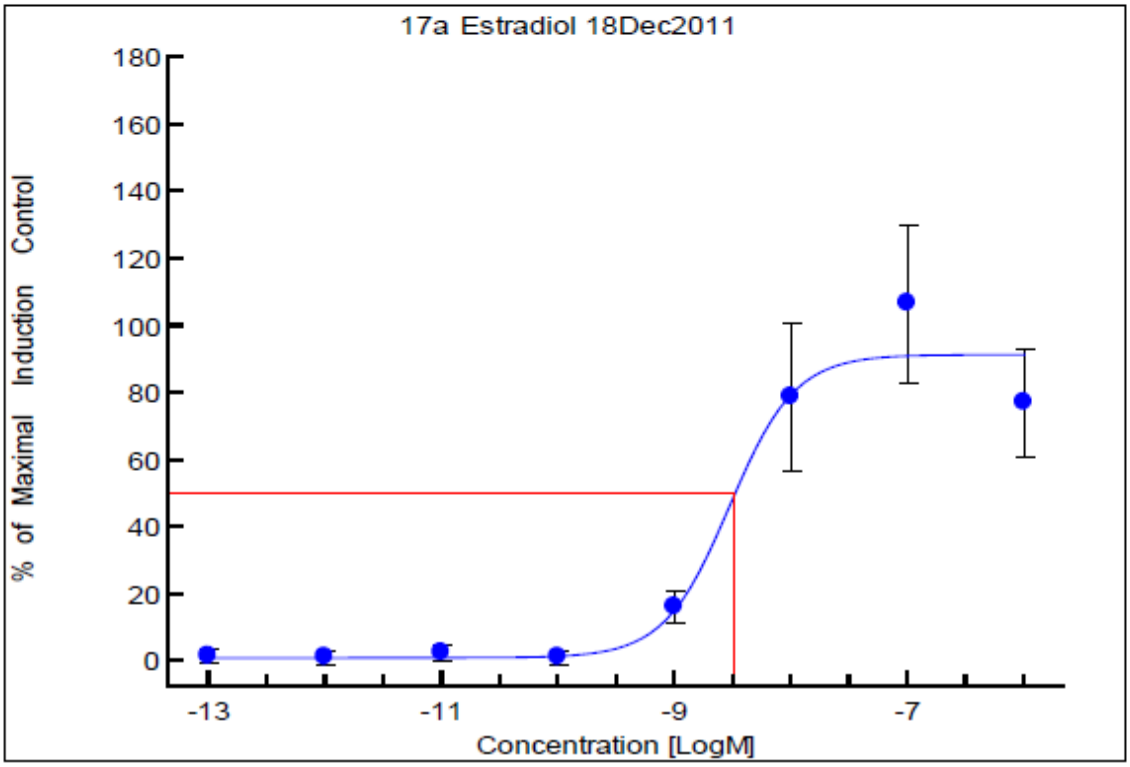


The two separate graphs represent the data (Means ± standard deviation) from the two different independent runs of the assay in the absence of antagonist (n = 6/concentration).

Figure B6.8.3-3: 17α-estradiol - relative transcriptional activity

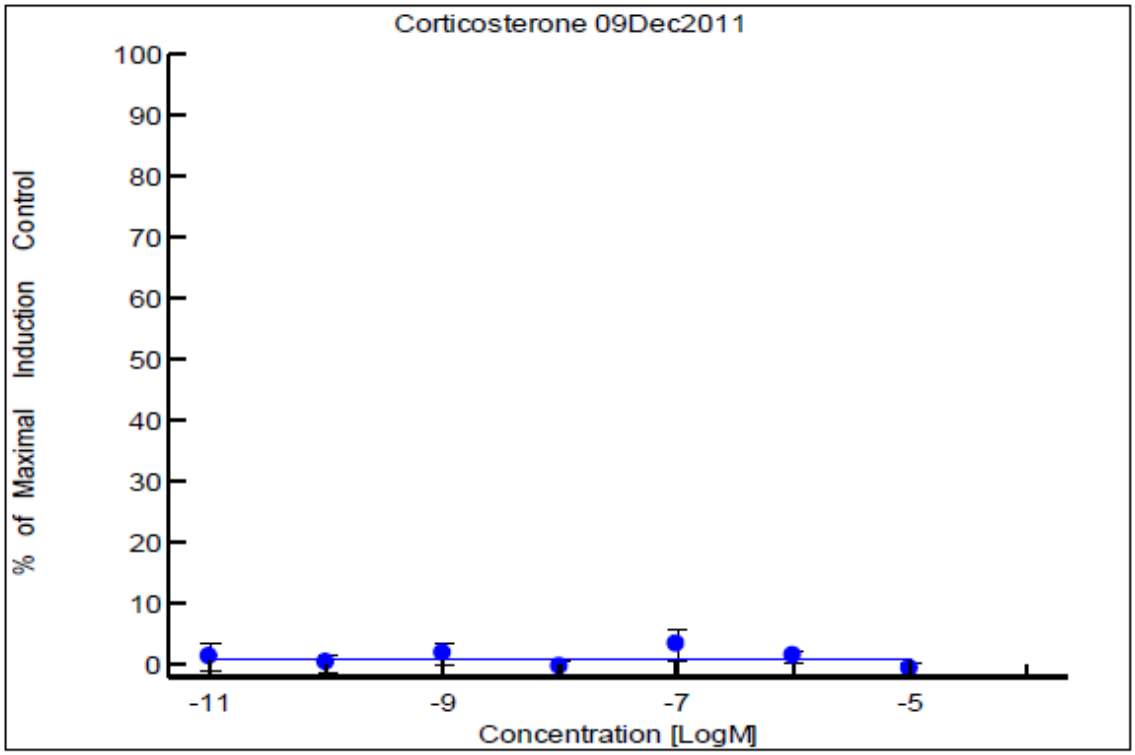


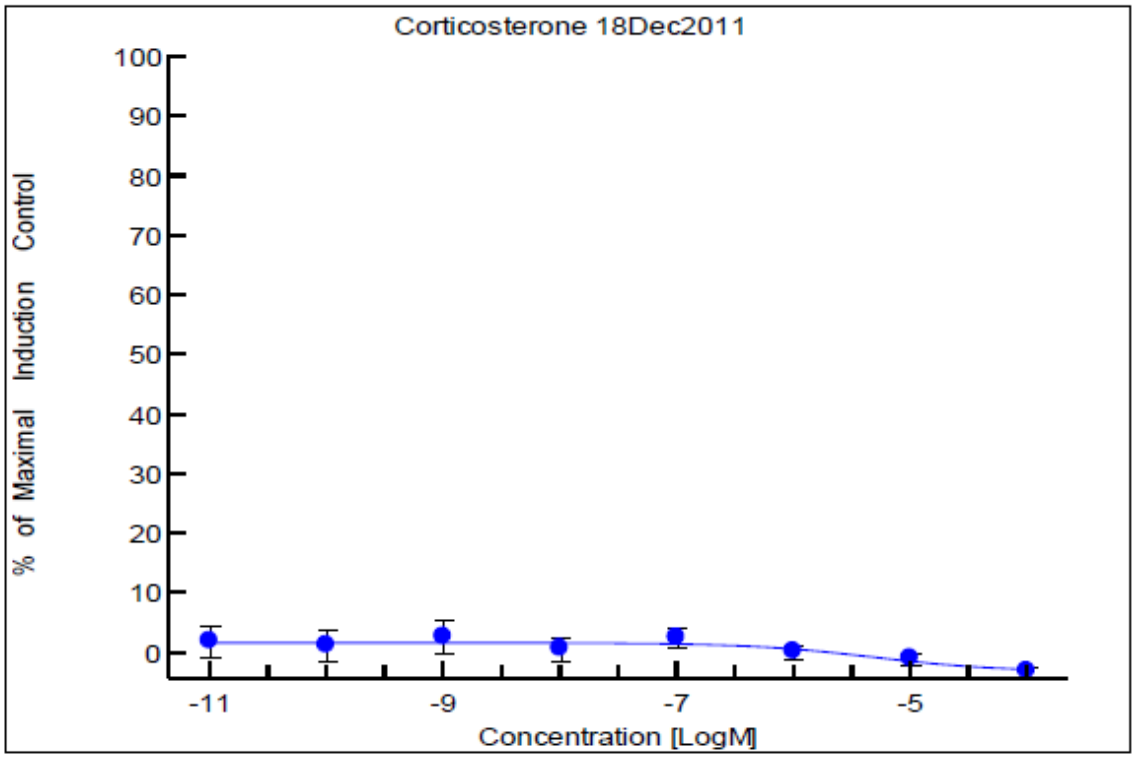
The German-McClure robust curve fitting (available in the XLfit data analysis software used by CeeTox) data set to generate the graph seen above (17α-Estradiol run December 09, 2011).



The two separate graphs represent the data (Means ± standard deviation) from the two different independent runs of the assay in the absence of antagonist (n = 6/concentration).

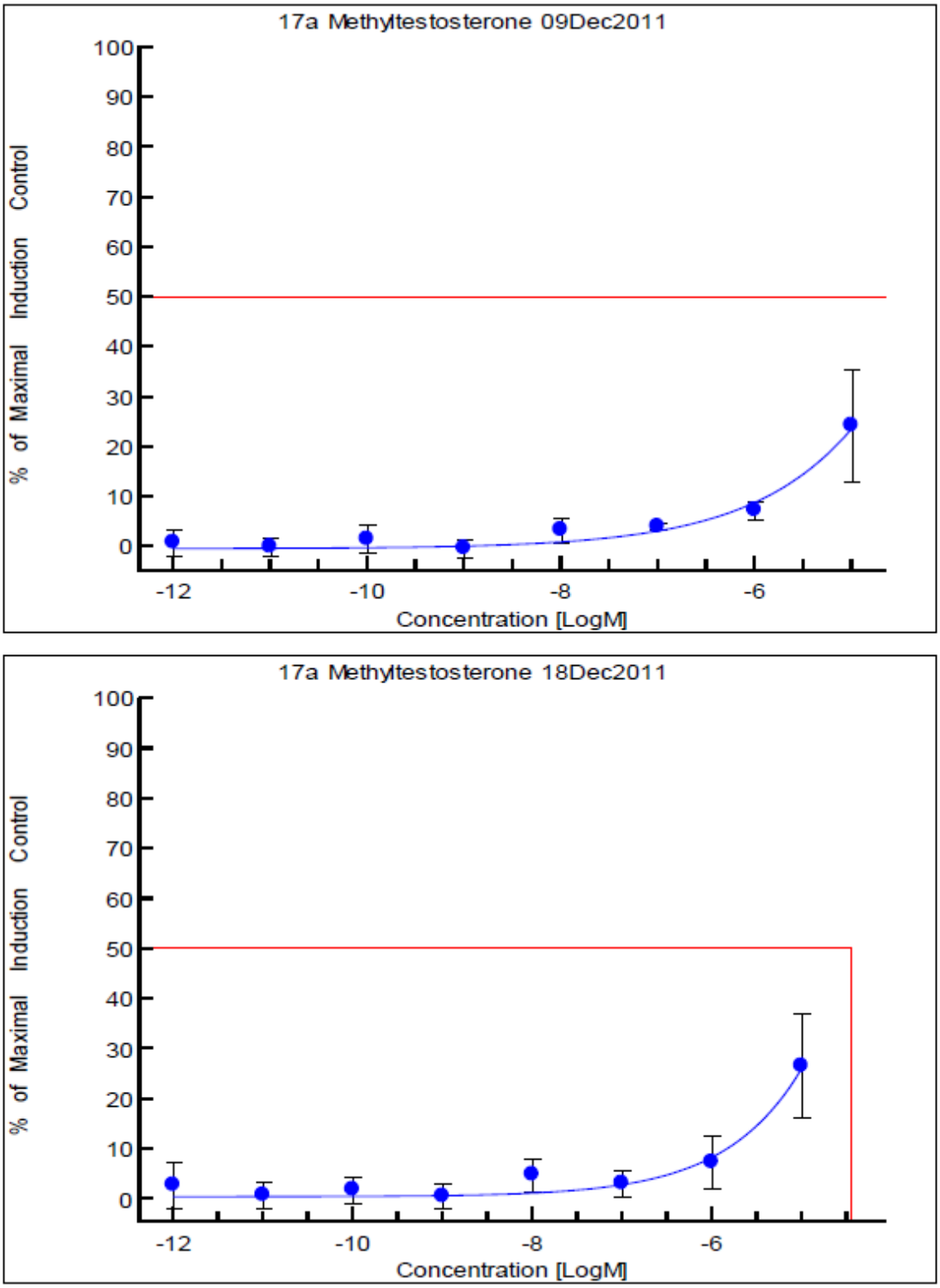
Figure B6.8.3-4: Corticosterone - relative transcriptional activity





The two separate graphs represent the data (Means ± standard deviation) from the two different independent runs of the assay in the absence of antagonist (n = 6/concentration).

Figure B6.8.3-5: 17α-methyltestosterone - relative transcriptional activity



The two separate graphs represent the data (Means ± standard deviation) from the two different independent runs of the assay in the absence of antagonist (n =6/concentration).

CONCLUSION

Esfenvalerate is not an agonist of human estrogen receptor alpha (hERα) in the HeLa-9903 assay.

3.9.3.10 [Study 10]

Study	IIA 5.10.1/02 Esfenvalerate (DPX-YB656) Technical: H295R Steroidogenesis Assay
Reference	Anonymous (2012c)
Date performed	November 2011 – December 2011
Test facility	E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, Delaware, USA.
Report reference	LLT-0233 (Laboratory ID DuPont-32096)
Guideline(s)	US EPA OPPTS 890.155, OECD 456
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Lot DPX-YB656-146, purity 85.7% as Aα
Study acceptable	Yes

METHODS

Esfenvalerate was tested for its potential to interact with the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors (FSHR and LHR) through the production of testosterone and estradiol/estrone via the H295R Steroidogenesis Assay. Three independent trials were conducted.

The positive controls were forskolin, a known inducer of testosterone and 17β-estradiol biosynthesis, and prochloraz, a known inhibitor of testosterone and 17β-estradiol biosynthesis). The vehicle was acetonitrile for esfenvalerate and DMSO for positive controls.

The cell line was H295R human adrenocortical carcinoma cells obtained from ATCC® Manassas, Virginia, USA. The cells were grown in supplemented media containing DMEM:F12 media base with ITS + Premix (insulin, transferrin, selenium, bovine serum albumin, and linoleic acid) and Nu-Serum. Cells were incubated at 37°C in an atmosphere of 5% CO_2.

Test concentrations of esfenvalerate ranged from 0.0001 to 100 µM for each of the 3 trials. Stock solutions were analysed for verification of concentration and stability when stored at room temperature for 6.5 hours. The positive control forskolin was tested at 1and 10 µM. Prochloraz was tested at 0.1 and 1 µM.

The test was conducted as follows. The H295R cells were plated in supplemented media at a density of 300,000 cells/mL in a 24-well plate (1 mL of cell suspension per well) to achieve 50-60% confluency in the wells at 24 hours. The appropriate concentrations of esfenvalerate in acetonitrile or the solvent control were added after the 24-hour pre-incubation of plated cells. The solvent acetonitrile was held constant at 0.05% in all dosing solutions. Quality control plates were dosed with the positive control compounds. The plates were then incubated for a further 48 hours after which they were observed for precipitation, cell viability and signs of cytotoxicity. The media from each well was removed and divided into 2 aliquots, transferred to microcentrifuge tubes, and either analyzed immediately or stored frozen at approximately -60 to -80°C until further processing for hormone measurements. Immediately after removing media, 300 µL of phosphate buffered saline (PBS), with Ca^{2}+ and Mg^{2+} was added to each well to prevent drying and a Live/Dead cell viability test was conducted on each exposure plate using a fluorescence assay (polyanionic dye calcein AM and ethidium homodimer 1(EthD-1)). Analysis of the production of testosterone and 17β-estradiol by H295R cells was conducted by HPLC/MS/MS following extraction of hormones from the media of each well.

The results of the hormone analyses were normalised to the mean solvent control value and then expressed as changes relative to the solvent control in each exposure plate. All doses that exhibited

cytotoxicity greater than 20% or exceeded the limits of solubility (precipitation) were omitted from further evaluation. The data were evaluated for evidence of a dose response. A test substance was judged to potentially affect steroidogenesis if the fold induction or inhibition was statistically different from the solvent control at concentrations falling within the increasing or decreasing portion of the dose-response curve.

RESULTS

Cell viability and precipitation

Cell viability results at all esfenvalerate concentrations were > 80% of the solvent control cell viability and thus considered acceptable. Data at esfenvalerate concentrations of 10 and 100 µM were excluded from further evaluation because of low solubility.

Hormone assays

Exposure of H295R cells to esfenvalerate did not produce concentration dependent changes in the levels of testosterone and 17β-estradiol when compared with the solvent control (Table B6.8.3-1). Statistically significant differences at 0.0001, 0.01 and 1 µM in the testosterone assay and at 0.01 µM in the 17β-estradiol assay were considered not to be indicative of a biologically significant effect in the absence of a dose response, the low magnitude of the difference compared with that of the positive controls, and the intrinsic variability of the data.

The positive control compounds produced the expected responses with increased hormone levels shown by forskolin and decreased hormone levels shown by prochloraz.

The mean hormone data from the three independent trials expressed as the fold difference from the solvent control are summarised in the table below.

Table B6.8.3-1: Fold difference of testosterone and 17β-estradiol concentrations from solvent control (mean ± SD)

Fold difference in hormone levels vs solvent control (mean ± SD)		
Compound / concentration (µM)	**Testosterone**	**17β-estradiol**
Solvent control[1]	1.00 ± 0.086	1.00 ± 0.057
Esfenvalerate / 0.0001	0.851* ± 0.038	0.926 ± 0.045
Esfenvalerate / 0.001	0.913 ± 0.067	0.900 ± 0.033
Esfenvalerate / 0.01	0.904* ± 0.042	0.865* ± 0.105
Esfenvalerate / 0.1	0.957 ± 0.049	0.967 ± 0.044
Esfenvalerate / 1.0	0.904* ± 0.098	0.977 ± 0.069
Positive control data		
Blank	1.079 ± 0.074	1.065 ± 0.090
Solvent control[2]	1.000 ± 0.054	1.000 ± 0.095
Forskolin / 1	1.676 ± 0.133	22.81 ± 2.365
Forskolin / 10	1.713 ± 0.187	42.13 ± 6.773
Prochloraz / 0.1	0.853 ± 0.047	0.771 ± 0.090
Prochloraz / 1.0	0.401 ± 0.044	0.105 ± 0.096

* significantly different from solvent control with Dunnett's test ($p \leq 0.05$)
[1] Acetonitrile [2] DMSO

CONCLUSION

Esfenvalerate did not induce or inhibit steroid biosynthesis when tested at concentrations up to 1 µM, which was the limit of solubility in this steroidogenesis assay.

3.9.3.11 [Study 11]

Study	IIA 5.10.1/03 Esfenvalerate (DPX-YB656) Technical: *In Vitro* Aromatase Inhibition Using Human Recombinant Microsomes.
Reference	Snajdr SI (2011a)
Date performed	September 2011
Test facility	E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, Delaware, USA.
Report reference	LLT-0229 (Lab. ID DuPont-32091)
Guideline(s)	U.S. EPA, OPPTS 890.1200
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, Lot DPX-YB656-146, purity 85.7% as Aα
Study acceptable	Yes

METHODS

The ability of esfenvalerate to inhibit human recombinant microsomal aromatase activity, an enzyme responsible for the conversion of androgens to estrogens, was evaluated in the US EPA guideline *in vitro* aromatase inhibition assay.

The vehicle was acetone for the test substance and DMSO for positive control. To ensure the assay system performed as expected, full enzyme activity controls (FAC) and background activity controls (BAC) were evaluated with acetone as the solvent at the beginning and end of each run. The positive controls were 4-hydroxyandrostenedione (4-OH ASDN), a known aromatase inhibitor and radiolabelled ASDN ([1β-^{3}H]-Androst-4-ene-3,17-dione, [^{3}H]-ASDN (26.3 Ci (0.974 TBq)/mmol): radiochemical purity 99.972%.

A mixture of non-radiolabelled androstenedione (ASDN) and radiolabelled [^{3}H]-ASDN was used to measure the release of tritiated water during the conversion of ASDN to estrone as a direct assessment of aromatase activity. The final substrate solution had a concentration of 2 µM of ASDN (non-radiolabelled and radiolabelled combined), and a concentration of 1 µCi/mL [^{3}H]-ASDN.

Human recombinant microsomes were selected as the test system based on the recommendation from the EPA test guideline. The supplier-provided information for the microsomes was 7.4 mg protein/mL, 290 nmole/min/mg protein cytochrome c reductase activity, and 6.0 pmole product/min/pmole P450 aromatase activity. The initial protein content of the recombinant microsomes was determined to be 7.136 mg/mL prior to analysis. Standards were prepared using bovine serum albumin with concentrations ranging from 0.01-0.1 mg/mL.

The test substance (adjusted for purity, 85.7% active isomer) was evaluated at 8 final concentrations in acetone targeted between 1 x 10^{-10} and 2.5 x 10^{-5} M, which was the limit of solubility achievable in the assay system. Test substance solutions were analyzed by gas chromatography (GC) with a micro-electron capture detector (µECD). All analyses met acceptance criteria. The positive control, 4-OH ASDN was used to verify test system performance at 8 final target concentrations targeted between 1 x 10^{-10} and 1 x 10^{-5} M.

The ability of the test substance to inhibit human recombinant microsomal cytochrome P450 (CYP19) aromatase activity using a mixture of ASDN and [^{3}H]-ASDN as the substrate was evaluated according to the EPA test guideline. The complete assay ("all test components") contained buffer, propylene glycol, microsomal protein, ASDN/[^{3}H]-ASDN substrate and NADPH. To this was added the solvent, test substance or positive control. After pre incubation for 5 minutes at 37ºC, 1000 µL of warmed microsomes were added to initiate the reaction. The reaction was stopped after incubation for 15 minutes at 37ºC and ^3H_2O was extracted by the addition of 3 mL of chloroform. The unbound [^{3}H]-ASDN was extracted with chloroform followed by a charcoal/dextran wash, which has been

historically used at the testing laboratory and is well documented in the literature, rather than 3 extractions with methylene chloride as described in the EPA test guideline. After centrifugation the supernatant was counted for 5 minutes on LSC and aromatase activity was reported as nmol ^3H_2O/g microsomal protein/minute. Three replicates were included for each test substance concentration, 4 replicates for each solvent control or 2 replicates for each positive control concentration, for each of 3 acceptable assay runs.

The concentration that inhibited 50% of maximum radioligand binding (IC_{50}) values was determined as appropriate using Origin 8.5.1 (OriginLab Corp., Northampton, Massachusetts, U.S.A.). ANOVA analysis for the logIC_{50} and slope values was determined using SAS Version 9.2. All statistical significance was at the 0.05 significance level.

RESULTS

Four independent aromatase inhibition runs were performed. In the first run, the initial acetone FAC had an activity of approximately 0.04 nmol ^3H_2O/min/mg protein, which did not meet the performance criteria as described in the test guideline. As a result, this run was excluded from the calculations and a fourth run was performed.

The results are summarised in Table B6.8.3-2. Aromatase activity was reported as % of the mean FAC value. No significant inhibition of aromatase activity was observed for esfenvalerate up to the limit of solubility. Therefore, no IC_{50} was calculated.

The positive control 4-OH ASDN produced a dose related inhibition of aromatase activity. The logIC_{50} values for 4-OH ASDN for each of the 3 reported runs were -7.2, -7.3, and -6.9 logM [^{3}H]-ASDN with an average of -7.2 ± 0.05 logM [^{3}H]-ASDN. The logIC_{50} values for each run were all statistically significantly different from each other.

Table B6.8.3-2: Aromatase activity assay: mean ± SD of the percent activity (nmol/min/mg protein) for each concentration

% aromatase activity (nmol/min/mg protein) (mean ± SD)				
Compound	**Final conc. (M)**	**Run 2 9 Sept 2011**	**Run 3 13 Sept 2011**	**Run 4 15 Sept 2011**
BAC (DMSO)	-	0.03 ± 0.05	-0.05 ± 0.04	0.04 ± 0.01
FAC (DMSO)	-	103.33 ± 1.93	97.32 ± 9.55	102.86 ± 2.05
4-OH ASDN	1×10^{-10}	107.48 ± 3.03	88.39 ± 2.92	101.28 ± 0.54
4-OH ASDN	1×10^{-9}	99.26 ± 3.04	95.15 ± 8.17	96.68 ± 5.19
4-OH ASDN	1×10^{-8}	85.36 ± 2.61	78.57 ± 1.16	91.12 ± 0.74
4-OH ASDN	3×10^{-7}	66.84 ± 1.87	63.01 ± 1.66	78.01 ± 7.74
4-OH ASDN	1×10^{-7}	38.85± 0.21	35.46 ± 0.19	58.96 ± 0.61
4-OH ASDN	3×10^{-6}	18.55 ± 0.48	15.38 ± 0.51	33.42 ± 1.61
4-OH ASDN	1×10^{-6}	7.36 ± 0.16	5.58 ± 0.04	14.60 ± 0.49
4-OH ASDN	1×10^{-5}	0.79 ± 0.13	0.83 ± 0.02	1.81 ± 0.13
BAC (DMSO)	-	-0.03 ± 0.08	0.05 ± 0.09	-0.04 ± 0.02
FAC (DMSO)	-	96.67 ± 9.84	102.68 ± 2.92	97.14 ± 1.53
BAC (acetone)	-	-0.02 ± 0.29	0.09 ± 0.28	-0.06 ± 0.31
FAC (acetone)	-	107.00 ± 3.83	103.88 ± 2.35	101.96 ± 1.66
Esfenvalerate	1×10^{-10}	84.49 ± 6.87	104.38 ± 2.94	89.72 ± 1.30
Esfenvalerate	1×10^{-9}	82.35 ± 7.10	104.85 ± 1.06	92.15 ± 2.59
Esfenvalerate	1×10^{-8}	81.11 ± 2.05	102.46 ± 2.75	90.63 ± 2.22
Esfenvalerate	1×10^{-7}	75.52 ± 3.82	92.34 ± 2.34	87.59 ± 2.67
Esfenvalerate	1×10^{-6}	75.92 ± 2.55	91.19 ± 1.50	83.44 ± 4.33

Esfenvalerate	3×10^{-6}	80.79 ± 3.25	92.01 ± 0.28	83.38 ± 5.34
Esfenvalerate	1×10^{-5}	78.42 ± 1.38	91.95 ± 3.09	83.56 ± 3.61
Esfenvalerate	2.5×10^{-5}	74.31 ± 9.06	86.56 ± 3.31	88.19 ± 3.00
BAC (acetone)	-	0.02 ± 0.14	-0.09 ± 0.30	0.06 ± 0.15
FAC (acetone)	-	93.00 ± 3.34	96.12 ± 0.42	98.04 ± 3.23

With the exception of run 1, all runs were within or very close to the suggested performance criteria, and therefore the assay was considered valid. Minor deviations from the guideline did not affect the integrity of the study.

CONCLUSION

Esfenvalerate did not inhibit aromatase activity when tested up to the limit of solubility in this human recombinant microsomal aromatase inhibition assay.

3.10 Reproductive toxicity

Not applicable.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

Note: study summaries of the acute oral toxicity studies in the rat (Anonymous, 1985a) and mouse (Anonymous, 1986a), and the acute inhalation toxicity study in the rat (Anonymous, 1985f) are summarised in sections 3.1 and 3.3, respectively.

3.11.1.1 [Study 1]

Study	IIA 5.7.1/001 Acute oral neurotoxicity study in rats
Reference	Anonymous (2000a)
Date performed	March 2000
Test facility	Anonymous
Report reference	LLT-0206 (Lab. Project ID DuPont-3874)
Guideline(s)	OECD 424
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate technical (also referred to as Asana technical). Batch no. DPX-YB656-84, purity 98.58% (as sum of all isomers)
Study acceptable	Yes

METHODS

Sprague-Dawley CD®(SD)(IGS)BR rats, about 53 days old on the day of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.7.1–1: Study design

Test group	Dose level of Esfenvalerate (mg/kg)	Number of animals	
		Males	Females

1	0 (corn oil vehicle control)	10	10
2	1.75	10	10
3	1.90	10	10
4	20	10	10
5	80	10	10

All selected rats had normal weight gains and clinical signs prior to dosing. The animals received a single gavage dose of esfenvalerate dissolved in corn oil and were observed for up to 16 days. Analysed samples of dosing formulations used on the study were all within 13% of nominal, demonstrating that the achieved concentrations, homogeneity and stability were satisfactory.

General clinical observations were recorded daily. Bodyweights were determined on study days 1 (day of dosing), 2, 8 and 15. Food consumption was monitored throughout the study period. A standard functional observational battery (observations made in home cage, while being handled and in open field, sensorimotor tests) was conducted prior to dosing, and again on study days 1 (7-8 h after dosing), 8 and 15. Motor activity measurements were conducted on the same days as the FOB.

The animals were killed on study days 16 or 17. Major organs were examined macroscopically at a necropsy. Six animals/sex/group were randomly selected for whole body perfusion and neuropathology. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were dissected, sampled and preserved. Only tissue samples from the control and high dose groups were examined microscopically.

RESULTS

There were no treatment related mortalities.

Clinical signs of toxicity were observed in males and females at 80 mg/kg. These signs were similar to those observed at the day 1 FOB (see below), and included abnormal gait and mobility, salivation, skin stains and diarrhoea, reported mainly 1-2 days after dosing; these signs had resolved by day 4.

Treatment related-FOB findings were present only at the day 1 assessment, as summarised in Table B.6.7.1-2. The following signs considered to be treatment related were present at 80 mg/kg: soiled fur, salivation, tremors, uncoordination, stereotypical grooming, abnormal gait, diarrhoea and paw shaking in both genders, together with slow righting reflex in males and increased reaction to touch or tail pinch in females. Also, stereotypical grooming and tremors were seen in occasional animals at 20 mg/kg and tremors were present in one female at 1.9 mg/kg; these observations are considered to be treatment related. Additionally, forelimb grip strength and hindlimb footsplay were significantly decreased in females at 80 mg/kg.

Table B.6.7.1–2 Selected FOB findings on day 1: signs (number of affected animals), forelimb grip strength (kg) and hindlimb footsplay (cm)

FOB obaservation	Dose level of Esfenvalerate (mg/kg)									
	Males					Females				
	0	1.75	1.9	20	80	0	1.75	1.9	20	80
Soiled fur (n)	0	0	1	1	7*	0	0	0	0	1
Salivation (n)	0	0	0	0	1	0	0	0	0	1
Slow righting reflex (n)	0	0	0	0	1	0	0	0	0	0
Tremors (n)	0	0	0	1	2*	0	0	1	2	9*
Uncoordinated (n)	0	0	0	0	1	0	0	0	0	3
Stereotypical grooming (n)	0	0	0	1	1	0	0	0	1	1
Abnormal gait (n)	0	0	1	0	4*	0	0	0	0	7*
Diarrhoea (n)	5	1	4	7	10*	1	0	0	2	5*
Paw shaking (n)	0	0	0	0	2*	0	0	0	0	4*
Increased reaction to touch (n)	0	0	0	0	0	1	0	0	1	3*
Increased reaction to tail pinch (n)	0	0	0	0	0	0	0	0	0	2
Forelimb grip strength (kg)	0.73	0.72	0.86	0.69	0.65	0.69	0.71	0.65	0.67	0.46*
Hindlimb footsplay (cm)	7.1	7.6	8.1	5.9	6.3	6.6	6.9	6.6	5.7	4.9*

* significantly different from control, $p<0.05$

The motor activity measurements revealed differences considered to be treatment-related only at the day 1 assessment, as summarised in Table B.6.7.1-3. For males motor activity, measured as duration of movement and numbers of movements was decreased during the 1st 10 minute interval, and slightly reduced over the entire 60 minute observation period. For females, the number of movements in the 1st 10 minute interval was significantly lower than controls, but this is considered not to be a treatment-related change as a dose-related response was not present. However, the number of movements for females at 80 mg/kg was significantly reduced in the 2nd 10 minute interval and slightly reduced over the entire 60 minute observation period, differences which probably are treatment-related.

Table B.6.7.1–3 Selected motor activity assessment findings on day 1: mean duration of movements (sec) and mean number of movements

Motor activity finding	Dose level of Esfenvalerate (mg/kg)									
	Males					Females				
	0	1.75	1.9	20	80	0	1.75	1.9	20	80
Duration: 1st 10 min. interval	316	334	339	286	229*	332	296	343	319	259
Duration: over 60 min. period	847	664	916	722	703	928	840	1086	910	750
Number: 1st 10 min. interval	123	137	126	122	119	144	142	127*	136*	126*
Number: 2nd 10 min. interval	112	102	105	94	99	131	136	123	114	88*
Number: over 60 min. period	430	346	419	419	465	533	511	514	496	422

* significantly different from control, $p<0.05$

Group mean bodyweights are presented in Table B.6.7.1-4. Transient reductions in bodyweight after dosing were observed for both genders at 80 mg/kg and males at 20 mg/kg. The statistical analysis of female bodyweight revealed significantly lower gains over the entire study period at 1.9, 20 and 80 mg/kg, but in the absence of a dose dependant response this was considered to be a chance finding.

Table B.6.7.1–4 Group mean bodyweights (g) and bodyweight gains (g)

Day	Dose level of Esfenvalerate (mg/kg)									
	Males					Females				
	0	1.75	1.9	20	80	0	1.75	1.9	20	80
Bodywt. d 1	266	268	270	271	269	187	186	187	189	189
Bodywt. d 2	266	270	272	270	252	188	189	188	189	187
Bodywt.. d 8	309	314	314	315	306	208	205	202	201	204
Bodywt. d 15	348	360	357	360	350	226	219	215	215	219
Gain d 1-2	0.4	2.6	2.4	-1.2	-17.0*	0.2	3.0	0.8	0.2	-2.1
Gain d 2-8	42.6	44.0	41.9	44.9	53.2*	20.3	16.3	14.3	11.6*	17.3
Gain 8-15	38.8	45.8	42.5	45.3	44.3	18.2	13.2	12.6	14.3	14.5
Gain d 1-15	81.8	92.4	86.8	89.0	80.4	38.7	32.5	27.7*	26.2*	29.7*

* significantly different from control, p<0.05

The only treatment-related effect on food consumption was a transient reduction after dosing in males at the highest dose level, as shown in Table B.6.7.1-5.

Table B.6.7.1–5 Group mean food consumption (g/animal/day)

Day	Dose level of Esfenvalerate (mg/kg)									
	Males					Females				
	0	1.75	1.9	20	80	0	1.75	1.9	20	80
1-2	17.7	18.7	20.0	16.5	9.4*	11.5	13.4	11.3	12.2	12.0
2-8	25.6	25.6	25.1	26.0	25.6	18.0	17.5	17.3	17.1	17.4
8-15	26.3	27.2	26.5	27.5	28.0	19.0	18.0	18.1	17.7	17.7
1-15	25.6	25.9	25.4	26.1	25.6	18.0	17.5	17.3	17.1	17.2

*significantly different from control, p<0.05

There were no treatment related macroscopic necropsy findings. The microscopic examination of the nervous system tissues did not reveal any treatment related changes.

CONCLUSION

A single dose of esfenvalerate caused an number transient changes, namely changes in clinical condition (including soiled fur, salivation, tremors, uncoordination, stereotypical grooming, abnormal gait, diarrhoea, paw shaking in both genders, slow righting reflex and increased reaction to touch or tail pinch), reduced motor activity, reduced forelimb grip strength and hindlimb footsplay, reduced bodyweight gain and reduced food consumption. These effects were present mainly at the high dose level of 80 mg/kg, but some effects were present at 20 mg/kg and tremors were observed in one female at 1.9 mg/kg. However, there were no microscopic neurological lesions.

Overall, study NOAELs of 1.9 mg/kg for males and 1.75 mg/kg for females were identified.

3.11.1.2 [Study 2]

Study	IIA 5.7.1/002 Comparative neurotoxicity of S-1844 and S-5602: effects of single oral administration
Reference	Anonymous (1985e)
Date performed	July – August 1985
Test facility	Sumitomo Chemical Co., Ltd., Japan
Report reference	Report no. LLT-50-0003
Guideline(s)	Similar to OECD 424
Deviations from the guideline	Detailed clinical observations and functional testing appeared not to be as comprehensive as specified in the test guideline
GLP	GLP status not reported

Test material	S-5602 (fenvalerate, purity 95.5% as sum of all isomers, batch no. LH028); S-1844 (esfenvalerate, purity 94.5 as sum of all isomers batch no. PKG-85306). Information of purity & batch numbers provided by Applicant June 2013.
Study acceptable	Yes

METHODS

The neurotoxicity potential of esfenvalerate (S-1844) was tested in CD rats by single oral administration in comparison with fenvalerate (S-5602). Groups of male and female rats were orally administered with 5, 20 and 90 mg/kg of S-1844 in corn oil as well as with 20, 80 and 360 mg/kg of S-5602 in corn oil. The animals were observed for 2 weeks after dosing.

Clinical observations, body weight measurements, functional testing using inclined plane (slip angle test) were conducted during the observation period. All animals, whether found dead or sacrificed at termination, were subject to light microscopic observation for nerve tissues.

RESULTS

Eight animals, two males and one female receiving 90 mg/kg S-1844 and one male and four females receiving 360 mg/kg S-5602, were found dead within 24 hours after dosing. Toxic signs from 2 hours after dosing such as muscular fibrillation, hunched posture and ataxia were observed in the intermediate and high dose groups of both compounds; tremor and limb paralysis were also observed in some animals from the high dose groups. Clinical signs of toxicity had disappeared by 2 days after dosing. No toxic signs related to be compound-treatment were observed in any rat dosed with 5 mg/kg S-1844 and 20 mg/kg S-5602.

Body weight depression was observed in males and females receiving 90 mg/kg S-1844 and 360 mg/kg S-5602. Slip angle test did not reveal any functional deficit in the treated groups.

Slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves were recognized in many males and females treated with 90 mg/kg S-1844 and 360 mg/kg S-5602 on histopathology. There were no remarkable differences in the incidence and severity of nerve damages between high dose groups of S-1844 and S-5602. There was a relationship between the esfenvalerate content and the dose required to produce clinical signs or nerve lesions in peripheral nerves (the Aα isomer content of esfenvalerate was approximately four-fold higher than fenvalerate). No remarkable changes in other nervous tissues were observed in any rat of the low and intermediate groups of both compounds.

CONCLUSION

It was demonstrated that both compounds caused peripheral nerve lesions at lethal doses, but did not produce the pathological effect at the non-lethal dosages where the clinical signs were clearly noticed.

3.11.2 Human data

No relevant data available.

3.11.3 Other data

3.11.3.1 [Study 1]

Study	IIA 5.2.2/01 Acute dermal toxicity of S-1844 in rats
Reference	Anonymous (1985m)
Date performed	May-July 1985
Test facility	Sumitomo Chemical Co. Ltd.

Report reference	Report nº LLT-50-0006
Guideline(s)	OECD 402
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (S-1844). Lot PKG-85036, purity 94.5% as sum of all isomers.
Study acceptable	Yes

METHODS

Esfenvalerate (S-1844; ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity as total isomer was 94.5%) dissolved in corn oil was dermally applied in concentrations of 500, 1000, 2000, 3200 and 5000 mg/kg to ten male and ten female Sprague Dawley rats, for each dosage group, and the animals received food and water ad libitum except for a period of 20 hours before dosing and four hours after dosing. The a.i. was kept in contact with the skin by means of an occlusive dressing which was removed after 24 hours. The skin was cleaned with diethyl ether and the animals were observed for mortality and signs of toxicity after 10 and 30 min., 1, 2 and 4 hours after treatment and daily thereafter for a period of 14 days. Body weights were recorded at the beginning, mid point and end of the study; at the end also, the rats were sacrificed and subjected to a gross mortem examination. Statistical method: T-test for the comparison of body weights, and the Fischer's Exact probability test for the incidence of gross pathological findings.

RESULTS

There were no mortalities at any concentration of the test material. Signs of toxicity in animals exposed to dose levels of 2000 mg/kg and above included, muscular fibrillation, decrease of spontaneous activity, ataxia, irregular respiration and urinary incontinence.

Muscular fibrillation was also observed in animals exposed to 1000 mg/kg. These signs of toxicity developed 2 - 4 hours after application, but had disappeared within eight days. The mean body weights and/or the mean body weight gains for male rats treated at 2000 mg/kg and above were slightly lower than in the control group. There were no significant treatment-related findings at post mortem, and no evidence of skin-irritation at the application sites.

The acute dermal LD_{50} value of esfenvalerate in both male and female rats was found to be greater than 5000 mg/kg. The NOEL was 500 mg/kg.

CONCLUSION

Esfenvalerate is of low acute dermal toxicity to rats in this study.

3.11.3.2 [Study 2]

Study	IIA 5.2.2/02 Acute dermal toxicity of MO70616 in the rabbit
Reference	Anonymous (1985j)
Date performed	November 1985
Test facility	Anonymous
Report reference	Report nº LLT-61-0041
Guideline(s)	OECD 402
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate (MO70616; 83.7 a.i.). Batch number not reported.
Study acceptable	Study judged to be acceptable at original EU review. However, at the AIR2 Renewal Review this study was considered to be unacceptable because of 20% mortality in the vehicle (water) control group

METHODS

Esfenvalerate (MO70616, 83,7% a.i.) undiluted was applied dermally at a concentration of 2000 mg/kg to groups of five male and five female New Zealand White Rabbits; to a further group of five female control animals 2000 mg/kg of deionised water was dermally applied. The test substance was kept in contact with the skin by means of an occlusive dressing during 24 hours. Food and water were available ad libitum throughout the study.

Body weights were recorded at the beginning, midpoint and end of the study. The animals were observed for mortality and signs of toxicity at 1, 2, 4, 6 and 24 hours after treatment and at least twice daily thereafter for a period of 14 days. At the end of the study the animals were sacrificed and subjected to a gross post mortem examination. Statistical method - none.

RESULTS

There was 20% mortality in the group of control animals and 10% mortality in the esfenvalerate treated group of rabbits. However, these were not believed to be treatment-related. Signs of toxicity included decreased activity, ataxia, body tremors, constricted pupils, decreased defecation and urination, diarrhoea, emaciation, muscle tremors, poor hindlimb co-ordination and small faeces. Very slight erythema and very slight to slight oedema was observed in rabbits 24 hours after application of the test material, however, all signs of irritation has disappeared after seven days. Body weights of treated animals were comparable to those of the control group.

At post mortem, the animal that died in the treated group showed signs of diarrhoea, emaciation, nasal discharge, salivation, gastrointestinal tract distended with gas and discoloration of the gastrointestinal tract. One other animal showed signs of diarrhoea, and although these findings were possibly related to treatment, similar symptoms were observed in the control animals that died during the study.

The acute dermal LD_{50} of esfenvalerate to both male and female rabbits was found to be greater than 2000 mg/kg.

CONCLUSION

Esfenvalerate was of low acute dermal toxicity to rabbits.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

Note: study summaries of the 90-day studies in the rat (Anonymous, 1984a and Anonymous, 1987), 21-day dermal study in the rat (Anonymous, 2000b), the 2-year study in the rat (Anonymous, 2011a), 18-month study in the mouse (Anonymous, 1997), 2-generation and 1-generation studies in the rat (Anonymous, 1994 and Anonymous, 1999b) are presented in section 3.9.1.

Additional studies relevant for STOT RE are summarised below.

3.12.1.1 [Study 1]

Study	IIA 5.3.2/03 Comparative subacute toxicity in B6C3F1 mice treated with S-1844 and S-5602 for 3 months.
Reference	Anonymous (1985h)
Date performed	April – July 1985
Test facility	Sumitomo Chemical Co. Ltd.
Report reference	Report no. LLT-50-0004
Guideline(s)	OECD 408
Deviations from the guideline	None
GLP	Yes
Test material	S-1844 (esfenvalerate, lot PKG-85036, purity 94.5%) and S-5602 (fenvalerate, lot 41028, 95.5%). Batch number not reported.
Study acceptable	Yes

METHODS

This study was performed in order to evaluate the subacute toxicity of S-1844 (esfenvalerate) and to compare its toxicity with S-5602 (fenvalerate). S-1844 had a ratio of optical isomers, Aα : Aβ : Bα : Bβ = 87.2 : 7.4 : 4.8 : 0.6; the purity of total isomer was 94.5%; S-5602 had a ratio of optical isomers, Aα : Aβ : Bα : Bβ = 24.2 : 25.4 : 26.3 : 24.1; the purity of total isomer was 95.5%.

Groups of 12 male and 12 female B6C3F1 mice (SPF)/dose were fed diets containing 0, 50, 150 or 500 ppm S-1844 for 90 days (these doses were selected according to a preliminary study in mice -not presented- where clinical signs such as hypersensitivity and alopecia, depression of body weight and decreased concentration of plasma glucose and cholesterol were observed for the group of 500 ppm). An additional group of 12 male and 12 female B6C3F1 mice was fed the diet containing 2000 ppm S-5602 (in order to compare S-1844 to S-5602, this study was so designed that the diets for both compounds contained the same concentration of the active isomer (Aα)). The compounds were first dissolved in corn oil and then mixed with basal diet. The concentration of corn oil in all diets was adjusted to two percent; stability of S-1844 and homogeneity of S-1844 and S-5602 were determined at every dosage taken from every two preparations of the freshly prepared diet.

Statistical analysis of body weight, food consumption, water intake, organ weight, haematological and blood biochemical examination data, the difference or variances between control group data and each treatment group data was analysed by F-test. In the case where the difference was not significant, Student's t-test was used. Fisher-Behrens test was applied when the difference was significant. U-test was employed for the statistical analysis of urinalysis. Gross pathological and histopathological findings were analysed by Fisher's exact test when needed.

RESULTS

No death was found in any group. The following toxic signs were observed in mice receiving 500 ppm S-1844 and 2000 ppm S-5602 : fibrillation, tremor, convulsion, hypersensitivity to sounds (during early stage of the study), abnormal gait (hunched posture and unsteady gait), salivation (week 1 of the study), higher grooming activities such as scratch and licking, leading to higher incidence of external lesions such as alopecia, scab and sore formation.

A similar depression of body weight gain and water intake was observed in these two groups.

Both these groups showed some effects on some parameters of urinalysis : decreased pH, elevated protein, ketone, bilirubin, urobilinogen concentrations and specific gravity; haematology : decreased erythrocyte counts, haemoglobin concentration and haematocrit values but increased neutrophil ratio; clinical biochemistry : decreased concentration of total protein, albumin, glucose, cholesterol, triglyceride and phospholipid were observed in mice receiving 500 ppm S-1844, the same depression for glucose, triglyceride and phospholipid concentration was also seen in mice receiving 2000 ppm S-5602, glucose and triglyceride concentration were also lower in males receiving 150 ppm S-1844 (but these changes are considered not to be of toxicological significant because of the absence of other changes at this level), increased levels of lactate dehydrogenase (LDH), GPT activities and blood urea nitrogen concentration (BUN) were observed in both 500 ppm S-1844 and 2000 ppm S-5602 groups. In respect to leucine aminopeptidase activity (LAP), mice receiving 500 ppm S-1844 showed lower activity, while 2000 ppm S-5602 showed higher activity, this effect was considered to be related to differences in Bβ isomer content.

Higher liver and spleen weight were observed in mice receiving 2000 ppm S-5602 and were considered to be related to the formation of granulomatous changes observed in the liver and spleen; the absolute liver weight was decreased in mice receiving 500 ppm S-1844 probably related to the decreased body weight gain; higher salivary gland weight was seen in both S-1844 500 ppm and S-5602 2000 ppm groups, but there were no indication of any histopathological change in these glands.

Compound-related histopathological changes were observed in liver, spleen, lymph nodes, thymus, skin, kidney and stomach. They were divided into five categories : 1) microgranulomatous changes in liver, spleen and lymph nodes (only observed in mice receiving 2000 ppm S-5602); 2) inflammatory changes in skin (in both high dose compounds); 3) reactive changes in lymphatic tissues (both high dose compounds); 4) ulcerative changes in stomach (slight effect in a few male receiving 500 ppm S-1844); and 5) decrease of fat deposition in liver and kidney (both high dose compounds).

CONCLUSION

The principal difference between the toxicity of fenvalerate and esfenvalerate was the granuloma formation observed in mice receiving fenvalerate at 2000 ppm. The granuloma formation has been studied with four chiral isomers of fenvalerate using B6C3F1 mice and was considered to be dependent on the content of the Bα-isomer within the test chemicals. In accordance with this conclusion, the microgranulomatous changes were not observed in any tissues of mice treated with S-1844 (esfenvalerate) which has a very low content of Bα-isomer (less than 5%).

Leucine aminopeptidase lower activity was observed only in mice receiving 500 ppm S-1844. Another difference between the two compounds was the ulcerative changes in glandular stomach in the 500 ppm group of S-1844, but with a low toxicological significance.

It was concluded that there were no remarkable toxicological difference between mice fed with S-1844 and S-5602 for three months except the granuloma formation and higher leucine aminopeptidase activity observed only in mice receiving S-5602. The NOAEL for S-1844 (esfenvalerate) was considered to be 150 ppm for both males and females (intakes of ~30.5 and 36.5 mg/kg/day, respectively).

3.12.1.2 [Study 2]

Study	IIA 5.3.2/04 One-year oral study in dogs with MO 70616 Technical
Reference	Anonymous (1986e)
Date performed	January 1985 – January 1986
Test facility	Anonymous
Report reference	Report no. LLT-61-0063
Guideline(s)	OECD 452
Deviations from the guideline	None
GLP	Yes
Test material	MO 70616 Technical (esfenvalerate, purity 98.7% as sum of all isomers). Batch WRC Tox. Sample No. 730C
Study acceptable	Yes

METHODS

MO 70616 Technical (esfenvalerate, purity 98,7% a.i.) was administered to groups of six male and six female Beagle dogs in the diet over a period of one year at concentrations of 0, 25, 50, 100 and 200 ppm (350 g diet of powdered dog chow moistened with equal parts of water was presented on a four hour period daily). Nanograde acetone was the vehicle used for preparation of all diets. Determinations were made to establish the stability, homogeneity and isomer ratio of MO 70616, which did not change from pre-study values.

Doses were selected on the basis of a previous three weeks study (not presented) in which beagle dogs were administered 0, 100, 300 or 500 ppm MO 70616 in the diet, and neurological clinical signs and lower body weight appeared in the 300 and 500 ppm dose groups; in addition, food consumption was lower and absolute and relative adrenal weights were higher in the 500 ppm dose group.

ANOVA's one-way analysis of variance and ANCOVA's analysis of covariance were used in statistical analysis. If the ANOVA or ANCOVA was significant, comparisons between control and treatment groups were analysed by one-tailed Dunnett's t-test except relative organ weights were analysed by two-tailed test.

RESULTS

No signs of toxicity were observed during the study and there were no mortalities. There were no treatment-related effects on mean body weight, mean food consumption, ophthalmic examination,

organ weights, macroscopic and microscopic findings. Differences noted between treated and control animals in clinical pathology parameters were considered to be normal biological variations, not important toxicologically, and not related to treatment (inorganic phosphorus, lactate dehydrogenase, total bilirubin and reticulocyte count).

CONCLUSION

The NOEL was 200 ppm, equivalent to approximately 5 mg/kg/day, the highest dose tested in the study.

3.12.1.3 [Study 3]

Study	IIA 5.7.4/001 Subchronic oral neurotoxicity study in rats
Reference	Anonymous (2000c)
Date performed	June - September 1999
Test facility	Anonymous
Report reference	LLT-0205 (Lab. Project ID DuPont-3081)
Guideline(s)	OECD 424
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate technical (also referred to as Asana technical). Batch no. DPX-YB656-84, purity 98.58% (as sum of all isomers)
Study acceptable	Yes

METHODS

Sprague-Dawley CD®(SD)(IGS)BR rats, about 48 days old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.7.2–1: Study design

Test group	Dietary concentration of Esfenvalerate (ppm)	Number of animals	
		Males	Females
1	0	12	12
2	50	12	12
3	100	12	12
4	300	12	12

Pelletised test and control diets were prepared using Rodent LabDiet® 5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of Penflufen in analysed samples of test diet used on the study were all within 15% of the nominal, demonstrating satisfactory concentration and homogeneity.

Some males at 300 ppm received skin treatment with an antiseptic agent, chlorohexadine (1:1 dilution), following the development of skin sores.

General clinical observations were recorded at least once daily. Bodyweights were measured weekly. Food consumption was monitored throughout the study. A standard functional observational battery (observations made in home cage, while being handled and in open field, sensorimotor tests) was conducted prior to dosing, and again on study weeks 4, 8 and 13. Motor activity measurements were conducted on the same days as the FOB.

The animals were killed after approximately 13 weeks exposure. Major organs were examined macroscopically at a necropsy. Six animals/sex/group were randomly selected for whole body perfusion and neuropathology. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were dissected, sampled and preserved. Tissues only from the control and high dose groups were examined microscopically.

RESULTS

Received doses were calculated in terms of mg Esfenvalerate/kg body weight. Mean values are shown below:

Table B.6.7.2–2 Mean dose received (mg/kg/day)

Dietary concentration of Esfenvalerate (ppm)	50	100	300
Males	3.2	6.4	20.1
Females	3.7	7.3	22.8

Unscheduled deaths, considered to be treatment-related, occurred among males at 300 ppm. Two were killed prior to the scheduled 3 month termination because of the presence of serious skin sores, one on day 52 and the other on day 88.

Clinical signs considered to be treatment-related were observed at the daily general clinical examination. Abnormal gait was observed in all males and females at 300 ppm (mean onset days 3/4), which correlated with observations made in the FOB (as described in the paragraph below). Identical observations were made in the earlier esfenvalerate 90-day repeated dose toxicity at dietary concentrations of 300 ppm (IIA 5.3.2/02) and in the esfenvalerate multigeneration reproductive toxicity study (IIA 5.6.1/01). Additionally, at 300 ppm there was an increased incidence of skin sores, affecting 6 out of the 12 males; as stated above, for 2 males the wounds were so severe that the animals were killed prematurely. Possibly the skin lesions were related to dermal contact with test substance in the diet, though the use of pelleted diet will have minimised dermal contact.

The key FOB findings are summarised in Table B.6.7.2-3 which highlights treatment-related effects on the incidence of abnormal gait, reductions in forelimb and hindlimb grip strength and a decrease in footsplay. In the open field assessment a number of males and females at 300 ppm were observed with abnormal gait (dragging, hopping) at the 4, 8 and 13 week FOB. Treatment-related reductions in forelimb grip strength were observed in males at 100 and 300 ppm. Among females, forelimb grip strength was statistically significantly reduced in comparison with controls at week 4, but this difference can not be conclusively attributed to treatment as the control value appeared to be unusually high. Treatment-related reductions in hindlimb grip strength were observed at 300 ppm in both males and females. Footsplay was reduced at 300 ppm, only among males.

Table B.6.7.2–3 Selected FOB findings: signs (number of affected animals), forelimb and hindlimb grip strength (kg) and hindlimb footsplay (cm)

FOB finding	Week	Dietary concentration of Esfenvalerate (ppm)							
		Males				Females			
		0	50	100	300	0	50	100	300
Abnormal gait (dragging, hopping) (n)	-1	0	0	0	0	0	0	0	0
	4	0	1	0	7*	0	0	0	9*
	8	0	0	0	5*	0	0	0	8*
	13	0	0	0	3*	0	0	0	6*
Forelimb grip strength (kg)	-1	0.56	0.54	0.56	0.54	0.64	0.57	0.58	0.57
	4	1.15	1.06	1.02	0.71*	1.00	0.82	0.81	0.73*
	8	1.29	1.17	0.95*	0.86*	0.79	0.77	0.72	0.69
	13	1.04	1.04	0.90	0.89	0.79	0.69	0.58	0.70
Hindlimb grip strength (kg)	-1	0.59	0.55	0.52	0.53	0.56	0.53	0.55	0.56
	4	0.75	0.73	0.69	0.58*	0.65	0.63	0.64	0.54*
	8	0.87	0.83	0.77	0.78	0.77	0.75	0.73	0.63
	13	0.91	0.88	0.88	0.86	0.82	0.75	0.76	0.72*
Footsplay (cm)	-1	7.2	7.6	6.4	6.1	7.3	6.2	6.8	6.8
	4	8.5	8.5	8.2	5.8*	7.1	6.0	6.3	7.4
	8	9.3	9.7	9.0	6.5*	7.6	7.1	6.4	7.3
	13	8.9	8.8	8.1	7.1	7.8	7.8	5.9	6.3

* significantly different from control, $p<0.05$

The motor activity assessment revealed marginal treatment-related effect in males at 300 ppm, only at week 8 (see Table B.6.7.2-4). The normal pattern of declining motor activity over the 60 minute observation period was less pronounced than observed in the control group, with the number of movements being significantly increased for the 6^{th} 10 minute interval and when analysed as total number of movements over the 60 minute observation period. The duration of movements for males at 300 ppm at week 8 was also increased towards the end of the observation period, although statistical significance was not achieved.

Table B.6.7.2–4 Selected motor activity assessment findings at week 8: mean number of movements and mean duration of movements (sec)

Motor activity finding	Week	Dietary concentration of Esfenvalerate (ppm)							
		Males				Females			
		0	50	100	300	0	50	100	300
Number: 1st 10 min interval	8	132	129	133	133	136	126	133	132
Number: 2nd 10 min. interval		135	133	137	138	133	131	124	135
Number: 3rd 10 min. interval		115	125	128	128	104	118	121	118
Number: 4th 10 min. interval		84	121	113	120	99	123	106	105
Number: 5th 10 min. interval		61	95	94	106	104	106	81	89
Number: 6th 10 min. interval		59	63	66	111*	86	80	95	107
Number: over 60 min. period		586	665	671	737*	662	684	660	687
Duration: 1st 10 min interval		406	424	396	409	385	403	387	404
Duration: 2nd 10 min. interval		335	353	325	352	303	301	277	307
Duration: 3rd 10 min. interval		238	294	280	303	192	249	229	255
Duration: 4th 10 min. interval		165	239	211	278	179	228	175	182
Duration: 5th 10 min. interval		112	165	187	212	179	178	115	158
Duration: 6th 10 min. interval		97	131	122	195	133	142	150	181
Duration: over 60 min. period		1353	1606	1519	1748	1371	1500	1332	1487

* significantly different from control, $p<0.05$

Adverse effects on bodyweights were present in males at 100 and 300 ppm and females at 300 ppm, as shown in Table B.6.7.2-5. For males, bodyweight and bodyweight gains were significantly reduced throughout the study, with mean bodyweight being 12.5% less than controls at termination. At 100 ppm, mean bodyweight was 7% less than controls. For females at 300 ppm, bodyweight gain was significantly reduced at the start of the study and over the study period; at termination mean bodyweight was 10% lower than controls.

Table B.6.7.2–5 Selected group mean bodyweights (g) and bodyweight gains (g)

Day	Dietary concentration of Esfenvalerate (ppm)							
	Males				Females			
	0	50	100	300	0	50	100	300
Bodyweight day 0	211	207	209	212	169	169	170	167
Bodyweight day 7	278	269	270	248*	199	198	195	183
Bodyweight day 49	515	501	493	457*	285	284	277	258
Bodyweight day 91	617	598	573	540*	319	319	304	286
Gain days 0-7	66.8	62.9	61.4*	35.9*	30.1	29.2	25.2	16.0*
Gain days 0-91	406	392	364*	328*	150	150	134	119*

*significantly different from control, $p<0.05$

Adverse effects on food consumption were present in males at 100 and 300 ppm and females at 300 ppm, as shown in Table B.6.7.2-5. Consumption for males at 300 ppm was significantly reduced for most weeks of the study, with consumption over the entire study being about 7% less than controls. At 100 ppm the reduction among males was less marked. For females at 300 ppm consumption was less than controls during the first few weeks of the study, though statistical significance was not achieved. Food efficiency was significantly reduced for both males and females at 300 ppm.

Table B.6.7.2–4 Selected group mean food consumption (g/rat day) and food efficiency (g bodyweight gain/g food consumed) values

Day	Dietary concentration of Esfenvalerate (ppm)							
	Males				Females			
	0	50	100	300	0	50	100	300
Consumption, d 0-7	28.0	26.4	24.9*	20.9*	19.0	19.3	18.2	15.7
Consumption, d 7-15	30.0	28.4	28.4	27.1*	19.8	19.9	19.2	18.9
Consumption, d 42-49	31.7	29.9	29.0*	28.1*	18.8	20.6	18.7	19.0
Consumption, d 84-91	29.5	29.1	27.4	29.1	18.1	19.2	18.0	18.6
Consumption, d 0-91	30.2	28.7	28.4	28.0*	19.0	20.0	18.9	18.7
Efficiency, d 0-91	0.147	0.148	0.140	0.128*	0.087	0.082	0.078	0.069*

* significantly different from control, $p<0.05$

There were no treatment related macroscopic necropsy findings. The microscopic examination of the nervous system tissues did not reveal any treatment-related changes.

CONCLUSION

Dietary administration of esfenvalerate for 90 days to the rat at concentrations of 100 ppm and above caused adverse effects, observed as reductions in forelimb grip strength, and marginal reductions in bodyweights and food consumption in males. At the highest concentration tested, 300 ppm, similar adverse effects were also present in females, accompanied by abnormal gait and reduced hindlimb grip in both genders. Additionally, in males at the highest concentration, skin sores were present and footsplay was decreased. However, there were no microscopic neurological lesions. Overall, study NOAELs of 50 ppm (intake of about 3.2 mg/kg/day) for males and 100 ppm (intake of about 7.3 mg/kg/day) for females were identified.

3.12.1.4 [Study 4]

Study	IIA 5.7.4/002 13-Week dietary neurotoxicity study of esfenvalerate TG in the Rat
Reference	Anonymous (1999c)
Date performed	November 1998 – March 1999
Test facility	Anonymous
Report reference	Laboratory Project ID 97524, Report no. LLT-0189
Guideline(s)	OECD 424
Deviations from the guideline	None
GLP	Yes
Test material	Esfenvalerate TG, purity 86.0% S,S isomer; 97.3% total fenvalerate isomers, batch no. 60610G. Information on batch no. provided by Applicant June 2013
Study acceptable	Yes

METHODS

Groups of 12 Sprague-Dawley (CD) rats/sex were treated for 13 weeks, by dietary incorporation, with esfenvalerate TG at dose levels of 0, 40, 120 or 360 ppm (groups 1 to 4 respectively), corresponding to mean achieved intakes of 0.0, 3.0, 8.9 and 28.8 mg/kg/day for males, and 0.0, 3.7, 10.7 and 35.0 mg/kg/day for females respectively. Purity of esfenvalerate was 86.0% as S,S isomer and 97.3% as total isomers, during dose formulation no correction was made for the purity of the test article. The control group received pelleted basal diet only.

The dose levels were selected based on the results of a previous 2-week range-finding dietary toxicity study (CTBR Project No. 97495) - not presented.

All animals were examined twice daily for mortality and clinical signs, in addition a complete detailed examination was performed weekly. Body weights and food intake were measured weekly. A functional observational battery (FOB) both qualitative and quantitative –grip strength and hindlimb splay- and motor activity test were performed prior to treatment initiation and during weeks 2, 5, 9 and 13, and an ophthalmological examination was conducted prestudy and during week 13.

At study completion (day 93), five rats/sex/group were given a whole-body perfusion (with brain dimensions later measured) and those animals in the control and high dose groups subsequently underwent a neuropathological examination. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were examined.

Group variances for body weight, food consumption and quantitative FOB data were compared using Bartlett's test. When the differences between group variances were not significant ($P>0.001$), a one-way analysis of variance (ANOVA) was performed. If significant differences ($P<0.05$) were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. When the differences between group variances were significant ($P<0.001$) by Bartlett's test, the Kruskal-Wallis test was then performed. Where significant differences ($P<0.05$) between the groups were indicated by the Kruskal-Wallis test, the values for the control and treated groups were compared using Dunnett's test (or Wilcoxon/Mann Whitney "U" test). Motor activity data were analysed using a repeated measures analysis. The repeated measures values were collapsed into two parameters, total counts and a linear constructed variable (LCV) which evaluated the rate of linear change within the test session. Group variances for total counts and the LCV were compared using Bartlett's test. The differences between group variances were not significant ($P>0.001$) using Bartlett's test, therefore, an analysis of variance (ANOVA) for the prestudy data and an analysis of covariance (ANCOVA) with the prestudy data as the covariate for the week 2, 5, 9 and 13 data was then performed. If significant ($P<0.05$) differences were indicated by the ANCOVA, a t-test was used to compare the control and treated groups. Qualitative FOB data were analysed by comparing the control group to the treated groups using Fisher's exact probability test. Initially, the comparison included all scores within a category, where appropriate, for all groups together. If a significant ($P<0.05$) difference was detected, then comparisons were made using all scores within a category between the control group and each of the treated groups

RESULTS

There were no treatment related mortalities. The only clinical signs attributed to treatment were observed in a small number of 360 ppm group males, which showed lesions/scabbing at the inguinal/sacral/urogenital/scrotal regions.

The body weight of the 360 ppm males and females were significantly ($P<0.05$ or $P<0.01$) reduced throughout the treatment period. For males in the 120 ppm group, decreases, occasionally significant ($P<0.05$), were also observed, while females of this dose group showed values slightly lower than the control group without statistical significance. No significant differences were observed between the control and the 40 ppm groups.

Males and females in the 360 ppm group showed a significant ($P<0.01$) decrease in food intake during the first week of treatment. Other differences noted (a significant decrease for the 120 ppm females from days 50 to 57 and significant increases for the 40 ppm males and females from days 71 to 78 and 78 to 85) were considered of no toxicological significance.

At the week 2 FOB, the forelimb grip strength was significantly ($P<0.05$) decreased for males and females in the 360 ppm group. In addition, the ease of removal from the home cage was significantly ($P<0.05$) reduced for the 360 ppm females at this assessment. No significant differences were noted for the subsequent testing occasions.

At the FOB, one 360 ppm male showed from the week 5 onwards a slight ataxic gait and overall gait incapacity. This was considered to be associated with the lesions this animal had at the scrotal/hindlimb area. Additional findings noted for this animal at the week 13 assessment included signs such as slight tremors at the head and body, altered air righting reflex, limited usage of limbs, and a lesion at the scapular region. Due to the severity of the lesion on the hindlimbs, it was not

possible to conduct grip strength or hindlimb splay measurements for this animal at the week 13. Another 360 ppm male showed a slight ataxic gait and overall gait incapacity at the week 13 assessment, as this animal had a lesion at the tail region, this was considered likely to be the cause of its abnormal gait.

At the week 2 motor activity assessment, females in the 120 and 360 ppm groups showed a significant ($P<0.05$ or $P<0.01$) decrease in total activity counts when compared to the control and treated groups.

There were no ocular changes considered related to treatment.

There were no gross or histopathological changes attributed to treatment. A few males in the 360 ppm groups showed skin ulcerations. There were no significant differences in brain measurements (weight, length or width) between the control and treated groups.

CONCLUSION

Treatment of male and female rats with esfenvalerate TG for 13 weeks by dietary incorporation at concentrations of 0, 40, 120 and 360 ppm resulted in the following:

At the 360 ppm level, lower body weight, decreased food consumption, decreased grip strength in both sexes at week 2, decreased ease of removal from the home cage and reduced motor activity at week 2 in females, and scabbing in inguinal/sacral/urogenital/scrotal regions were observed.

For the 120 ppm group, decreased body weight occurred in males, and females showed a reduction in motor activity at the week 2 assessment.

No neuropathological lesions attributed to treatment were observed.

The NOAEL of this study was the dose level of 40 ppm.

Given the transient and generalised nature of the behavioural changes, together with the absence of treatment-related lesions in nervous tissues, esfenvalerate TG was considered not to be neurotoxic.

Additional comment from UK RMS

It is noted the skin lesions were present only at the highest dose levels in the two esfenvalerate 90-day dietary neurotoxicity studies (Malley 2000b, IIA 5.7.4/001; Beyrouty 1999, IIA 5.7.4/001), both apparently conducted using pelleted diet. The use of pelleted diet will reduce the potential for dermal contact with the diet, so it is less clear whether these dermal lesions occurring at high doses can be regarded as a local effect due to dermal contact with the diet, as was the case for the skin lesions seen the dietary chronic and reproductive studies. Possibly, the effects of minimal dermal contact test substance the diet were exacerbated by the presence of systemic neurobehavioral effects.

3.12.2 Human data

No relevant data available.

3.12.3 Other data

No other relevant data available.

3.13 Aspiration hazard

Not applicable.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

[Study 1]

Study reference:

R. Graham, A. Flenley (2011) Esfenvalerate: Assessment of ready biodegradability by measurement of CO_2 evolution. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-0058

Detailed study summary and results:

UK activated sewage sludge was collected from a sewage treatment works and added to a buffered mineral medium. Esfenvalerate was then added to give a nominal concentration of 15 mg carbon/L. Five additional vessels were also prepared; two reference substance control vessels containing sodium benzoate, two blank control vessels containing only the buffer medium and a single toxicity control vessel containing both the esfenvalerate and sodium benzoate.

The test system was incubated at 22 ± 2ºC in the dark. Trap analysis for evolved CO_2 was performed at regular intervals (10 sample intervals in total) for up to 28 days after treatment. The theoretical yield of CO_2 from the vessels was calculated and the cumulative values for the test substance, reference substance and toxicity control vessels were corrected against the blank controls.

The theoretical yield of evolved CO_2 from esfenvalerate was 0% at 28 days. Hence, esfenvalerate was considered as being classified as ‘not readily biodegradable’.

Test type:

OCED guideline 301B (Revised 1992)

Yes (laboratory certified by UK National Authority)

Test substance:

Esfenvalerate (purity: 100%)

Materials and methods:

UK activated sewage sludge was collected from a sewage treatment works which had a predominantly domestic waste-water catchment. The suspended solids concentration of the sludge was determined before use. A buffered, aqueous, synthetic, mineral salts medium was prepared and inoculated with microorganisms derived from a sample of activated sludge not exposed to esfenvalerate, to give a nominal final solids concentration of 30 mg suspended solids/L. The activated sludge was not acclimatised or adapted to esfenvalerate before exposure to the test conditions. The inorganic carbon concentration of the inoculated buffer medium was determined using an InnovOx carbon analyser before test initiation.

The carbon content of esfenvalerate was 71.5%, with no adjustment made for purity. Due to the low solubility of esfenvalerate (<1 µg/L), a suspension trial was performed before test initiation. Esfenvalerate was shown to provide an acceptable dispersion in ultrapure water to enable direct addition to the test system.

Two test substance vessels were prepared using inoculated buffered medium (1 litre), which had been sealed and aerated overnight with CO_2 free air, and esfenvalerate (24 mg/L) was added to give a nominal concentration of 15 mg carbon/L. Five additional vessels were also prepared; two reference substance control vessels using sodium benzoate to give a nominal concentration of 15 mg carbon/L; two blank control vessels, containing only the buffer medium and a single toxicity control vessel, using both the esfenvalerate and sodium benzoate, to give a nominal concentration of 15 mg carbon/L of both. All test vessels were made up to 3 litres by addition of ultrapure water and each vessel was sealed, connected to a series of three traps containing aqueous barium hydroxide and connected to a

CO_2-free air supply. The test system was incubated at 22 ± 2ºC in the dark for up to 28 days. pH measurements were taken at test initiation and completion from the blank controls and reference vessels. Trap analysis for evolved CO_2 was performed at regular intervals (10 sample intervals in total) for up to 28 days after treatment.

The theoretical yield of CO_2 from the vessels were calculated and the cumulative values for the test substance, reference substance and toxicity control vessels were corrected against the blank controls.

Results:

The inorganic carbon concentration of the inoculated buffer medium was determined to be 0 mg carbon/L, therefore the validity criteria of <5% carbon loading was achieved. The total CO_2 evolved from both blank controls was 78 mg, therefore the validity criteria of <120 mg was achieved. The rate of biodegradation of the reference substance in the presence of esfenvalerate (87% in 28 days) was comparable to that of the reference substance alone (89% in 28 days), therefore it can be concluded that esfenvalerate does not have an inhibitory effect on the sludge microorganisms under test conditions. The pH values were in the range of 7.47 to 7.63 throughout the study period.

The amount of evolved CO_2 from the test substance vessels was ≤0.7 mg (corrected against the blank controls) throughout the study period. The theoretical yield of evolved CO_2 from esfenvalerate was 0% at 28 days. To be considered readily biodegradable, a test substance must achieve 60% biodegradation by the end of the test. Hence, esfenvalerate was considered as being classified as 'not readily biodegradable'.

4.1.2 BOD_5/COD

No information provided.

4.1.3 Aquatic simulation tests

[Study 1]

Study reference:

R. Graham, J. Gilbert (2012) [^{14}C]-Esfenvalerate: Hydrolytic Stability. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-0068

Study summary and results:

A preliminary study (Tier I) was performed at 50ºC with sterile buffer solutions at pH 4, pH 7 and pH 9 using two radiolabelled forms of esfenvalerate. Esfenvalerate was found to be hydrolytically stable at pH 4, however at pH 7 and pH 9, > 10% hydrolysis occurred after five days, triggering a tier II aqueous hydrolysis test for pH 7 and pH 9. In the Tier II test, buffer solutions were incubated at pH 7 at 40, 50 and 60ºC and at pH 9 at 25, 40 and 50°C in the dark for up to 32 days.

Mean mass balances were in the range 88.9 – 104.2% AR (applied radioactivity) for all sampling points and all tier II incubation temperatures at pH 4, 7 and 9. Sterility and pH (within ± 2) of the samples were maintained during the study period.

Two major hydrolytic degradates were CPIA and PBald, which were formed via ester cleavage of esfenvalerate. CPIA was observed at maximum levels in the range of 41.6% to 93.4% AR and PBald was observed at maximum levels of 36.0% to 90.9% AR.

The incubations at higher temperatures at pH 7 (50 and 60°C) resulted in $CONH_2$-Fen levels exceeding 10%. AR The maximum levels observed were in the range of 10.1 to 11.1% AR. Under alkaline conditions, the $CONH_2$-Fen further degraded to 3-phenoxymandelic acid and CPIA-carboxamide across all three temperature ranges. 3-Phenoxymandelic acid was observed at maximum levels in the range of 8.6% to 11.3% AR and CPIA-carboxamide was observed at maximum levels in

the range of 10.2% to 12.2% AR. All other degradates including PBacid and PBCN accounted for less than 10% AR throughout the study.

Chiral analysis of samples also showed that esfenvalerate was rapidly converted to its 2*S*α*R*-isomer under neutral and alkaline conditions to a 1:1 ratio, and remained at almost the same ratio for the entire incubation period at all tested temperatures.

The activation energy values for hydrolysis at pH 7 and 9 were calculated to be 98.6 kJ/mol and 103.3 kJ/mol, respectively. The DT_{50} values at pH 7 and pH 9 ranged from 3.3 to 427.7 days and 2.7 hours to 5.3 days, respectively.

Test type:

OECD Guideline 111 (April 2004)

GLP (laboratory certified by UK National Authority)

Test substance:

[Phenoxyphenyl-^{14}C]-esfenvalerate (purity: 99.3%, optical purity 98.7%)

[Chlorophenyl-^{14}C]-esfenvalerate (purity: 99.8%, optical purity 99.1%)

Materials and methods:

A preliminary study (tier I) into the hydrolytic stability of esfenvalerate (two radiolabels) was investigated at 50ºC. Solutions of aqueous buffers were prepared at pH 4, pH 7 and pH 9, using a sodium acetate buffer (0.01 M), a potassium dihydrogen orthophosphate buffer (0.01 M) and a sodium tetraborate buffer (0.02 M), respectively, at 50ºC. A sorption test was performed to investigate the potential for sorption of esfenvalerate to the test vessels. After dispensing to the test vessels, buffers were de-oxygenated by sonication, followed by sparging with nitrogen for *ca* 5 minutes. Vessels were sterilised by autoclaving before being treated with esfenvalerate (with a co-solvent of acetonitrile of 10%) under sterile conditions to give a final concentration of 0.004 µg/mL. Units were incubated in a water bath at 50ºC in the dark for up to 5 days.

Samples analysis was performed in duplicate at 0 and 5 DAT. On analysis, samples were acidified to <pH 2, partitioned with dichloromethane (2-3 times, 100 mL) and quantified by LSC. A sub-sample of the organic fraction (*ca* 150 mL) was diluted with acetonitrile (*ca* 50 mL) before being concentrated by rotary evaporation (*ca* 20mL). For the tier I test, the sample was again diluted with acetonitrile (*ca* 20 mL) and concentrated (*ca* 5 mL) before being analysed by LSC and HPLC. For the tier II test, a subsample of the original first concentrate was taken and concentrated (*ca* 1 mL), before being quantified by LSC and analysed by HPLC. Additional units were also prepared for pre- and post-treatment pH measurements and confirmation of sterility. Esfenvalerate was found to be hydrolytically stable at pH 4. At pH 7 and 9, >10% hydrolysis occurred after 5 days, consequently a Tier II test was performed at pH 7 and 9.

For the tier II test, solutions of the appropriate aqueous buffers were prepared at pH 7 at 40, 50 and 60ºC, and at pH 9 at 25, 40 and 50ºC. Test preparation was performed in the same manner as the tier I test, with vessels being incubated in the dark at their respective temperatures for up to 32 DAT. Sample analysis was performed as descried previously.

Sub-samples of the concentrated extracts (300 µL) that contained >5% AR were concentrated to dryness and re-constituted in hexane (30 µg/L), and quantified by LSC and analysed by chiral HPLC.

Results:

Sterility and pH (within ± 2) of the samples were maintained during the study period. Sorption of esfenvalerate to the test vessels was found not to occur at the test concentration of 0.004 µg/L containing 10 % acetonitrile as a co-solvent.

In the preliminary study (tier I), esfenvalerate was found to be hydrolytically stable at pH 4, with levels of esfenvalerate in the range of 90.9 - 98.6% AR at 5 DAT. At pH 7 and 9, >10% AR

hydrolysis occurred after 5 days, with levels of esfenvalerate in the range of 50.1% to 62.7% and 0.8% to 0.9% AR, respectively. Consequently a Tier II test was performed at pH 7 and 9.

Mean mass balances were in the range 88.9 – 104.2% for all sampling points and all tier II incubation temperatures at pH 4, 7 and 9.

The results of the incubations of pH 7 at 40°C and pH 9 at 25°C are shown in Tables B.8.4-1 and B.8.4-2. The incubations undertaken for pH 7 at 50 and 60°C and pH 9 at 40 and 50°C were considered not to provide any further useful data and so are not presented in detail.

Two major hydrolytic degradates were CPIA and PBald, which were formed via ester cleavage of esfenvalerate. CPIA was observed at maximum levels of 41.6, 72.0 and 74.8% AR in the pH 7 samples at 40, 50 and 60°C, respectively, and 93.4, 91.1 and 88.1% AR in the pH 9 samples at 25, 40 and 50°C, respectively. PBald was observed at maximum levels of 36.0, 58.8 and 67.7% AR in the pH 7 samples at 40, 50 and 60°C, respectively, and 90.9, 80.9 and 79.3% AR in the pH 9 samples at 25, 40 and 50°C, respectively.

The incubations at higher temperatures at pH 7 (50 and 60°C) resulted in the degradate $CONH_2$-Fen exceeding 10% AR. The maximum levels observed were in the range of 10.1 to 11.1% AR. Under alkaline conditions, the degradate $CONH_2$-Fen further degraded to 3-phenoxymandelic acid and CPIA-carboxamide across all three temperature ranges. 3-Phenoxymandelic acid was observed as maximum levels of 8.6, 10.9 and 11.3% AR in the pH 9 samples at 25, 40 and 50°C, respectively. CPIA-carboxamide was observed as maximum levels of 10.6%, 10.2% and 12.2% AR in the pH 9 samples at 25, 40 and 50°C, respectively.

All other degradates including PBacid and PBCN accounted for less than 10% AR throughout the study.

Chiral analysis of samples also showed that esfenvalerate was rapidly converted to its 2*S*α*R*-isomer under neutral and alkaline conditions to a 1:1 ratio, and remained at almost the same ratio for the entire incubation period at all tested temperatures.

Table B.8.4-1: Product balance following hydrolysis of esfenvalerate in pH 7 buffer at 40°C

Time (days)	Phenoxyphenyl label			Chlorophenyl label		
	Esfenvalerate	PBald	$CONH_2$-Fen	Esfenvalerate	CPIA	$CONH_2$-Fen
0	95.8	ND	ND	97.7	ND	ND
0	95.7	ND	ND	99.1	ND	ND
5	90.9	8.5	ND	90.5	6.6	ND
5	86.7	8.8	ND	87.3	7.9	ND
12	79.4	17.9	ND	81.8	17.9	ND
12	78.0	18.5	ND	77.5	17.7	ND
15	76.5	24.3	ND	73.5	23.6	3.4
15	76.1	22.5	ND	66.7	25.7	3.3
20	65.0	31.9	ND	58.2	39.6	ND
20	63.9	34.4	ND	60.1	34.2	4.7
25	54.8	36.5	ND	57.4	37.6	5.1
25	60.6	35.4	ND	56.0	35.3	4.7
29	54.6	37.2	6.3	52.8	41.4	5.5
29	52.4	31.2	4.1	49.5	41.8	5.5

Table B.8.4-2 Product balance following hydrolysis of esfenvalerate in pH 9 buffer at 25°C

Time (days)	Phenoxyphenyl label			Chlorophenyl label		
	Esfenvalerate	PBald	3-Phenoxy-mandelic acid	Esfenvalerate	CPIA	CPIA-Carboxamide
0	94.6	ND	ND	100.6	ND	ND
0	101.4	ND	ND	100.9	ND	ND
1	78.4	15.8	ND	-	-	-
1	74.4	20.9	ND	-	-	-
2	65.9	32.8	ND	-	-	-
2	65.3	34.6	ND	-	-	-
3	53.9	42.8	ND	-	-	-
3	48.5	48.5	ND	-	-	-
5	23.1	64.4	3.5	28.7	62.9	4.1
5	24.4	65.0	4.3	26.2	66.4	4.1
11	6.2	82.2	5.0	4.6	88.7	8.0
11	6.7	85.2	7.7	4.1	89.8	7.6
15	1.8	85.0	8.3	1.2	92.9	10.1
15	1.5	87.8	8.5	1.6	91.2	8.1
20	0.7	86.5	7.8	ND	92.8	8.5
20	0.6	87.7	7.9	ND	92.9	9.8
25	0.9	86.6	9.2	ND	92.2	9.8
25	0.2	81.3	8.0	ND	94.7	8.9
32	ND	92.7	6.8	ND	92.6	10.8
32	ND	89.1	8.4	ND	90.9	10.4

* Single replicate only

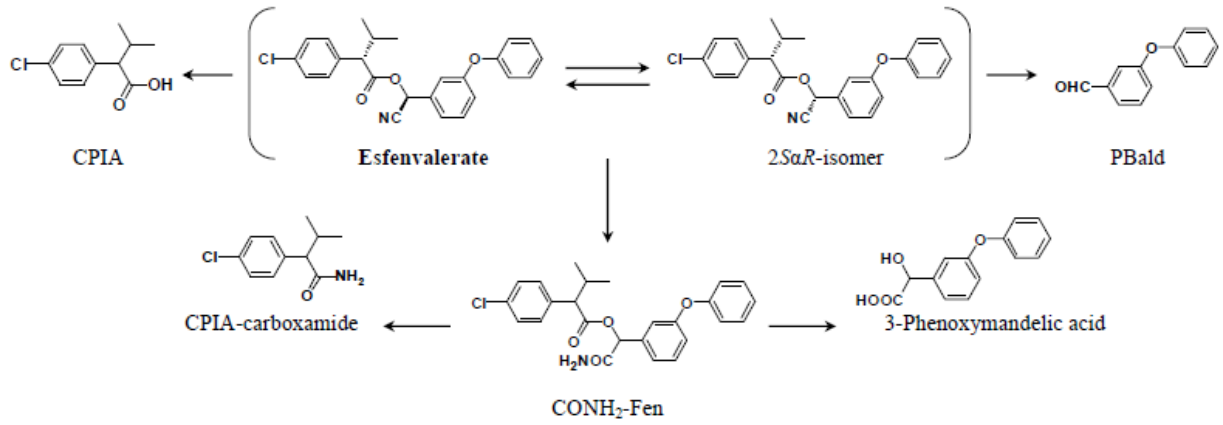
DT_{50} and DT_{90} values (SFO) calculated by the applicant (validated and accepted by the RMS) are presented in Table B.8.4--3. The activation energy values for hydrolysis at pH 7 and 9 were calculated to be 98.6 kJ/mol and 103.3 kJ/mol, respectively. The proposed route of degradation is shown in Figure B.8.4-1.

Table B.8.4-3: Hydrolytic degradation rates of esfenvalerate

pH	Temperature (°C)	DT_{50} (day)	DT_{90} (day)
4	50	Stable	Stable
7	20*	427.7	1421.2
7	40	32.3	107.2
7	50	10.0	33.2
7	60	3.3	11.0
9	20*	5.3	17.7
9	25	2.8	9.2
9	40	7.3 hr	24.4 hr
9	50	2.7 hr	9.0 hr

* extrapolated using Arrhenius plot and activation energy

Figure B.8.4-1 Proposed hydrolysis degradation route of esfenvalerate



[Study 2]

Study reference:

R. Graham, R. Dove (2012) [^{14}C]-Esfenvalerate: Photodegradation and quantum yield in sterile, aqueous solution. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-0065

Study summary and results:

The aqueous photolysis of esfenvalerate in sterile aqueous buffer at pH 4 ± 2 was investigated using two radiolabelled forms of esfenvalerate. Esfenvalerate was applied under sterile conditions to a sodium acetate buffer (with acetonitrile as a co-solvent) and vessels were sealed with quartz lids. Dark control vessels were dispensed into glass jars. All vessels contained polyurethane bungs in the inlet/outlet arms, attached to bacterial air filters, and a security trap, followed by two sodium hydroxide traps for volatiles. Samples were irradiated for up to 30 days at 25 ± 2°C, using UV filtered light with the average intensity being adjusted to *ca* 25 W/m^2 over the 300 – 400 nm range, so that light received within 30 days was equivalent to 30 days of UK/US summer sunlight. Dark controls were maintained at 25 ± 2°C in the dark. A PNAP/PYR actinometer was also included for the determination of quantum yield.

Sampling occurred in duplicate at 0, 1, 3, 7, 14, 21 and 30 DAT. On analysis, samples were acidified to <pH 2, partitioned with dichloromethane and the organic layer concentrated. Volatiles were analysed using the polyurethane foam bungs and sodium hydroxide traps. A vessel rinse was also performed after removal of the buffer. All samples were quantified by LSC and the concentrated organic extract analysed by HPLC. Chiral HPLC analysis was also used to investigate possible fenvalerate isomers.

Mean mass balances for each sampling point ranged from 96.2% to 103.8%. Radioactivity was found mainly in the organic layer. In the irradiated samples, radioactivity decreased in the organic layer to 78.5% to 79.0% AR, with the aqueous layer increasing to 13.0% to 13.4% AR by the end of the study period. Radioactivity in the dark controls stayed in the organic layer. Little radioactivity was detected in the vessel rinses, with maximum levels observed at <1% AR. Volatilisation was low, with maximum levels at 5.5% AR by 30 DAT.

Esfenvalerate was seen to degrade under photolytic conditions to <1.7% AR by 21 DAT. The major degradates observed were PBacid, Dec-fen A, Dec-fen B and PA-Fen, with the mean maximum levels >10% AR. The degradates Dec-fen A, Dec-fen B and PA-Fen all reached maximum levels at 7 DAT, before declining, while PB-acid reached maximum levels at 14 DAT, before declining slightly.

Chiral analysis of selected samples showed that esfenvalerate was not isomerised under irradiation.

The SFO DT_{50} value for esfenvalerate under irradiated conditions equivalent to UK/US summer sunlight was 2.0 days. The dark controls showed no significant degradation of esfenvalerate. The quantum yield for esfenvalerate was determined to be 0.016.

Test type:

OECD Guideline 316 (October 2008)

GLP (laboratory certified by UK National Authority)

Test substance:

[Phenoxyphenyl-^{14}C]-esfenvalerate (specific activity 10.84 MBq/mg, radiochemical purity 99.3%)

[Chlorophenyl-^{14}C]-esfenvalerate (specific activity 10.22 MBq/mg, radiochemical purity 99.8%)

Materials and methods:

The aqueous photolysis of esfenvalerate in sterile aqueous buffer at pH 4 was investigated using two radiolabelled forms of esfenvalerate. The sodium acetate buffer (0.01 M) was prepared at pH 4 ± 2 and sterilised by autoclaving before use, and dispensed to test vessels (450 mL) under sterile conditions, along with acetonitrile (50 mL). The test vessels contained an injection port, and inlet and outlet arms, which contained polyurethane bungs, and were then attached to bacterial air filters. After dispensing, the irradiated vessels were sealed with a quartz lid and attached to a security trap, and two sodium hydroxide (2 M) traps. Dark control vessels were dispensed into glass jars. Additional vessels were also dispensed for pH measurements and sterility checks.

Esfenvalerate was applied under sterile conditions so as to give a nominal concentration of 0.004 µg/mL. Due to the low solubility of esfenvalerate in water, the test solutions were prepared with 10% acetonitrile as a co-solvent. The irradiated units were placed under continuous irradiation for up to 30 days at a temperature of 25 ± 2ºC, which was maintained by cooling tank. The light source was from Suntest Accelerated Exposure instruments equipped with xenon lamps, which were adjusted to *ca* 25 Watts/m^2 (300 – 400 nm) so that the light received within 30 days was equivalent to 30 days of UK/US summer sunlight. The dark controls were maintained in the dark at 25 ± 2ºC.

Sampling occurred in duplicate at 0, 1, 3, 7, 14, 21 and 30 DAT. On analysis, samples were acidified to <pH 2, partitioned with dichloromethane (2 times, 100 mL), with the aqueous layer stored separately, and concentrated by rotary evaporation and under nitrogen. Volatile compounds were trapped with polyurethane foam bungs, which were extracted using acetonitrile (*ca* 9 hours) and sodium hydroxide traps. A vessel rinse with acetonitrile (50 mL) was also performed after removal of the buffer. All samples were quantified by LSC and the concentrated organic extract analysed by HPLC. Chiral HPLC analysis was also used to investigate possible fenvalerate isomers.

A PNAP/PYR actinometer was also included for the determination of quantum yield.

Results:

Mean mass balances for each sampling point ranged from 96.2 to 103.8%. Radioactivity was found mainly in the organic layer, with levels in the range of 102.0% to 103.6% AR at 0 DAT. In the irradiated samples, radioactivity decreased to 78.5% to 79.0% AR in the organic layer, with the aqueous layer increasing to 13.0% to 13.4% AR by the end of the study period. Radioactivity in the dark controls stayed in the organic layer. Little radioactivity was detected in the vessel rinses, with maximum levels observed at <1% AR. Volatilisation was low, with maximum levels at 5.5% AR by 30 DAT.

The results of HPLC analyses are shown in Tables B.8.4-4 and B.8.4-5. Small amounts of radioactivity reported in undifferential regions and unresolved backgrounds have not been included in the tables which results in small discrepancies between mass balance and the sum of the components listed in the tables. This is not considered to be of concern.

Esfenvalerate was seen to degrade under photolytic conditions to <1.7% AR by 21 DAT. The major degradates observed were PBacid, Dec-fen A, Dec-fen B and PA-Fen, with the mean maximum levels

>10% AR. The degradates Dec-fen A, Dec-fen B and PA-Fen all reached maximum levels at 7 DAT, before declining, while PB-acid reached maximum levels at 14 DAT, before declining slightly.

The dark controls showed no significant degradation of esfenvalerate. The quantum yield for esfenvalerate was determined to be 0.016.

Chiral analysis of selected samples showed that esfenvalerate was not isomerised under irradiation. The proposed route of degradation is shown in Figure 4.1.3-2.

Table B.8.4-4 Product balance following the aqueous photolysis of [phenoxyphenyl-^{14}C]esfenvalerate in pH 4 buffer

Time (days)	esfenvalerate	PBacid	PBald	Desphenyl -fen	Dec-fen A	Dec-fen B	PA-Fen	Total unknown*	Total in buffer
0	101.3	ND	ND	ND	ND	ND	ND	ND	102.3
0	101.5	ND	ND	ND	ND	ND	ND	ND	101.6
1	85.9	2.0	ND	ND	5.8	3.6	2.1	1.3	102.6
1	69.2	2.4	1.3	ND	11.5	8.7	5.2	2.5	102.0
3	35.7	5.0	4.8	2.2	20.8	14.1	9.8	7.6	101.5
3	27.9	7.8	5.3	ND	22.5	13.2	8.8	13.1	100.7
7	21.2	6.8	1.3	2.1	25.0	15.6	11.1	13.1	99.7
7	21.7	7.0	1.7	2.2	23.6	15.9	11.8	11.3	99.2
14	0.5	17.8	1.7	9.6	6.2	5.3	3.3	41.1	95.0
14	0.8	17.7	5.6	4.0	8.8	7.0	4.1	44.0	96.5
21	1.1	13.8	3.0	1.4	16.3	9.7	7.7	34.1	94.4
21	1.3	13.1	2.2	2.6	14.1	9.0	7.5	34.5	92.5
30	ND	15.4	2.1	2.2	10.1	9.4	5.7	46.3	93.5
30	ND	12.8	3.2	0.7	ND	1.6	0.8	67.8	90.3
Dark control									
30	98.5	ND	ND	ND	ND	ND	ND	ND	98.8
30	99.0	ND	ND	ND	ND	ND	ND	ND	100.6

* largest single unknown = 6.9%, ND = Not detected

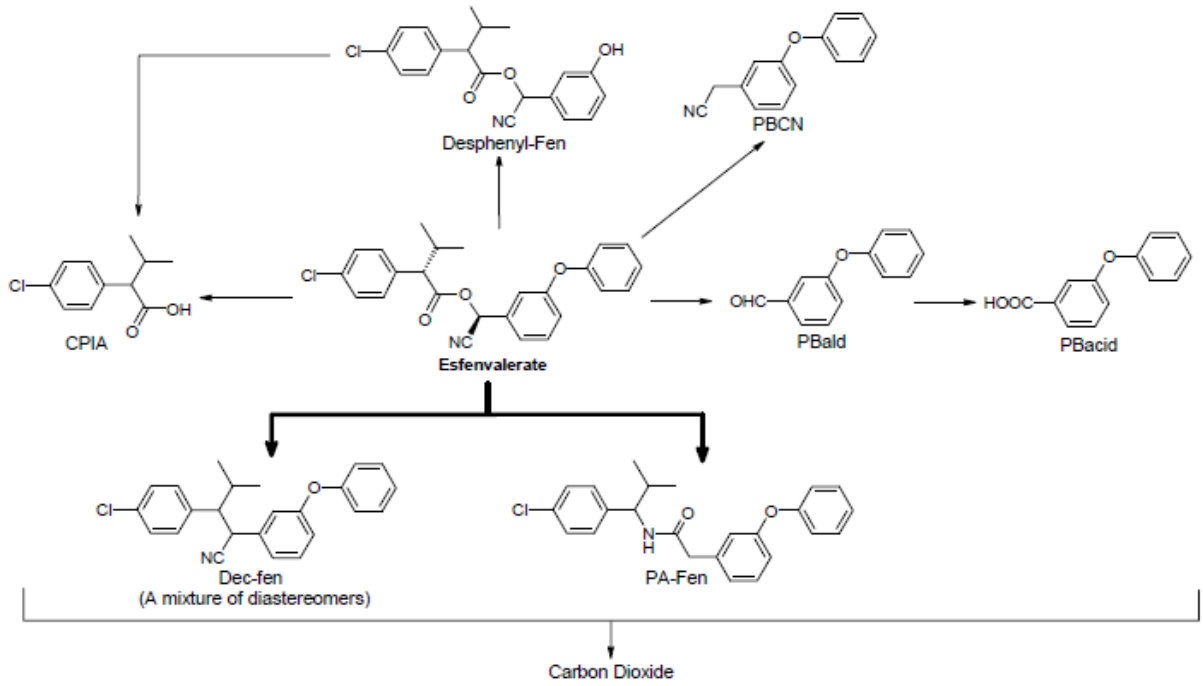
Table B.8.4-5 Product balance following the aqueous photolysis of [chlorophenyl-^{14}C]esfenvalerate in pH 4 buffer

Time (days)	esfenvalerate	CPIA	Desphenyl -fen	Dec-fen A	Dec-fen B	PA-Fen	Total unknown*	Total in buffer
0	101.7	ND	ND	ND	ND	ND	ND	102.7
0	103.1	ND	ND	ND	ND	ND	ND	104.4
1	72.9	1.1	ND	10.9	7.7	4.7	3.8	102.5
1	70.0	2.2	ND	13.9	8.7	5.1	2.0	102.5
3	24.7	5.3	ND	24.3	15.5	10.8	20.2	100.9
3	31.2	5.0	ND	21.2	13.1	9.8	17.5	99.4
7	7.6	8.6	1.8	22.6	13.7	7.8	36.3	99.5
7	16.0	6.3	0.8	24.1	14.3	11.1	25.2	99.2
14	0.5	7.3	10.8	6.3	5.2	4.2	60.3	95.7

Time (days)	esfenvalerate	CPIA	Desphenyl -fen	Dec-fen A	Dec-fen B	PA-Fen	Total unknown*	Total in buffer
14	ND	7.6	6.4	7.3	6.7	5.8	60.6	97.7
21	1.7	6.5	5.2	15.8	10.3	8.0	45.9	94.3
21	ND	8.1	5.0	3.4	3.1	2.2	70.9	94.1
30	ND	9.8	1.7	1.2	1.6	0.8	72.4	89.9
30	ND	8.6	1.4	0.8	1.0	ND	77.6	93.8
Dark control								
30	93.8	ND	ND	ND	ND	ND	ND	94.5
30	97.2	ND	ND	ND	ND	ND	ND	98.2

* largest single unknown = 9.8%, ND = Not detected

Figure B.8.4-3 Proposed photolysis degradation route of esfenvalerate (Graham and Dove, 2012)



The SFO DT_{50} value for esfenvalerate under irradiated conditions equivalent to UK/US summer sunlight was 2.0 days (Chi^2 error = 9.51).

[Study 3]

Study reference:

Y. Suzuki, T. Fujisawa, T. Katagi (2012) Esfenvalerate: Calculation of aqueous photolysis rates in near surface water at north latitudes 10º and 80º using GCSOLAR programme. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLP-0103

Study summary and results:

The aqueous photolysis rate of esfenvalerate in near surface water at northern latitudes of 10º and 80º were calculated using the GCSOLAR (ver. 1.2) programme.

Test type:

GLP: No (calculation only)

Test substance:

Not applicable

Materials and methods:

The aqueous photolysis rate of esfenvalerate in near surface water at northern latitudes of 10 to 80ºN were calculated using the GCSOLAR (ver. 1.2) programme. The half-lives were calculated as a function on season, latitude, time of day, depth of water bodies and ozone layer thickness. The input data were obtained from the report on photodegradation and quantum yield in sterile, aqueous solution of esfenvalerate (Graham and Dove, 2012).

Results:

The photolytic half-life values of esfenvalerate at latitudes 10 to 80ºN in the four seasons are presented below.

The calculated photolytic half-lives of esfenvalerate were 1.28 to 1.36 days at latitudes of 30, 40 and 50ºN in summer.

Half-life of esfenvalerate at latitude 10 to 80 in the four seasons - spring, summer, autumn and winter

Latitude (º)	**Half-life in days**			
	Spring	**Summer**	**Autumn**	**Winter**
10	1.37	1.37	1.52	1.71
20	1.38	1.30	1.71	2.08
30	1.44	1.28	2.04	2.78
40	1.55	1.30	2.65	4.31
50	1.76	1.36	3.94	8.47
60	2.13	1.46	7.27	24.1
70	2.80	1.60	19.4	NC
80	3.90	2.06	NC	NC

NC – not computed because number is too large (out of range)

4.1.4 Other degradability studies

[Study 1]

Study reference:

C. Lewis (1995) [14C]-Esfenvalerate: Biodegradation in natural water/sediment systems at 10ºC.. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-0040

Study summary and results:

The degradation of esfenvalerate was investigated in two aquatic systems using one radiolabelled form of esfenvalerate. The systems were set up with natural sediment and associated waters, and CO_2 free air was bubbled gently into the water layer. Suitable traps for collecting volatile compounds were connected to the system. The systems were equilibrated for up to 76 days, before esfenvalerate was applied to the water layer. Samples were incubated for up to 100 days at 10ºC in the dark.

At nine appropriate time point, duplicate flasks were taken and the water and sediment separated. The water layer was partitioned with dichloromethane concentrated to dryness and re-constituted in

methanol. The sediment was extracted with acetone and then extracted by Soxhlet. The resulting extract was acidified, partitioned with dichloromethane, then concentrated to dryness and re-constituted in methanol.

Radioactivities in the aqueous and combined organic phase from surface water as well as the radioactivity from the sediment were determined by LSC. Evolved volatile radiolabelled material was also collected for analysis at the same sampling intervals and the radioactivity was determined by LSC. In both systems esfenvalerate and its degradation products were determined by TLC and HPLC. DT_{50} values for the total systems were determined from the intersection point of the 50% line with the plot of mean esfenvalerate concentrations. DT_{90} values were estimated by linear regression analysis using the 0, 14, 30, 61 and 100 day time points.

Recoveries in the two systems were 80.74% to 98.51% of applied radioactivity. The lower recoveries at 0.25 to 14 days were attributed to loss of radioactivity on dip tubes and probes.

In two water - sediment systems esfenvalerate dissipated very rapidly from the water phase (first order DT_{50} 5.3 to 8.9 days, not taking into consideration that >50% esfenvalerate had already partitioned into prior to the t=0 sampling point). This was due to both partitioning into sediment and to degradation. For both aquatic systems after 100 days, water contained mainly CPIA (44% to 48% AR) with small amounts of esfenvalerate (2.7% to 3.4% AR) and sediments contained mainly esfenvalerate (26% to 27% AR) with only small amounts of CPIA (4.1% to 5.4% AR). A number of minor degradation products were also produced but in relatively small amounts. Low levels of volatile radioactivity were evolved, one of the components probably being carbon dioxide. Low levels of bound residues were formed.

Test type:

Proposed UK guideline for the Conduct of Biodegradability T**e**sts on Pesticides in Natural Sediment-Water Systems

GLP: Yes (certified laboratory)

Test substance:

[Chlorophenyl-^{14}C]-esfenvalerate (>97.5% radiochemical purity, 164 µCi/mg specific activity)

Materials and methods:

Two systems (Mill Stream Pond and Site B, Table 4.1.4-7) were set up containing natural sediment (2.5 cm depth) and associated water (25 cm depth) and CO_2-free air was bubbled gently into the water layer. A security trap, followed by an Amberlite XAD-2 trap, a paraffin (2%) in xylene trap and two ethanolamine traps for collecting volatile compounds were connected to the system. The flasks were allowed to equilibrate for up to 69-76 days in the dark at 10 ± 2ºC while being slightly agitated on an orbital shaker. Esfenvalerate was added to the water phase at *ca* 8 µg/unit, a rate equivalent to 50 g a.i./ha, and the flasks were incubated in the dark at 10 ± 2ºC for up to 100 days (Table B.8.4-7a).

Table B.8.4-7a Characterisation of sediments

Parameter	**Mill stream pond**	**Site B**
Particle size distribution		
63 µm to 2 mm (sand)	20.3	1.7
2 µm to 63 µm (silt)	53.1	34.2
< 2 µm (clay)	26.6	64.0
UK Texture Classification	Clay loam	Clay
pH (1:2.5) extract in water	7.6	7.3
pH (1:2.5) extract in 1M KCI	7.2	7.0
Organic Carbon (%)	5.8	1.2

Parameter	Mill stream pond	Site B
Cation exchange capacity (CED) (mEq/100g)	43.0	32.9
Total nitrogen (%)	0.68	0.19
Total phosphorus (%)	0.25	0.11
Dry mass (%)	22.82	41.46

At nine appropriate time points (0, 6, 24 and 48 hours and 7, 14, 30, 61 and 100 days after application) duplicate flasks were taken and the water and sediment separated. The water layer was acidified with HCl (1M, 45 mL) and partitioned with dichloromethane (3 times, *ca* 100 mL), concentrated to dryness and re-constituted in methanol. The sediment was extracted with acetone (3 times, 15 mins, *ca* 100 mL), then extracted by Soxhlet (*ca* 18 hrs) with acetone. The resulting extract was acidified with HCl (1M, 3 mL) and partitioned with dichloromethane (3 times, *ca* 50 mL), concentrated to dryness and re-constituted in methanol.

Radioactivity in the aqueous and combined organic phase from surface water as well as the radioactivity from the sediment were determined by LSC. Evolved volatile radiolabelled material was also collected for analysis at the same sampling intervals and the radioactivity was determined by LSC. In both systems esfenvalerate and its degradation products were determined by 2D-TLC and HPLC. DT_{50} values for the total systems were determined from the intersection point of the 50% line with the plot of mean esfenvalerate concentrations. DT_{90} values were estimated by linear regression analysis using the 0, 14, 30, 61 and 100 day time points.

Results:

Recoveries in the two systems were 80.74% to 98.51% of applied radioactivity. The lower recoveries at 0.25 to 14 days were attributed to loss of radioactivity on dip tubes and probes. The distribution of radioactivity for both systems are shown in Tables B.8.4-7b and B.8.4-7c.

In the Mill stream pond aquatic system, the proportion of applied radioactivity in surface water decreased from 33% immediately after application to 15% after 24 hours and increased to 51% after 100 days. In site B aquatic system, levels of radioactivity were variable and in the vicinity of 18 - 45% during the first seven days after application but then increased steadily to 55% after 100 days.

Radioactivity in the sediment extract of Mill stream pond aquatic system was 61% AR immediately after application, rising to 70%AR after 24 hours but then decreasing to 34% AR after 100 days. Levels in the sediment extract from site B aquatic system were 75% AR at time zero, falling to 35% AR after 6 hours; rising to 70% AR at seven days and falling again to 33% AR after 100 days.

Radioactivity not extracted from the sediment was low for both systems (< 5% AR). For both water sediment systems the mean total volatile radioactivity accounted for 5.2% and 3.2% AR for Mill stream pond and site B, respectively.

In Mill stream pond aquatic system, esfenvalerate levels decreased from 91% AR at time zero to 30% ARafter 100 days. There was a corresponding increase in CPIA during this period from 0.4% to 48% AR after 100 days. The compound 4'-OH-fen increased to a maximum of 5% AR at 14 days then decreased to 4% after 100 days. Minor levels of desphenyl-fen (up to 0.5% AR) and seven unknown compounds were also detected by TLC. Unknown compounds accounted for < 1.5%AR individually and < 2.5% AR in total. Results from site B aquatic system were similar. Levels of esfenvalerate decreased from 93% AR at time zero to 29% AR after 100 days while levels of CPIA increased from 0.5% to 54% AR over the same period. 4'-OH-fen (maximum of 6% AR), desphenyl-fen (maximum of 0.5%AR) and six unknown compounds (total maximum < 1.5% AR) were detected also.

For both aquatic systems after 100 days, water contained mainly CPIA (44% - 48% AR) with small amounts of esfenvalerate (2.7 - 3.4% AR) and sediments contained mainly esfenvalerate (26 - 27% AR) with only small amounts of CPIA (4.1 - 5.4% AR).

Esfenvalerate quickly re-distributed into the sediment phase of the aquatic systems at 10ºC where it was hydrolysed to CPIA. A number of minor degradation products were also produced but in relatively small amounts. Low levels of volatile radioactivity were evolved being one of the components probably carbon dioxide. Low levels of bound residues were formed.

Table B.8.4-7b Characterisation of radioactivity within the Mill Stream pond system following [chlorophenyl-^{14}C]esfenvalerate application (mean of duplicates)

	% of the applied radioactivity								
	Days after application								
	0	**0.25**	**1**	**2**	**7**	**14**	**30**	**61**	**100**
Organic Volatiles	NA	0.17	0.71	0.54	1.41	1.57	1.52	2.15	2.95
CO_2	NA	ND	ND	ND	0.16	0.81	0.39	0.40	2.26
Aqueous phase	33.67	32.22	14.83	17.37	20.19	19.94	31.98	40.35	50.93
Esfenvalerate	30.30	28.68	12.87	13.01	14.73	3.95	4.31	4.12	2.65
CPIA	0.42	0.61	1.62	2.51	4.57	14.75	24.44	34.84	44.04
4'-OH-fen	ND	ND	ND	0.19	0.25	0.46	0.45	0.45	0.84
Desphenyl-fen	ND	ND	ND	ND	0.32	0.45	0.53	0.31	ND
Sediment phase extract	61.42	56.38	70.38	68.36	64.32	68.01	57.98	50.06	33.71
Esfenvalerate	60.91	55.32	68.70	66.88	61.49	59.73	52.02	44.12	27.12
CPIA	ND	ND	ND	ND	0.66	2.12	1.46	2.97	4.06
4'-OH-fen	ND	0.43	0.66	1.02	1.39	4.53	4.10	2.35	3.02
Desphenyl-fen	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unextractable radioactivity	1.5	0.19	0.11	0.34	0.41	1.32	0.57	0.79	1.33
Unit rinse	0.25	ND	0.73	0.50	0.71	1.13	2.00	2.61	3.44
Total	96.84	88.94	86.76	87.10	87.17	92.76	94.42	96.35	94.59

ND - not detected; NA – not analysed

Up to 5 unknown components were additionally found but each accounted for <1.5% AR in the whole system

Table B.8.4-7c Characterisation of radioactivity within the Site B system following [chlorophenyl-^{14}C]esfenvalerate application (mean of duplicates)

	% of the applied radioactivity								
	Days after application								
	0	**0.25**	**1**	**2**	**7**	**14**	**30**	**61**	**100**
Organic Volatiles	NA	0.03	0.27	0.40	0.34	0.91	0.97	1.11	1.66
CO_2	NA	ND	ND	ND	0.13	0.87	0.49	0.92	1.51
Aqueous phase	22.99	45.24	27.61	37.46	18.37	20.34	30.35	33.94	55.16
Esfenvalerate	20.19	41.90	20.66	32.20	9.07	10.66	8.59	4.61	3.42
CPIA	0.46	0.95	5.19	3.08	7.43	8.84	17.22	26.66	48.44
4'-OH-fen	ND	ND	ND	0.54	0.56	0.41	0.86	0.65	0.47
Desphenyl-fen	ND	ND	ND	0.24	0.30	0.07	0.50	0.25	ND
Sediment phase extract	75.48	35.44	55.34	47.05	70.03	65.01	61.80	57.69	33.49
Esfenvalerate	72.82	35.82	53.28	46.17	66.12	62.04	53.79	49.74	25.59
CPIA	ND	ND	ND	ND	0.64	0.50	2.04	2.80	5.36
4'-OH-fen	ND	ND	0.38	0.69	2.01	2.14	4.76	4.60	2.75
Desphenyl-fen	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unextractable radioactivity	0.04	0.04	0.10	0.09	0.39	0.20	0.89	1.09	3.65
Unit rinse	ND	ND	0.18	ND	ND	0.55	1.74	2.21	1.46
Total	98.51	80.74	83.49	85.00	89.24	87.87	96.23	96.94	96.91

ND - not detected; NA – not analysed
Up to 5 unknown components were additionally found but each accounted for <1.5% AR in the whole system

The rates of dissipation/degradation of esfenvalerate in both systems are shown in Table B.8.4-7d.

Table B.8.4-7d Rates of dissipation/degradation of esfenvalerate (days)

System	**Water**				**System**			
	Kinetic method	**DT_{50}**	**DT_{90}**	**r^2**	**Kinetic method**	**DT_{50}**	**DT_{90}**	**r^2**
Mill Stream Pond	observation	-	<14	-	observation	ca 54	212^{+}	-
Site B	observation	-	<30	-	observation	ca 68	215^{+}	-

$^{+}$ rates of degradation calculated by linear regression of selected datapoints

In two water/sediment systems esfenvalerate dissipated very rapidly from the water phase (first order dissipation DT_{50} 5.3 to 8.9 days, not taking into consideration that >50% esfenvalerate had already partitioned into prior to the t=0 sampling point)). This was due to both partitioning into sediment and to degradation.

[Study 2]

Study reference:

T. Jarvis, A. Mamouni (2011c) Recalculation of esfenvalerate sediment water kinetics according to FOCUS (2006) guidance. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-0059

Study summary and results:

A kinetic re-evaluation of the water sediment studies (Takahashi & Oshima, 1988; Lewis, 1995) was undertaken in accordance with FOCUS kinetics guidance (2006). The first study (Takahashi & Oshima, 1988) was not performed to current guidance and does not meet the data requirements. Therefore the results from this study have not been summarised.

The assessment on visual and statistical fit, residual plot and χ^2 error was undertaken using the recommendations of FOCUS kinetics guidance (2006). Kinetic modelling was performed using KinGUI version 2.0.

In the overall water/sediment systems at 10°C the first order DT_{50} and DT_{90} values ranged from 65.3 to 79.4 days, and 216.9 to 263.3 days, respectively, demonstrating significant degradation even in the sediment phase. The DT_{50} values (normalised to 20ºC) ranged from 25.3 to 30.7 days.

Test type:

GLP: No (calculation only)

Test substance:

[Chlorophenyl-^{14}C]-esfenvalerate

Materials and methods:

A kinetic re-evaluation of the water sediment studies (Takahashi & Oshima, 1988; Lewis, 1995) in accordance with FOCUS kinetics guidance (2006). The first study (Takahashi & Oshima, 1988) was not performed to current guidance and does not meet the data requirements. Therefore the results from this study have not been summarised.

The assessment on visual and statistical fit, residual plot and χ^2 error using the recommendations of FOCUS kinetics Guidance (2006). Kinetic modelling was performed using KinGUI version 2.0.

Since only *ca* 30% radioactivity remained in the water phase at the day 0 sampling point, then P-I water dissipation rates were not determined.

Results:

The results of esfenvalerate distribution and degradation over time are shown in Table B.8.4-8.

Table B.8.4-8 Distribution of esfenvalerate following incubation of [chlorophenyl-^{14}C]esfenvalerate in Millstream or System B water/sediment system

Time (days)	Millstream			Time (day)	System B		
	Water	Sediment	Total		Water	Sediment	Total
0	26.27	63.18	89.45	0	24.01	71.53	95.54
0	34.34	58.63	92.97	0	16.38	74.10	90.48
0.25	27.56	59.06	86.62	0.25	46.04	35.23	81.27
0.25	29.80	51.57	81.37	0.25	37.76	36.40	74.16
1	15.45	66.30	81.75	1	19.75	58.49	78.24
1	10.28	71.10	81.38	1	21.57	48.08	69.65
2	10.16	68.07	78.23	2	36.40	44.83	81.23
2	15.87	65.68	81.55	2	28.01	47.50	75.51

Time (days)	Millstream			Time (day)	System B		
	Water	Sediment	Total		Water	Sediment	Total
7	15.28	65.35	80.63	7	9.07	66.59	75.66
7	14.19	57.62	71.81	7	n.s.	65.66	n.s.
14	6.30	64.28	70.58	14	4.53	65.75	70.28
14	1.60	55.18	56.78	14	16.78	58.34	75.12
30	6.32	71.16	77.48	30	7.63	45.59	53.21
30	2.30	32.88	35.18	30	9.55	61.99	71.54
61	3.61	29.88	33.49	61	1.85	50.11	51.96
61	4.64	58.37	63.01	61	7.37	49.37	56.74
100	2.27	14.99	17.27	100	4.95	26.64	31.59
100	3.03	39.25	42.28	100	1.88	24.54	26.42

For the Millstream pond and System B SFO kinetics provided an acceptable fit with no systematic bias of the residuals, even though there were significant differences in the replicate values at some timepoints. For the Pond and River systems, clear bias was shown with SFO kinetics. Improved fit was afforded by DFOP which was chosen. The kinetic fits are presented in Table B.8.4-9. The best fit kinetic is shown in bold. The visual fits are presented in Figures B.8.4-5 and B.8.4-6.

Table B.8.4-9 Summary of results of the kinetic determinations for esfenvalerate in sediment/water systems (P-I)

Parameter	Millstream system	System B
Model	**SFO**	**SFO**
$\chi 2$ error (%)	5.4	6.7
k (day^{-1}) *	0.0106 ($7.0x10^{-5}$)	0.00875 ($1.1x10^{-6}$)
DT_{50} (days)	**65.3**	**79.3**
DT_{90} (days)	216.9	263.3
Model	FOMC	FOMC
$\chi 2$ error (%)	4.3	6.9
α Lower CI Upper CI	0.6150 -0.7053 1.935	151.7 $-2.790x10^{-3}$ 3093.4
β Lower CI Upper CI	28.871 -74.948 132.69	17310 $-3.191x10^{+5}$ $3.5368x10^{+5}$
DT_{50} (days)	60.2	79.3
DT_{90} (days)	>1000	264.7
Model	DFOP	DFOP
$\chi 2$ error (%)	3.6	7.1
k1*	0.2230 (0.3927)	0.00875 ($5.4x10^{-6}$)
k2*	0.00833 (0.0307)	0.000334 (NA)

Parameter	Millstream system	System B
g*	0.1530 (0.1956)	1 (NA)
DT_{50} (days)	63.3	79.3
DT_{90} (days)	256.6	263.3

*P value from the t-test is given in brackets.

Figure B.8.4-5 Millstream system SFO kinetics (χ2 error 5.4%)

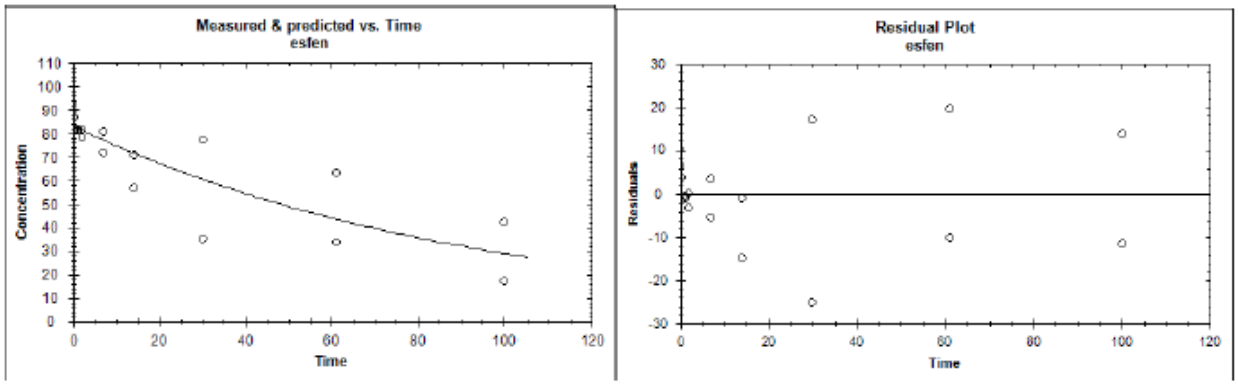
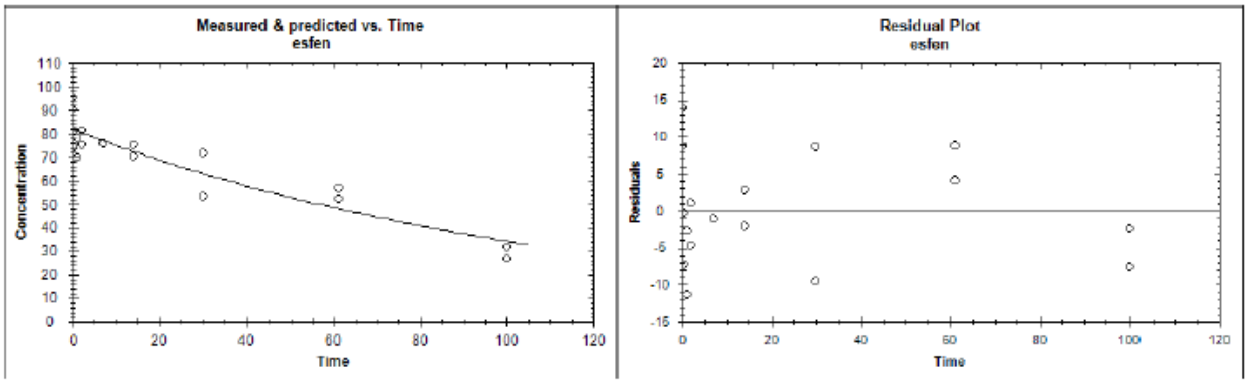


Figure B.8.4-6 System B - SFO kinetics (χ2 error 6.7%)



Since the water sediment studies were conducted at 10°C, the results were normalised to 20°C using the Arrhenius constant value of 65.4 kJ/mol. Since the Arrhenius constant was determined from soil studies, it is not entirely appropriate to use this value. Since the FOCUS models used in calculation of PECsw are not sensitive to the whole system DT_{50}, during the EU review it was considered that it was acceptable to use the normalised DT_{50} values in modelling and that it would not significantly affect the outcome of the modelling. The whole system DT_{50} values normalised to 20°C are presented in Table B.8.4-10.

Table B.8.4-10 Normalisation of P-I DT_{50} values for whole system

Parameter	Incubation Temperature (°C)	Kinetic Model	DT_{50} (day) as SFO	DT_{50} normalised to 20°C (day)
Millstream system	10	SFO	65.3	25.3
System B	10	SFO	79.3	30.7

Since esfenvalerate remains predominantly in the sediment, in line with FOCUS guidance, it is appropriate to use the geometric mean DT_{50} value from the whole system as a surrogate for the

sediment phase. In this case the DT_{50} in the water phase is set as the default value of 1000 days (Note: This is not relevant for aquatic hazard classification).

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

[Study 1]

Study reference:

Anonymous (1991) Accumulation and Metabolism of ^{14}C-Esfenvalerate in Carp (*Cyprinus carpio*). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLM-10-0031

Study summary and results:

A dynamic 42-day study (28 days exposure and 14 days depuration phases) was conducted to evaluate the bioconcentration potential of **^{14}**C-esfenvalerate to carp (*Cyprinus carpio*). On the basis of the results from this study, it can be concluded that esfenvalerate is rapidly taken up, extensively metabolised and readily eliminated by carp. Residues of total radioactivity and total fenvalerate isomers in whole fish at the end of the exposure period with [^{14}C-chlorophenyl] esfenvalerate were 161 and 110 μg/l, respectively (bioconcentration factors after 28 days exposure were 2850 and 3110, respectively). Residues of total radioactivity and total fenvalerate isomers in these fish at the end of the exposure period with [^{14}C-phenoxyphenyl] esfenvalerate were 225 and 168 μg/l, respectively (bioconcentration factors after 28 days exposure were 3340 and 3650, respectively). After transfer to untreated water there was a rapid decrease in levels of total radioactivity and total fenvalerate isomer residues in the fish with time. The percentage of total radioactivity and total fenvalerate isomers eliminated after 14 days depuration were 74.4% and 68.7% for [^{14}C-phenoxyphenyl] esfenvalerate and 76.5% and 69.3% for [^{14}C-chlorophenyl] esfenvalerate, respectively. The depuration half-lives for total radioactivity and total fenvalerate isomers were calculated to be 6.89 and 7.80 days for [^{14}C-phenoxyphenyl] esfenvalerate and, 6.50 and 7.88 days for [^{14}C-chlorophenyl] esfenvalerate, respectively.

Test type:

USA EPA 165 – 4; not GLP compliant

Test substance:

[**^{14}**C-phenoxypheny] esfenvalerate; Lot No. C-85-068; Specific Activity; 60.3 mCi/mmol; Purity 99.4% (radiochemical) and >99% (chemical)

[**^{14}**C-chlorophenyl] esfenvalerate; Lot No. C-85-078; Specific Activity; 53.8 mCi/mmol; Purity 99.4% (radiochemical) and >99% (chemical)

Materials and methods:

A dynamic 42-day study (28 days exposure and 14 days depuration phases) was conducted to evaluate the bioconcentration potential of **^{14}**C-esfenvalerate to carp (*Cyprinus carpio*). Groups of 42 fish were exposed to either [**^{14}**C-phenoxypheny] or [**^{14}**C-chlorophenyl] esfenvalerate being respectively 60.3 and 53.8 μCi/mmol the specific activity. The radiochemical purity of both samples was 99.9% and chemical purity > 99%.

Fish were exposed the nominal concentration of 0.1 μg/l in a flow through system at 25ºC for 28 days. After 1, 3, 7, 10, 14, 21 and 28 days of exposure groups of three fish were sampled for analysis of radioactivity in the whole fish. Three additional fish were removed and dissected for analysis of radioactive residues in viscera and carcass after 28 days exposure. Water samples were also removed

on these days and additionally on days 17 and 24 for determination of metabolites. After 28 days exposure the remaining 18 fish were transferred to tanks containing clean water for a further 14 days. Groups of fish were sampled after 1, 3, 7, 10 and 14 days of depuration for analysis of radioactivity in the whole fish. Water quality parameters (temperature and pH) were measured throughout the study and the level of radioactivity in the water was monitored daily.

Fish were observed daily for mortality and any adverse behaviour throughout the uptake and depuration phase.

Extractable radioactivity was measured by LSC and unextractable radioactivity was measured by LSC after combustion. Radioactivity was characterised on TLC plates by comparison with reference materials and quantified by LSC.

The bioconcentration factors (BCF), uptake rate constant (k1) and depuration rate constant (k2) were determined by computer analysis.

Results:

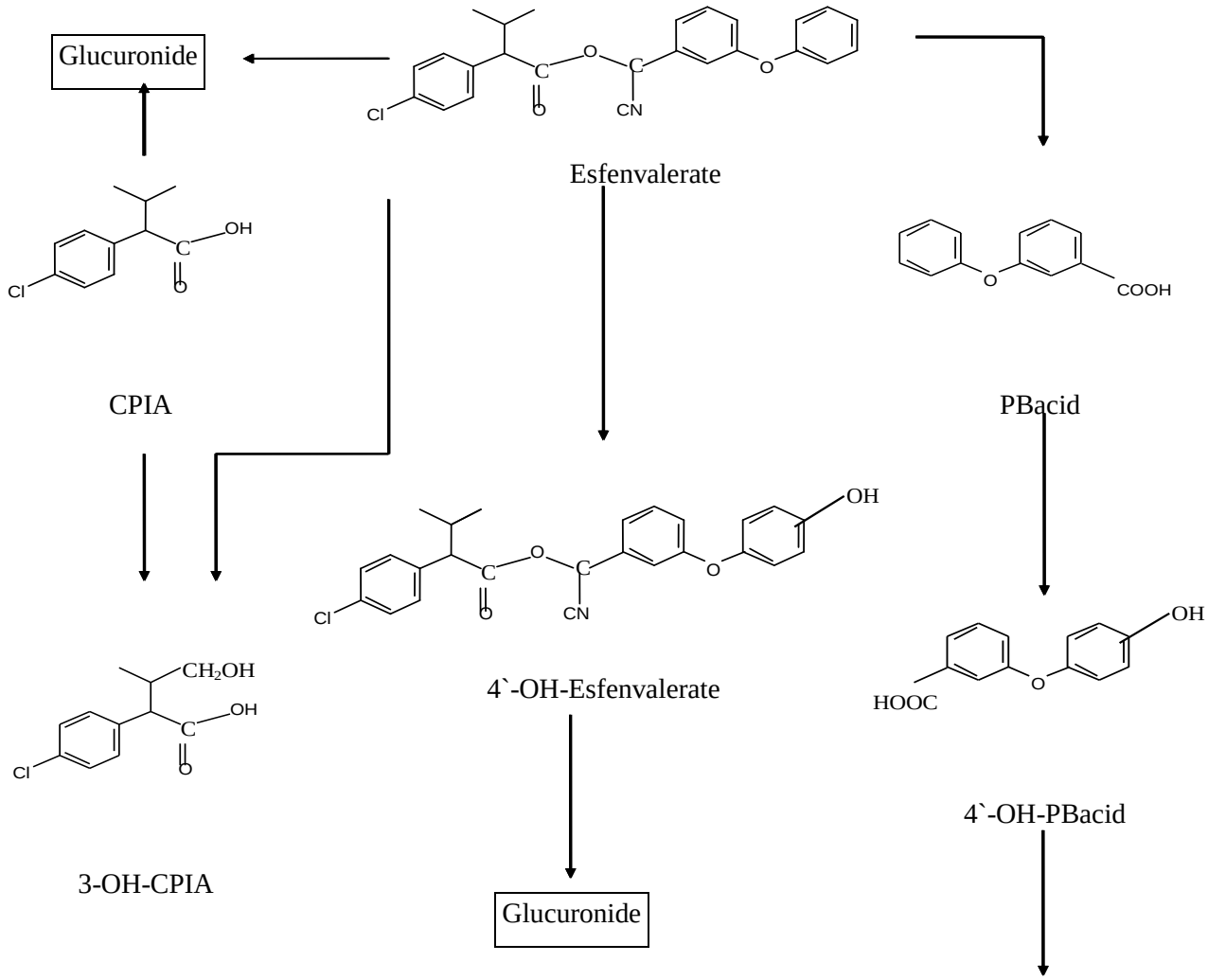
No abnormal behaviour or mortalities were observed throughout the study. The actual concentration of esfenvalerate remained relatively constant during the study (0.035 - 0.061 µg/l). A rapid initial uptake of radioactivity by fish was observed during the first 7 days of exposure. The level of [^{14}C-chlorophenyl] esfenvalerate reached a "plateau" and was relatively stable from 7 to 28 days of exposure. Residues of total radioactivity and esfenvalerate in the whole fish after 28 days of exposure were 161 and 110 µg/kg, respectively, and the corresponding bioconcentration factors (BCF) were 2850 and 3110.

Radioactivity in fish exposed to [^{14}C-phenoxypheny] esfenvalerate increased throughout the entire exposure period. Residues of total radioactivity and esfenvalerate in whole fish at the end of the exposure period were 225 and 168 µg/kg, respectively. The corresponding BCF values were 3340 and 3650. When compared, the tissue residue level and the bioconcentration factors between the two radiolabelled materials, revealed no significant difference.

After transfer of fish to untreated water there was a rapid decrease in levels of ^{14}C and esfenvalerate residues in fish with time. The percentage of total radioactivity and esfenvalerate eliminated after 14 days depuration were respectively 74.4 and 68.7% for phenoxypheny-labelled material and 76.5 and 69.3% for chlorophenyl-labelled material. The depuration half-lives for total radioactivity and esfenvalerate were calculated to be 6.89 and 7.80 days for phenoxypheny-labelled material and 6.50 and 7.88 days for chlorophenyl-labelled material. The uptake rate constant (k_1) was in the range 317 - 495 µg/kg/day and the depuration rate constant (k_2) was calculated to be 0.1 day^{-1}

Esfenvalerate in the water accounted for approximately 60% of the radioactivity. The major degradation products identified were: PBacid (up to 10% AR), 4`- OH-Esf (up to 12% AR) and CPIA (up to 21% AR). Total esfenvalerate accounted for 40 - 75% of the total radioactive residues in the whole fish. The major metabolites present were: 4`-OH-Esf glucoronide (< 0.045ppm), 4`-OH-Esf (< 0.007ppm), 4`-OH-PBacid-sulfate (< 0.008ppm) and CPIA (< 0.026ppm). For molecular structure and chemical name of compounds see Annex δ. None of these metabolites was present in the whole fish at levels of 0.05 ppm or greater.

Figure B.4.2.1-1: Proposed Metabolic Pathways for Esfenvalerate in Carp (Anonymous, 1991)



The highest levels of radioactivity were found in viscera (661 - 1094 ppb) in comparison to the remaining carcass (95 - 136 ppb). In these tissues, esfenvalerate amounted to 69 - 91% and 12 - 18% of total radioactivity residues in the carcass and viscera, respectively. The presence of these metabolites indicates that the metabolic pathway for esfenvalerate in fish involves oxidation at the 4`-position of the alcohol moiety and 3-position of the acid moiety, cleavage of the ester linkage and conjugation of the resultant phenol and acid with glucuronic acid and sulphuric acid. Figure 1 presents the proposed metabolic pathway of esfenvalerate in carp.

This study showed that esfenvalerate is rapidly taken up, extensively metabolised and readily eliminated by fish with a half-life of approximately 7 - 8 days.

4.2.2 Bioaccumulation test with other organisms

[Study 1]

Study reference:

H. Ohkawa, R. Kikuchi, J. Miyamoto (1980) Bioaccumulation and biodegradation of the (*S*)-Acid isomer of fenvalerate (Sumicidin) in an Aquatic Model Ecosystem. Published; *J. Pestic. Sci.* **5**, 11-22 (1980), Sumitomo Chemical Co., Ltd. report No.: AM-00-0108

Study summary and results:

Carp were exposed to ^{14}CN-S-fenvalerate for 7 days. Additionally, two model ecosystem studies in a semi-field design (water-sediment system) were carried out in which carp as well as *Daphnia* and field collected snails were added. On the basis of the results from these studies, it can be concluded that (*S*)-fenvalerate (esfenvalerate and its [2*S*, α*R*] isomer) is rapidly taken up, extensively metabolised and readily eliminated by carp. Also, the bioconcentration factors for (*S*)-fenvalerate in fish is significantly reduced when more realistic exposure conditions of a water-sediment system are used, even when taking into account the possibility of food-chain magnification (algae - daphnids - fish in this test system).

Test type:

Guideline and GLP compliance not specified in study report

Test substance:

^{14}CN-S-fenvalerate; Specific Activity; 15.7 mCi/mmol; Purity >99% (radiochemical)

^{14}CO-S-fenvalerate; Specific Activity; 5.0 mCi/mmol; Purity >99% (radiochemical)

^{14}CH-S-fenvalerate; Specific Activity; 20.0 mCi/mmol; Purity >99% (radiochemical)

Materials and methods:

The studies were conducted with three ^{14}C-labelled preparations of S-fenvalerate: ^{14}CN-S-fenvalerate (specific activity 15.7 μCi/mmol), ^{14}CO-S-fenvalerate (specific activity 5.0 μCi/mmol) and ^{14}CH-S-fenvalerate (specific activity 20.0 μCi/mmol), and the radiochemical purity was > 99%. Sorpol 3005 and xylene, diluted in water or mixed with soil were used as vehicles.

Ten carp (*Cyprinus carpio*) of 8 - 20 g weight were exposed to ^{14}CN-S-fenvalerate at 24 ± 2ºC for 7 days at a nominal concentration of 0.8 μg/l. During a 7-day exposure period fish were transferred to freshly prepared solutions at 6 and 24 hours after their addition and then at 24 hour intervals. After a 7-day exposure period, some of the fish were transferred to clean water and held for further 25 days with the fish again being sampled at intervals for analysis. Water samples were taken before and after replacement of the test solution for analysis of radioactivity.

Additionally, two model ecosystem studies in a semi-field design were carried out. In one set of experiments air-dried sandy loam soil was treated with ^{14}CN-S-fenvalerate at a rate of approximately 0.3 ppm. After 7 day equilibration periods three carp were added as well as *Daphnia* (*Daphnia pulex*) and ten field collected snails (*Cipangopaludina japonica*). Water samples were taken at 2-day intervals for analysis. The ecosystem was terminated after 7 days. By this time fish, snails and daphnids were collected for residue analysis. In a further series of experiments the soil was treated with each of the three radio-labelled ^{14}C-S-fenvalerate preparations at the same rate and conditions as mentioned above and the ecosystem was terminated after 30 days.

Results:

In the first study all of the fish behaved normally and appeared to be in good condition throughout the experiment. The S-fenvalerate concentration declined to 0.5 - 0.6 μg/l immediately before the water changes. There was a rapid initial uptake of radioactivity by fish with [^{14}C] levels of 0.92 μg/kg S-fenvalerate equivalents after seven days (81% S-fenvalerate). The bioconcentration factor of S-

fenvalerate at this time, based on the measured 24 hour concentrations, was in the range 1245 - 1494 (1537 -1844 for total radioactivity). The levels of S-fenvalerate in fish were still increasing after seven days indicating that the "plateau" level of bioconcentration would be higher, although the rate of uptake had greatly slowed down. On transfer to clean water, the [^{14}C] level in fish decreased rapidly with a half-life of about five days. Approximately 87% of the incorporated [^{14}C] was eliminated from the fish body during the depuration period. S-fenvalerate accounted for about 81% and 89% of the [^{14}C] in fish during the exposure and depuration periods, respectively.

In the aquatic model ecosystem studies, S-fenvalerate and its degradation products were desorbed in part into water and then taken up into organisms either directly or through the food chain. The concentration of S-fenvalerate in the water, in an initial test using ^{14}CN-S-fenvalerate, after the 7-day equilibration period was 0.57 μg/l. The level of radioactivity in the various components of the system after the subsequent 7-day exposure period is shown in Table 4.2.1-1.

Table 4.2.1-1: Levels of ^{14}C and S-fenvalerate in Various Components of an Aquatic Model Ecosystem after 7 Days Exposure (Ohkawa *et al*, 1980)

fraction	S-fenvalerate equivalents (μg/l)					
	soil	water	fish	snail	*Daphnia*	Algae
S-fenvalerate	234	0.35	42.8	216	239	167
extractable ^{14}C	246	0.49	67	291	243	180
unextractable ^{14}C	30.5	< 0.01	10.5	60	3	10
total ^{14}C	277	0.49	86	320	246	190

The bioconcentration factor of S-fenvalerate in fish at seven days was 122 (176 for total radioactivity). The corresponding figures for snails, daphnids and algae were 617 (651), 683 (502) and 477 (386), respectively.

Similar results were obtained after 30-day exposure periods with ^{14}CN-, ^{14}CO- and ^{14}CH-labelled S-fenvalerate. The S-fenvalerate concentration in the water was down to 0.14 - 0.21 μg/l after 30 days.

Concentrations of S-fenvalerate in fish, snails, *Daphnia* and algae ranged from 34 to 42, 139 to 167, 103 to 132 and 100 to 248 μg/kg, respectively after 30 days of exposure. The bioconcentration factors in the various organisms in the aquatic model ecosystem, using the average water concentration over the 30 day exposure period, are shown in Table 4.2.1-2.

Table 4.2.1-2: Bioconcentration Factors for S-fenvalerate and Total ^{14}C in Various Organisms in an Aquatic Model Ecosystem after 30 Days Exposure (Ohkawa *et al*, 1980).

organism	^{14}CN		^{14}CO		^{14}CH	
	^{14}C	S-fen	^{14}C	S-fen	^{14}C	S-fen
fish	221	109	174	69	257	117
snail	472	491	-	-	428	386
Daphnia	191	303	229	269	234	322
algae	328	412	516	506	283	278

In conclusion S-fenvalerate was rapidly taken up by carp and readily excreted with a half-life of about 5 days. These results show that the potential bioaccumulation of esfenvalerate in fish in the semi-field system will be lower than under laboratory conditions, even when taking into account the possibility of food chain magnification.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

[Study 1]

Study reference:

Anonymous (1985k) The acute toxicities of esfenvalerate and S-5602 (fenvalerate) to bluegill (*Lepomis macrochirus*). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-50-0004

Detailed study summary and results:

Twenty bluegill sunfish (*Lepomis macrochirus*) juveniles were exposed to each solution of 0.14, 0.20, 0.28, 0.40 and 0.57 µg esfenvalerate/L, and of 0.5, 0.63, 0.79, 1.0, 1.26, 1.59 and 2.0 µg fenvalerate/L in a flow-through system for 96 hours. Mortalities were observed at 24-hr intervals, and the 96-hr LC_{50} values were found to be 0.21 µg/L (0.18 - 0.28 for 95% confidence limits) and 0.70 µg/L (0.66 - 0.74 for 95% confidence limits) for esfenvalerate and fenvalerate, respectively, based on nominal concentrations.

Test type:

Guideline not specified in study report; not GLP compliant

Test substance:

Esfenvalerate; lot No. 20601; purity not specified

S-5602 (fenvalerate); lot No. 30114; purity 94.7%

Materials and methods:

Juveniles (2.07 ± 0.38 mg, 55.9 ± 3.5 mm) of bluegill sunfish (*Lepomis macrochirus*) were exposed under flow through conditions at 25º ± 1ºC for 96 hours to esfenvalerate (purity not specified) and fenvalerate (94.7% purity), which were mixed with ten parts of Tween-80 solvent, and then diluted with dechlorinated tap water, to give nominal concentrations of 0.14, 0.20, 0.28, 0.40 and 0.57 µg esfenvalerate/L, and of 0.5, 0.63, 0.79, 1.0, 1.26, 1.59 and 2.0 µg fenvalerate/L.

Five fish were introduced to each glass aquarium (30 x 30 x 30 cm) containing 20 L of graded levels of the test solutions. A micro glass pump was used to deliver the chemical solution to the test aquarium at a flow rate of 40 L/day. There were four replicates for each test solution, solvent control and water control. Concentrations of esfenvalerate (0.2 µg/L) and fenvalerate (0.5 and 0.79 µg/L) were measured daily during the test. Fish were observed for mortality and symptoms of toxicity at 24-hr intervals for up to 96 hours and LC_{50} values were calculated by Probit analysis.

Results:

Results are based on nominal concentrations. The actual concentrations of esfenvalerate were between 80 and 105% of the nominal value of 0.2 µg/L throughout the study. For fenvalerate, the actual concentrations were between 64-72% of the nominal values of 0.5 and 0.79 µg/L.

The symptoms of toxicity noted for esfenvalerate and fenvalerate included abnormal respiration, loss of equilibrium, lethargy and death. Mortality was observed for all esfenvalerate concentrations (from 10% to 100%) and for fenvalerate concentrations above 0.5 µg/L (20% and 100%). Fish in the controls showed no mortality and no abnormal behaviour.

Table 4.3.1-1: Cumulative mortality data for bluegill (*Lepomis macrochirus*) exposed to esfenvalerate and fenvalerate (S-5602).

Compound	Nominal concentration (µg/L)	No. of fish	Cumulative mortality (%)			
			24-hr	48-hr	72-hr	96-hr
Esfenvalerate	0.14	20	0	0	5.0	5.0
	0.20	20	0	5.0	20.0	45.0
	0.28	20	0	30.0	75.0	100
	0.40	20	25.0	60.0	90.0	100
	0.57	20	65.0	100	100	100
Fenvalerate (S-5602)	0.50	20	0	0	0	0
	0.63	20	0	10.0	15.0	20.0
	0.79	20	0	10.0	45.0	85.0
	1.0	20	0	45.0	95.0	100
	1.26	20	5.0	90.0	100	100
	1.59	20	35.0	100	100	100
	2.0	20	95.0	100	100	100
Tween-80	20	20	0	0	0	0
Control	-	20	0	0	0	0

The 96-hr LC_{50} values of esfenvalerate and fenvalerate to bluegill sunfish were 0.21 µg/L (0.18 - 0.28 for 95% confidence limits) and 0.70 µg/L (0.66 - 0.74 for 95% confidence limits), respectively. No NOEC values were reported.

[Study 2]

Study reference:

Anonymous (1985l) Acute toxicity of MO 70616 technical to rainbow trout (*Salmo gairdneri*). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-51-0010

Detailed study summary and results:

Ten rainbow trout (*Salmo gairdneri*) juveniles were exposed to each solution of 0.032, 0.056, 0.10, 0.18 and 0.32 µg/L µg esfenvalerate/L, along with a dilution water and solvent control (acetone), in a static system for 96 hours. Mortalities and symptoms of toxicity were observed at 24-hr intervals. The 96-hr LC_{50} value were found to be 0.26 µg/L (0.20 - 0.38 for 95% confidence limits), based on nominal concentrations. The NOEC was determined to be 0.10 µg/L based on lack of mortality and abnormal effects.

Test type:

Guideline EPA 660/3-75-009 (1975); GLP compliant

Test substance:

Esfenvalerate; lot No.2-4-0-0; purity 98.8%

Materials and methods:

Juveniles (0.56 ± 0.17 g, 41 ± 3.3 mm) of rainbow trout (*Salmo gairdneri*) were exposed under static conditions at 12º ± 1ºC to esfenvalerate (98.8% purity) for 96 hours at nominal concentrations of 0.032, 0.056, 0.10, 0.18 and 0.32 µg/L, along with a dilution water and acetone control.

Ten fish were introduced to each glass vessels containing 15 L of soft reconstituted water prepared to yield a total hardness of 40-45 mg/L as $CaCO_3$, a total alkalinity of 30-35 mg/L as $CaCO_3$, and a initial pH of 7.2 to 7.6. The test vessels were held in a water bath at 12º ± 1ºC. The test fish were acclimated to the dilution water and test temperature, and held without food for 48-96 hours prior to testing. Fish were observed for mortality and abnormal effects such as surfacing, loss of equilibrium and dark discoloration at 24 hour intervals up to 96 hours.

Analytical samples were taken at 0 and 96 hours from each test chamber. The LC_{50} and its 95 percent confidence values were estimated using a Binomial method, Moving Average method and Probit analysis.

Results:

Results are presented as nominal concentrations. There was 20 and 70% mortality respectively at test groups 0.18 and 0.23 µg/L after 96 hours of exposure. Almost all surviving fish showed symptoms of toxicity that included loss of equilibrium, quiescence, surfacing, erratic movement and fish in the bottom of the aquarium. Fish in the controls showed no mortality and no abnormal behaviour.

The dissolved oxygen concentration and pH ranged from 7.7 to 8.7 mg/L and 7.1 to 7.7, respectively, throughout the test.

Table 4.3.1-2: Mortality rates for rainbow trout (*Salmo gairdneri*) exposed to esfenvalerate (MO 70616 technical).

Compound	Nominal concentration (µg/L)	No. of fish	Cumulative mortality (%)			
			24-hr	48-hr	72-hr	96-hr
Esfenvalerate	0.32	10	10	50	60	70
	0.18	10	0	0	10	20
	0.10	10	0	0	0	0
	0.056	10	0	0	0	0
	0.032	10	0	0	0	0
Acetone	-	10	0	0	0	0
Control	-	10	0	0	0	0

The 96 hour LC_{50} value of esfenvalerate to rainbow trout was estimated to be 0.26 µg/l (0.20 - 0.38 for 95% confidence limits) and the NOEC was 0.10 µg/L, based on lack of mortality and abnormal effects. LC_{50} values at 24 and 48 hours were also calculated, and were determined to be >0.32 and >0.18 µg/L, respectively.

[Study 3]

Study reference:

Anonymous (1986f) The acute toxicity of S-1844 (esfenvalerate) and S-5602 (fenvalerate) to rainbow trout. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-60-0009

Detailed study summary and results:

Ten rainbow trout (*Salmo gairdneri*) juveniles were exposed to each solution of 0.010, 0.032, 0.056, 0.075, 0.100, 0.180, 0.240, 0.320, 0.560 and 0.750 µg esfenvalerate/L, and of 0.056, 0.075, 0.100,

0.180, 0.320, 0.560, 0.750 and 10 µg fenvalerate/L in a flow-through system for 96 hours. Mortalities and abnormal behaviour were observed at 24-hr intervals, and the 96-hr LC_{50} values were found to be 0.10 µg/L (0.05 - 0.17 for 95% confidence limits) and 0.22 µg/L (0.13 - 0.32 for 95% confidence limits) for esfenvalerate and fenvalerate, respectively, based on nominal concentrations.

Test type:

Guideline OECD 203 (1981); not GLP compliant

Test substance:

S-1844 (esfenvalerate); lot No. PKG 85036; purity 94.5%

S-5602 (fenvalerate); lot No. 30114; purity 94.7%

Materials and methods:

Juveniles (0.9 ± 0.13 g, 41.2 ± 2.1 mm) of rainbow trout (*Salmo gairdneri*) were exposed under flow through conditions at 14º ± 1ºC for 96 hours to esfenvalerate (94.5% purity) and fenvalerate (94.7% purity), which were mixed with five parts of Tween-80 solvent, and then diluted with distilled water, to give nominal concentrations of 0.01, 0.032, 0.056, 0.075, 0.1, 0.18, 0.24, 0.32, 0.56 and 0.75 µg esfenvalerate/L, and 0.056, 0.075, 0.1, 0.18, 0.32, 0.56, 0.75, 1.0 µg fenvalerate/L. Sodium pentachlorophenoxide was tested as a positive control.

Ten fish were introduced to each glass aquarium (30 x 30 x 30 cm) containing 20 L of graded levels of the test solutions. A micro glass pump was used to deliver the chemical solution to the test aquarium at a flow rate of 40 L/day. There were two replicates for each test solution, solvent control and positive control, and four replicates for water control. The actual concentrations of esfenvalerate (at 0.032 and 0.056 µg/L) and fenvalerate (at 0.10, 0.18 and 0.32 µg/L) were determined at 0, 48 and 96 hr after introducing the fish. Fish were observed for mortality and abnormal behaviour at 24 hour intervals up to 96 hours and LC_{50} values were calculated by Probit analysis.

Results:

Results are expressed as nominal concentrations. The two measured concentrations of esfenvalerate were between 107 and 125% of the nominal value of 0.032 and 0.056 µg /L.

The symptoms of toxicity (abnormal respiration, swimming at the surface, convulsions, lethargy) were observed at all concentrations except the lowest of esfenvalerate and fenvalerate. Mortality in the test systems was recorded above test concentration of 0.075 µg/L (30% at 96 h) for esfenvalerate and above 0.18 µg/L (30% at 96 h) for fenvalerate. Fish in the controls showed no mortality and no abnormal behaviour.

The dissolved oxygen concentration and pH ranged from 8.05 to 10.3 mg/L and 7.4 to 7.6, respectively, throughout the test.

Table 4.3.1-3: Cumulative mortality data for rainbow trout (*Salmo gairdneri*) exposed to esfenvalerate (S-1844) and fenvalerate (S-5602).

Compound	Nominal concentration (µg/L)	No. of fish	Cumulative mortality (%)			
			24-hr	48-hr	72-hr	96-hr
Esfenvalerate (S-1844)	0.010	10	0	0	0	0
	0.032	10	0	0	0	0
	0.056	10	0	0	0	0
	0.075	10	0	0	20	30
	0.100	10	0	0	10	50
	0.180	10	0	40	60	80
	0.240	10	30	80	90	100
	0.320	10	60	90	100	100
	0.560	10	80	100	100	100
	0.750	10	100	100	100	100
Fenvalerate (S-5602)	0.056	10	0	0	0	0
	0.075	10	0	0	0	0
	0.100	10	0	0	0	0
	0.180	10	0	10	20	30
	0.320	10	10	50	70	80
	0.560	10	10	80	100	100
	0.750	10	40	90	100	100
	1.000	10	50	100	100	100
Positive control (PCP.Na)	56	10	0	0	0	0
	75	10	10	30	30	40
	87	10	50	80	80	90
	100	10	90	100	100	100
	135	10	100	100	100	100
Tween-80	5.0	10	0	0	0	0
Control	-	20	0	0	0	0

The 96 hour LC_{50} values were calculated as 0.1 (0.05 - 0.17 µg/L for 95% confidence limits) and 0.22 µg/L (0.13 - 0.32 µg/L for 95% confidence limits), respectively for esfenvalerate and fenvalerate. The NOEC concentrations were 0.01 and 0.05 µg/L for esfenvalerate and fenvalerate, respectively.

[Study 4]

Study reference:

Anonymous (1984b) Acute toxicity of Sd47443, pydrin isomer, to the fathead minnow (*Pimephales promelas*). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-41-0021

Detailed study summary and results:

Ten fathead minnow (*Pimephales promelas*) juveniles were exposed to each solution of 0.13, 0.22, 0.36, 0.60 and 1.0 µg/L µg esfenvalerate/L, along with a dilution water and solvent control (acetone), in a static system for 96 hours. Mortalities and symptoms of toxicity were observed at 24-hr intervals. The 96-hr LC_{50} value were found to be 0.18 µg/L (0.13 - 0.36 for 95% confidence limits), based on nominal concentrations. The NOEC was determined to be 0.13 µg/L based on lack of mortality and abnormal effects.

Test type:

Guideline EPA 660/3-75-009 (1975); not GLP compliant

Test substance:

Esfenvalerate; lot No.SD47443; purity 98%

Materials and methods:

Juveniles (0.02 ± 0.01 g, 11.0 ± 2.0 mm) of fathead minnow (*Pimephales promelas*) were exposed under static conditions at 22 ± 1ºC and a 14:10 hour light dark cycle to esfenvalerate (98% purity) in acetone as a solvent, at nominal test concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 µg/L. Freshwater and solvent controls were tested concurrently.

Ten fish were introduced to each glass vessels containing 3 L of test solution or control freshwater. Two replicates were tested per treatment. Fish were observed for mortality and abnormal behaviour at 24 hour intervals up to 96 hours, and the LC_{50} values were determined by a Binomial probability method.

The test substance was measured in the highest concentration at test initiation and after two days (when 100% mortality was reached). Dissolved oxygen and pH were measured at the beginning and end of the study.

Results:

Results are based on nominal concentrations. However, although the initial measured concentration was 85% of the nominal value of 1.0 µg/L this had declined to 50% after two days, by which time all fish in the highest concentration were dead. This later drop in concentration is therefore not considered to have substantively affected the results based on nominals. The dissolved oxygen concentration and pH ranged from 3.3 to 8.7 mg/L and 6.4 to 7.4, respectively, throughout the test.

After 96 hours of exposure, mortality ranged from 0% in the 0.13 µg/L concentration to 100% for concentrations greater than or equal to 0.36 µg/L. There was no mortality in the controls.

Table 4.3.1-4: Mortality of fathead minnow (*Pimephales promelas*) exposed to pydrin (esfenvalerate).

Compound	Nominal concentration (µg/L)	No. of fish	Cumulative mortality (%)			
			24-hr	48-hr	72-hr	96-hr
Esfenvalerate	1.0	20	90	100	100	100
	0.60	20	75	85	100	100
	0.36	20	60	90	100	100
	0.22	20	5	35	45	55
	0.13	20	0	0	0	0
Acetone	-	20	0	0	0	0
Control	-	20	0	0	0	0

The 96 hours LC_{50} value for esfenvalerate was calculated to be 0.18 µg/L (0.13 - 0.36 µg/l for 95% confidence limit). The NOEC was determined to be 0.13 µg/L based on mortality.

4.3.2 Short-term toxicity to aquatic invertebrates

[Study 1]

Study reference:

L. E. Sayers (2005) Esfenvalerate - Acute toxicity to water fleas, *Daphnia magna*, under static-renewal conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-0120

Detailed study summary and results:

In an acute, static toxicity study, twenty *Daphnia magna* neonates were exposed to esfenvalerate technical at nominal concentrations of 0 (control and solvent control), 5, 10, 22, 49, 110, 240 and 540 µg/L for a duration of 48 hours. Observations of immobility were made at 24 hours and 48 hours. The toxicological endpoints were presented as mean measured concentrations. After 48 hours, 100% immobility was observed at the three highest test item concentrations of 130, 230, 500 µg esfenvalerate technical /L (mean measured); no immobility was observed in either control group and at the lowest concentration of 3.7 µg esfenvalerate technical/L (mean measured). The resulting EC_{50} value of esfenvalerate technical in *Daphnia magna* was 27 µg/L, based on mean measured concentrations.

Test type:

Guideline OECD 202 (1984); GLP compliant

Test substance:

Esfenvalerate; lot No. 312111G; purity 86.6%

Materials and methods:

Neonates of *Daphnia magna* STRAUS (< 24 hours old at test initiation) were exposed, under static conditions, for 48 hours to esfenvalerate (86.6% purity) at nominal test concentrations of 4.5, 10, 22, 49, 110, 240 and 540 µg/L. Water control and solvent control (DMF) were tested concurrently. The controls and treatment groups comprised four replicates. All replicates contained five daphnids. Immobility was assessed after 24 and 48 hours.

The study was conducted in 250 mL glass vessels, containing 200 mL of test solution. The study conditions were maintained at a temperature of 22 °C, a light intensity of 1200-1500 lux and a photoperiod 16 hours light: 8 hours dark. The animals were not fed for the duration of the study. The physico-chemical properties of the water were as follows: pH 7.7-8.3; oxygen content: 7.9 - 9.8 mg/L; total hardness: 180 mg/L (120 mg/L $CaCO_3$) during the 48 hour test, conductivity and 450-500 µmhos at test initiation.

Samples of test solution were taken at test initiation, 24 hours (new and aged solutions) and test termination for test item analysis. The test item concentrations were quantified by external calibration using GC/ECD, and the EC_{50} value was determined using Probit analysis.

Results:

Mean measured values for esfenvalerate technical ranged from 72 % to 120 % of nominal over 48 hours. The new solutions at test initiation ranged from 66-110% nominal; the aged solutions at 24 hours ranged from 69-130%; the new solutions at 24 hours ranged from 53-160% of nominal and the aged solutions at 48 hours ranged from 68-120% nominal. In some of the test solutions the analytically measured concentrations increased from test initiation to 24 hours (aged) and from 24 hours (new) to 48 hours (aged). The toxicological endpoints are based on geometric mean measured concentrations: 3.7, 7.2, 17, 59, 130, 230 and 500 µg/L.

All resultant exposure solutions were observed to be clear and colourless with no visible signs of undissolved test substance.

Following 48 hours of exposure (test termination), 0, 5, 10, 95, 100, 100 and 100% immobilisation was observed among daphnids exposed to 03.7, 7.2, 17, 59, 130, 230 and 500 µg/L, respectively. No immobility was observed in either control group. Surviving daphnids exposed to 59, 130, 230 and 500 µg/L treatment level were observed to be lethargic at 24 hours. No adverse effects were observed at 48 hours in the surviving daphnids. No adverse effects were observed among daphnids exposed to the controls.

Table 4.3.2-1: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate technical.

Nominal concentration (µg/L)	Control	Solvent control (DMF)	4.5	10	22	49	110	240	540
Measured concentration (µg/L)	--	--	3.7	7.2	17	59	130	230	500
Inmobile at 48 hr (%)	0	0	0	5	10	95	100	100	100

Based on mean measured concentrations, the 48 hours EC_{50} value to *Daphnia magna* for esfenvalerate technical was calculated to be 27 µg/L (21 - 36 µg/L for 95% confidence limit) and the NOEC 3.7 µg/L.

[Study 2]

Study reference:

L. E. Sayers (2011) Esfenvalerate A β Isomer- Acute toxicity to water fleas, *Daphnia magna*, under static-renewal conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-0142

Detailed study summary and results:

In an acute, static toxicity study, twenty *Daphnia magna* neonates were exposed to esfenvalerate Aβ at nominal concentrations of 0 (control and solvent control), 0.3, 0.66, 1.5, 3.2 and 7.0 µg/L for a duration of 48 hours. Observations of immobility were made at 24 hours and 48 hours.

In addition, a comparative toxicity test was conducted to compare the toxicity of the Aβ isomer of esfenvalerate with technical esfenvalerate. *Daphnia* neonates were exposed to esfenvalerate Aβ or esfenvalerate technical at nominal concentrations of 0 (control) 0 (solvent control) 0.1, 1.0 or 10 µg/L for a duration of 48 hours (equivalent to 0.05, 0.63 or 6.3 µg/L for the Aβ isomer and 0.049, 0.58 or 6.0 µg/L for the technical esfenvalerate). The control, solvent control and treatment groups comprised four replicates, each containing five daphnids. Observations of immobility were made 24 hours and 48 hours following study commencement.

The toxicological endpoints were presented as nominal and mean measured concentrations. After 48 hours, 100% immobility was observed at the highest test item concentration of 6.3 µg esfenvalerate Aβ isomer/L (nominal); no immobility was observed in either control groups and at concentrations of up to and including 0.05 µg esfenvalerate Aβ isomer /L (measured). In the comparative test, the EC_{50} for the Aβ isomer lay between 0.05 and 0.63 ug/L whereas the EC_{50} for the technical grade was below 0.049 ug/L (measured).

The resulting 48 hr-EC_{50} value of esfenvalerate Aβ in *Daphnia magna* was 0.21 µg/L, based on mean measured concentrations. The comparative toxicity test indicates that the technical grade esfenvalerate is more toxic than the Aβ isomer.

Test type:

Guideline OECD 202 (2004); GLP compliant

Test substance:

Esfenvalerate Aβ isomer; lot No. 09SC8090417-1; purity 98.8%

Technical grade esfenvalerate, Lot no. 60610G, purity: 87.3%

Materials and methods:

Neonates of *Daphnia magna* STRAUS (< 24 hours old at test initiation) were exposed, under static conditions, for 48 hours to esfenvalerate Aβ isomer (98.8% purity) at nominal test concentrations of 0.3, 0.66, 1.5, 3.2 and 7.0 µg/L. Water control and solvent control were tested concurrently. The controls and treatment groups comprised four replicates. All replicates contained five daphnids. Immobility was assessed after 24 and 48 hours.

The study was conducted in glass vessels, containing 200 mL of fortified well water for a period of 48 hours. The definitive and comparative study conditions were maintained at a temperature of 19.0 to 21 °C, a light intensity of 410 - 810 lux and a photoperiod 16 hours light: 8 hours dark. The vessels were not aerated and the animals were not fed for the duration of the study. The physico-chemical properties of the water were as follows: pH 7.8-8.3; oxygen content: 8.0 - 9.0 mg/L; total hardness: 180 mg/L $CaCO_3$ at test initiation, conductivity: 600 µS/cm at test initiation.

Samples of test solution were taken at test initiation, 24 hours (new and aged solutions) and test termination for test item analysis. The test item concentrations were quantified by external calibration using HPLC with MS detection, and the EC_{50} value was determined using Probit analysis.

Results:

Mean measured values for esfenvalerate Aβ ranged from 56 % to 100 % of nominal at test initiation and from 15% to 52% at test termination. The toxicological endpoints are based on geometric mean measured concentrations. Following 48 hours of exposure (test termination), 45, 60, 85, 95 and 100% immobilisation was observed among daphnids exposed to 0, 0.16, 0.34, 0.72, 1.8 and 3.1 µg/L, respectively. No immobility was observed in either control group. One surviving daphnid exposed to the 0.16 µg/L treatment level was observed to be on the bottom of the test vessel, while several other daphnids exposed to this treatment level were observed to be lethargic. All surviving daphnids exposed to the 0.34, 0.72, and 1.8 µg/L treatment level were observed to be on the bottom of the test vessel. No immobilization or adverse effects were observed among daphnids exposed to the controls.

Table 4.3.2-2: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate Aβ.

Nominal concentration (µg/L)	Control	Solvent control	0.3	0.66	1.5	3.2	7.0
Measured concentration (µg/L)	--	--	0.16	0.34	0.72	1.8	3.1
Inmobile at 48 hr (%)	0	0	45	60	85	95	100

Based on mean measured concentrations, the 48 hours EC_{50} value to *Daphnia magna* for esfenvalerate Aβ was calculated to be 0.21 µg/L (0.1 – 0.31 µg/L for 95% confidence limit) and the NOEC <0.16 µg/L.

In the comparative test, measured concentrations of new solutions at test initiation and at the 24-hour renewal interval ranged from 80 to 96% and from 85 to 99% of nominal concentrations for esfenvalerate Aβ isomer and esfenvalerate technical grade, respectively.

Measured concentrations of aged solutions at 24 hours and at test termination ranged from 26 to 48% and from 23 to 44% of nominal concentrations for esfenvalerate Aβ isomer and esfenvalerate technical grade, respectively. Geometric mean measured concentrations ranged from 50 to 63% and from 49 to 60% of nominal concentrations for esfenvalerate Aβ isomer and esfenvalerate technical grade, respectively, resulting in mean test concentrations of 0.050, 0.63 and 6.3 µg/L for esfenvalerate Aβ isomer and 0.049, 0.58 and 6.0 µg/L for esfenvalerate technical grade.

Following 48 hours of exposure, immobilization of 95 and 100% was observed among daphnids exposed to the 0.63 and 6.3 µg/L esfenvalerate Aβ isomer treatment levels, respectively. No immobilization was observed at 0.050 µg/L. In contrast, immobilization of 55, 85 and 100% was observed among daphnids exposed to the 0.049, 0.58 and 6.0 µg/L esfenvalerate technical grade treatment levels, respectively. Sublethal effects were not reported. No immobilization or adverse effects were observed among daphnids exposed to the controls.

Table 4.3.2-3: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate Aβ in a comparative toxicity test.

Nominal concentration (µg/L)	Control	Solvent control	0.1	1.0	10
Measured concentration (µg/L)	--	--	0.05	0.63	6.3
Inmobile at 48 hr (%)	0	0	0	95	100

Table 4.3.2-4: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate technical grade in a comparative toxicity test.

Nominal concentration (µg/L)	Control	Solvent control	0.1	1.0	10
Measured concentration (µg/L)	--	--	0.049	0.58	6.0
Inmobile at 48 hr (%)	0	0	55	85	100

The resulting 48 hr-EC_{50} value of esfenvalerate Aβ in *Daphnia magna* was 0.21 µg/L, based on mean measured concentrations.

The resulting 48 hr-EC_{50} value of technical esfenvalerate for *Daphnia magna* would be <0.049 µg/L based on mean measured concentrations. Looking at the results in table 4.3.2-4 there was 55% immobility at 0.049 µg/L, so the actual EC_{50} would be approximately 0.045 µg/L.

The comparative toxicity test indicates that the technical grade esfenvalerate is more toxic than the Aβ isomer. The *Daphnia* endpoint for technical esfenvalerate of <0.049 µg/L was mentioned but not used in the 2014 pesticides RAR for esfenvalerate. The reason given by the RMS was that the chronic toxicity endpoints for *Daphnia* and fish are several orders of magnitude lower and will therefore drive the overall risk assessment. Formulation and mesocosm endpoints were also used to determine the eventual risk assessment. This endpoint would however be relevant for the acute hazard classification of esfenvalerate.

[Study 3]

Study reference:

D. G. Hutton (1987) *Daphnia magna* static acute 48-hour EC_{50} of technical Asana® insecticide. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-71-0029

Detailed study summary and results:

In an acute, static toxicity study, twenty *Daphnia magna* neonates were exposed to esfenvalerate at nominal concentrations of 0 (control and solvent control), 0.11, 0.19, 0.31, 0.52, 0.86, 1.44, 2.4 and 4.0 µg/L for a duration of 48 hours. Observations of immobility were made at 24 hours and 48 hours. The toxicological endpoints were presented as nominal concentrations. The resulting 48-hour EC_{50} and NOEC values based on nominal concentrations of esfenvalerate in *Daphnia magna* were 0.9 µg/L (95% confidence limit 0.7-1.16 µg/L) and 0.11 µg/L, respectively.

Test type:

Guideline US-EPA 72-2 (1985); GLP compliant

Test substance:

Esfenvalerate; lot No. H-16,626; purity 98.6%

Materials and methods:

Twenty neonates of *Daphnia magna* (< 24 hours old at test initiation) were exposed, under static conditions, for 48 hours to esfenvalerate (98.6% purity) at nominal test concentrations of 0.11, 0.19, 0.31, 0.52, 0.86, 1.44, 2.4 and 4.0 µg/L. Water control and solvent control (acetone) were tested concurrently. The controls and treatment groups comprised two replicates. All replicates contained ten daphnids.

The study was conducted in 250 mL glass vessels, containing 200 mL of test solution. The study conditions were maintained at a temperature between 19.8 and 20.2°C and a photoperiod 16 hours light: 8 hours dark. The animals were not fed for the duration of the study. Dissolved oxygen and pH were measured in the water control and acetone control in the low, medium, and high exposure concentrations at the beginning and end of the exposure. The physico-chemical properties of the water at test initiation were as follow: pH 8.3; oxygen content 8.4 mg/L; total hardness 114 mg/L $CaCO_{3;}$ hardness 177 mg/L $CaCO_3$; and conductivity 560 µmhos.

Immobility counts and observations were made 24 and 48 hours after the exposure was initiated and EC_{50} values were calculated by Probit analysis.

Results:

The dissolved oxygen and pH ranged from 8.3 - 8.4 mg/l and 8.2 - 8.3, respectively throughout the test period. At the lowest concentration tested (0.11 µg/l) no *Daphnia magna* was immobilised but the highest concentration tested (4.0 µg/l) caused 100% immobilization after 48 hours. No immobilization was observed among daphnids exposed to the controls.

Table 4.3.2-5: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate.

Nominal concentration (µg/L)	Cumulative observed immobility (%)			
	24-hr		48-hr	
	Replicate A	Replicate B	Replicate A	Replicate B
4.0	70	40	100	100
2.4	30	20	70	80
1.44	10	30	60	60
0.86	10	20	60	50
0.52	0	0	50	20
0.31	0	0	10	20
0.19	0	0	10	0
0.11	0	0	0	0
Acetone	0	0	0	0
Control	0	0	0	0

Based on nominal concentrations, the resulting 48-hour EC_{50} and NOEC values based on nominal concentrations of esfenvalerate in *Daphnia magna* were 0.9 µg/L (95% confidence limit 0.7-1.16 µg/L) and 0.11 µg/L, respectively.

[Study 4]

Study reference:

D. G. Hutton (1987) Fed *Daphnia magna* static acute 48-hour EC_{50} of technical Asana® insecticide. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-71-0028

Detailed study summary and results:

In an acute, static toxicity study, twenty *Daphnia magna* neonates were exposed to esfenvalerate at nominal concentrations of 0 (control and solvent control), 0.11, 0.19, 0.31, 0.52, 0.86, 1.44, 2.4, 4.0 and 6.7 µg/L for a duration of 48 hours. Observations of immobility were made at 24 hours and 48 hours. The toxicological endpoints were presented as nominal concentrations. The resulting 48-hour EC_{50} and NOEC values based on nominal concentrations of esfenvalerate in *Daphnia magna* were 3.5 µg/L (95% confidence limit 2.7-4.9 µg/L) and 0.86 µg/L, respectively.

Test type:

Guideline US-EPA 72-2 (1985); GLP compliant

Test substance:

Esfenvalerate; lot No. H-16,626; purity 98.6%

Materials and methods:

Twenty neonates of *Daphnia magna* (< 24 hours old at test initiation) were exposed, under static conditions, for 48 hours to esfenvalerate (98.6% purity) at nominal test concentrations of 0.11, 0.19, 0.31, 0.52, 0.86, 1.44, 2.4, 4.0 and 6.7 µg/L. Water control and solvent control (acetone) were tested concurrently. The controls and treatment groups comprised two replicates. All replicates contained ten daphnids.

The study was conducted in 250 mL glass vessels, containing 200 mL of test solution. The study conditions were maintained at a temperature between 19.8 and 20.2°C and a photoperiod 16 hours light: 8 hours dark. Food was provided during the test. Dissolved oxygen and pH were measured in the water control and acetone control in the low, medium, and high exposure concentrations at the beginning and end of the exposure. The physico-chemical properties of the water at test initiation were as follow: pH 8.1; oxygen content 8.3 mg/L; total hardness 114 mg/L $CaCO_3$; hardness 177 mg/L $CaCO_3$; and conductivity 560 µmhos.

Immobility counts and observations were made 24 and 48 hours after the exposure was initiated and EC_{50} values were calculated by Probit analysis.

Results:

The dissolved oxygen and pH ranged from 6.2 - 8.3 mg/l and 7.5 - 8.1, respectively. At concentrations up to 0.86 µg/l at both 24 and 48 hours no *Daphnia magna* was immobilised. The highest concentration tested (6.7 µg/l) caused 40 and 65% immobilization at 24 and 48 hours respectively. No immobilization was observed among daphnids exposed to the controls.

Table 4.3.2-6: Immobility of *Daphnia magna* resulting from exposure to esfenvalerate.

Nominal concentration (µg/L)	Cumulative observed immobility (%)			
	24-hr		48-hr	
	Replicate A	Replicate B	Replicate A	Replicate B
6.7	40	40	70	60
4.0	10	40	50	60
2.4	20	20	50	40
1.44	10	0	40	30
0.86	0	0	0	0
0.52	0	0	0	0
0.31	0	0	0	0
0.19	0	0	0	0
0.11	0	0	0	0
Acetone	0	0	0	0
Control	0	0	0	0

Based on nominal concentrations, the resulting 48-hour EC_{50} and NOEC values based on nominal concentrations of esfenvalerate in *Daphnia magna* were 3.5 µg/L (95% confidence limit 2.7-4.9 µg/L) and 0.86 µg/L, respectively.

4.3.3 Algal growth inhibition tests

[Study 1]

Study reference:

J. W. Handley, I. G. Sewell, A. J. Bartlett (1991) The algistatic activity of esfenvalerate (SAG 303). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-01-0038

Detailed study summary and results:

In a static toxicity study, cultures of *Scenedesmus subspicatus* were exposed to esfenvalerate (SAG 303) at nominal concentrations of 0 (control and solvent control), 1.0, 2.0, 4.0, 8.0 and 16.0 µg/l for a duration of 96 hours to determine algal growth inhibition. Potassium dichromate was tested as a positive control concurrently. The cell density of the test vessels was measured at 0, 24, 48, 72 and 96 hours. The toxicological endpoints were presented as nominal concentrations. The resulting E_bC_{50}, E_rC_{50} and NOEC values of esfenvalerate were 6.5, 10.0 and 1.0 µg/L, respectively.

Test type:

Guideline OECD 201 (1984); GLP compliant

Test substance:

Esfenvalerate; lot No. 71219; purity 97%

Materials and methods:

Esfenvalerate was dosed as a solution in acetone diluted in water. Green algae *Scenedesmus subspicatus* were exposed at nominal concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0 µg/l of esfenvalerate (total isomer content 97% purity) in a static system at 24ºC for 96 hours. Potassium dichromate was tested as a positive control and acetone was tested as a solvent control. The study was conducted in an orbital incubator with 250 mL conical flasks containing 100 mL of test solution and controls in triplicates. The study conditions were maintained at a temperature of 24 °C and pH of the test solution ranged from 7.8 (at test initiation) to 8.6 (at test termination).

Samples were taken at 0, 24, 48, 72 and 96 hours and the absorbance measured at 665 nm. The cell densities of the control cultures at initiation and at termination were determined by direct counting with the aid of a haemacytometer. The effect of esfenvalerate on the biomass and growth rate was assessed from the area under the growth curve and the logarithmic growth rate. Samples of the solvent control and each test concentration were taken at 0 and 96 hours for analysis.

Results:

The results were based on nominal concentrations. Mean measured values for esfenvalerate ranged from 123 to 136 % of nominal at 0 hours and 113.8 to 171% after 96 hours. The culture of algae exposed to esfenvalerate at the concentration of 0.016 mg/l for 96 hours appeared to be colourless and clumped. No other abnormalities were observed in the treated or control cultures. The resulting E_bC_{50}, E_rC_{50} and NOEC values for the positive control potassium dichromate were 420, 370 and 125 µg/L, respectively. The resulting nominal E_bC_{50}, E_rC_{50} and NOEC (biomass and growth rate) values of esfenvalerate were 6.5, 10.0 and 1.0 µg/L, respectively.

4.3.4 *Lemna* sp. growth inhibition test

No data are available.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

No data are available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data are available.

4.4.3 Fish short-term toxicity test on embryo and sac-fry stages

[Study 1]

Study reference:

Anonymous (1991b) The prolonged toxicity of esfenvalerate (SAG 303) to rainbow trout using dynamic test conditions (*Salmo gairdneri*). Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-01-0036

Detailed study summary and results:

Ten rainbow trout (*Salmo gairdneri*) juveniles were exposed to each solution of 0.001, 0.0032, 0.01, 0.032 and 0.1 µg esfenvalerate/L, along with a dilution water control and solvent control (acetone), in a flow-through system for 21 days. Fish were observed for mortality and symptoms of toxicity at

various time intervals. The LC_{50} and NOEC values at day 21 (test termination) were found to be 0.013 µg/L (0.009 - 0.018 for 95% confidence limits) and 0.001 µg/L, respectively, based on nominal concentrations.

Test type:

Guideline OECD 204 (1984); GLP compliant

Test substance:

Esfenvalerate; lot No.71219; purity 97%

Materials and methods:

The prolonged toxicity of esfenvalerate (total isomer content 97% purity) to rainbow trout (*Salmo gairdneri*) was determined over 21 days in a flow-through system at 14ºC with a 16: 8-hour light-dark cycle

Groups of ten juvenile rainbow trout (2.14 ± 0.43g and 5.2 ± 0.4cm) were exposed to a range of five nominal concentrations of esfenvalerate: 0.001, 0.0032, 0.01, 0.032 and 0.1µg/L. Fish were observed for mortality and symptoms of toxicity at various time intervals throughout the study period. The lengths and weights of fish surviving to the end of the study were recorded and compared with control values. Temperature, dissolved oxygen and pH were recorded daily. LC_{50} values were determined by the method of Thompson W. R. (1947)[(1)].

Results:

Dissolved oxygen concentrations were in the range of 8.3 - 10.4 mg/L and pH of the solutions ranged from 7.4 - 7.8. Temperature remained constant at 14ºC during the study. The mean percentage concentration of the stock solutions was 121 - 123% of the nominal values. The results are presented as the nominal concentrations. No mortalities were observed in either control group or in test fish dosed 0.0032 µg/L or less. At the end of the study 30, 100 and 100% mortality was observed in the groups dosed at 0.01, 0.032 and 0.1 µg/L, respectively.

The most significant symptoms of toxicity observed at dose levels of 0.0032 µg/L and above included lying on the bottom, lethargy, loss of pigmentation, loss of equilibrium and coughing. Statistical analysis of the data showed that there were no significant effects on growth in terms of length of surviving test animals compared to controls. However, there was a significant ($p < 0.05$) reduction in mean weight of surviving fish exposed to 0.01 µg/L compared with the solvent control after 21 days which was attributed to reduced competition as a result of the smaller number of fish following the mortalities at this concentration.

1 Thompson, W. R. (1947) *Bacterial Reviews* **11** p: 115 - 145

Table 4.4.3-1: LC_{50} values for rainbow trout (*Salmo gairdneri*) exposed to esfenvalerate (SAG 303).

Time (days)	LC_{50} (μg/L)	95% confidence limits (μg/L)
Days 1-4	>0.10	-
Day 5	0.056	0.028-0.11
Days 6-9	0.032	0.022- 0.046
Days 10-11	0.025	0.018- 0.036
Day 12	0.020	0.016- 0.025
Days 13-14	0.016	0.013- 0.020
Day 15	0.014	0.011- 0.019
Days 15-21	0.013	0.009- 0.018

The $LC_{50,}$ LOEC and NOEC values at day 21 (test termination) were found to be 0.013 μg/L (0.009 - 0.018 for 95% confidence limits), 0.003 μg/L and 0.001 μg/L, respectively, based on nominal concentrations.

[Study 2]

Study reference:

Anonymous (1978b) Chronic toxicity of SD-43775 to the fathead minnow (*Pimephales promelas*). Sumitomo Chemical Co., Ltd. Unpublished report No.: AW-81-0071

Detailed study summary and results:

Fathead minnows (*Pimephales promelas*) were exposed to five concentrations of the pyrethroid insecticide SD-43775 (fenvalerate) throughout a complete life cycle. Mean measured concentrations during the 260 day exposure ranged from 0.020 to 0.21 μg/L. Most of the first generation fry, and all of the second generation fry transferred from control, died during 30 day exposure to a mean measured concentration of 0.21 μg/L. Five fish survived 260 days exposure to this concentration and spawned, although the number of eggs per female was substantially reduced. No significance differences in measured parameters of survival, growth and reproduction were observed between fathead minnows in the control, solvent control and those continuously exposed to mean measured concentrations of 0.020, 0.028, 0.056 and 0.090 μg/L.

Based on these observations, the maximum acceptable toxicant concentration of SD-43775 for this species is estimated to be >0.090 and <0.21 μg/L, and the NOEC value= 0.090 μg/L.

Test type:

Guideline modified US EPA "Recommended bioassay procedures for fathead minnow (*Pimephales promelas*, Rafinesque) chronic tests" (1971); not GLP compliant

Test substance:

Fenvalerate (SD-43775); lot No.4-1-0-0; purity 96%

Materials and methods:

Following a preliminary 96 hour acute toxicity test in static conditions and a 21 days flow through study to fry, duplicate groups of sixty eggs of fathead minnows were exposed at 25ºC to selected nominal concentrations of 0.013, 0.025, 0.05, 0.10 and 0.20 μg/L fenvalerate 96% purity in dimethyl

sulphoxide as a vehicle and observed until hatching was complete (≅ 4 days). Dead eggs were removed daily and the percentage hatch was calculated. Twenty newly hatched fry from each group were randomly selected, placed in growth chambers and exposed to the same concentrations of fenvalerate.

After 63 days fifteen fish from the combined fry groups were selected to continue exposure in a spawning chamber. After 168 days the number of fish in each group was reduced to three males and six females. The number of eggs in each spawn was recorded and groups of fifty eggs from a maximum of ten spawns were incubated to determine hatching success. Eggs spawned by control group were transferred to the highest treatment level where few first generation fish had survived.

Twenty fry from the first two egg groups incubated in each aquarium with 80% or greater hatching success were transferred to growth chambers, where they were exposed to the test solution for 30 days and then measured and weighed. Exposure of first generation fish was ended on test day 260 when spawning had ceased and the survivors were measured, weighed and examined for sex and gonadal conditions.

Six fish exposed to concentrations of 0.09 and 0.056 μg/L for 168 days were transferred to fresh water for a depuration study. Two fish were sampled after 14, 28 and 56 days for tissue analysis.

Biological parameters were subject to analysis of variance. For the effects the Dunnett's procedure (p=0.05) was used. Temperature, dissolved oxygen, total hardness, alkalinity and pH were measured on a regular basis.

Results:

The temperature and water quality parameters for hardness, alkalinity, pH and dissolved oxygen were found to be within the acceptable ranges. The mean measured concentrations throughout the study were between 90 - 112% of the nominal values, except at the lowest level which was 154%. The results are presented as the mean measured concentrations. There were no significant differences in the percentage of hatches in the control, solvent control and eggs exposed to fenvalerate at concentrations up to and including 0.21 μg/L. The percentage of fry surviving to 30 days was significantly lower in the group exposed to 0.21 μg/L compared to the other treatment and control groups but there was difference in survival from 30 to 60 days. There were no significant differences in the lengths of fry surviving to 60 days between the treated and control groups.

The majority of fish selected for continued exposure survived to 168 days when they were reduced in numbers for spawning.

During the spawning period five mortalities occurred, although they were not treatment related. No significant differences due to treatment were observed in the lengths and weights of fish surviving 260 days exposure between the control, solvent control and groups exposed to fenvalerate at concentrations up to 0.090 μg/l. The number of spawns per female and number of eggs per female from the few fish surviving exposure to 0.21 μg/L was lower than in the remaining treatments. Spawning was not affected by exposure concentration of fenvalerate up to and including 0.090 μg/L. The number of hatched eggs as percentage of spawned was similar in the treated and control groups.

Fry exposed to 0.21 μg/L did not survive to 30 days but fry exposed to lower concentrations of fenvalerate were comparable in length and weight to the controls 30 days after hatching.

Table 4.4.3-2: Survival and growth of second generation of fathead minnow after 30 days exposure to fenvalerate (SD-43775).

Mean measured concentrations (μg/L)	Replicate	30 day old fry		
		Survival (%)	Total length (mm)	Wet weight (g)
0.21	A	0	-	-
	B	0	-	-
0.090	A	78	23±3	0.115
	B	95	20±2	0.083
0.056	A	98	20±2	0.080
	B	93	20±2	0.087
0.028	A	100	20±2	0.081
	B	78	22±3	0.091
0.020	A	93	23±2	0.091
	B	100	19±4	0.074
Solvent control	A	100	20±2	0.093
	B	78	22±2	0.111
Control	A	68	22±2	0.127
	B	88	20±2	0.073

It was concluded that the most sensitive indicator of the toxicity of fenvalerate to the fathead minnow was reduced survival of fry. Fish surviving the initial exposure grew normally and matured sexually, although the number of eggs spawned per female was reduced. Based on these findings the Maximum Acceptable Toxicant Concentration (MATC) for fenvalerate in water to fathead minnow was estimated to be between 0.090 and 0.21 μg/L, and the NOEC value= 0.090 μg/L.

4.4.4 Chronic toxicity to aquatic invertebrates

[Study 1]

Study reference:

D. G. Hutton (1987) Chronic toxicity of technical Asana® insecticide to *Daphnia magna*. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-71-0027

Detailed study summary and results:

In a chronic study *Daphnia magna* neonates were exposed in a semi-static system to esfenvalerate at nominal concentrations 0.03, 0.06, 0.12, 0.25, 0.5 and 1.0 μg/L for a duration of 21 days. Acetone and water control groups were also run concurrently. Assessments were made throughout the study for survival, growth and reproduction. The NOEC for technical esfenvalerate to *Daphnia magna* over a 21-day period was reported to be 0.052 μg/L, based on mean measured concentrations.

Test type:

Guideline US-EPA 72-4 (1985); GLP compliant

Test substance:

Esfenvalerate; lot No. H-16,626; purity 98.6%

Materials and methods:

Groups of *Daphnia magna* less than 24 hours old were exposed for 21 days at nominal concentrations of 0.03, 0.06, 0.12, 0.25, 0.5 and 1.0 µg/L of esfenvalerate (98.6% purity) in a semi-static system at 20 ± 1ºC for 21 days with a 16: 8 hours light: dark cycle. The test solutions were changed three times per week over the exposure period. Acetone and water control groups were also used. The study was conducted in 250 mL beakers, containing 200 mL of test solution. Each test concentration was replicated three times with five *Daphnia magna* per beaker for survival measurements and seven times with one *Daphnia magna* per beaker for reproduction and growth measurements.

Assessments were made throughout the study for survival, growth and reproduction. The test solution was analysed at weekly intervals. Dissolved oxygen and pH were measured before and after each test material renewal. Results for each test concentration and water control were compared to the solvent control by Dunnett's test.

Results:

The dissolved oxygen and pH ranged from 3.0 - 8.6 mg/l and 7.4 - 8.7, respectively.

The mean measured concentrations over the course of the study (0.025, 0.052, 0.079, 0.15, 0.45 and 1.2 µg/L) ranged from 60 to 120% of the nominal concentrations. The results are expressed as mean measured concentrations.

All *Daphnia magna* exposed to concentrations of 0.45 µg/L and above died within seven days. Significant mortality was also seen in the group exposed to concentrations of 0.15 µg/L. There was a significant reduction in the total number of young produced, the number of young produced per day and the length of surviving *Daphnia magna* in the group exposed to concentrations of 0.079 µg/L. The group exposed to concentrations of 0.15 µg/L did not produce any young during the study and the length of the surviving *Daphnia magna* was significantly less than the controls. There was no effect on the size of surviving *Daphnia magna* over the 21-day period in groups exposed to concentrations of 0.052 µg/L and below. The highest concentration tested having no effect on reproduction was 0.052 µg/L.

Table 4.4.4-1: Chronic effects of technical Asana® insecticide on *Daphnia magna*.

Nominal concentration (µg/L)	Measured concentration (µg/L)	Survival (%)	Reproduction (mean number of young)	Mean length (mm)
Control	<0.001	100	83.9	4.1
Solvent control	<0.001	93	75.6	3.9
0.03	0.025	100	77.3	3.9
0.06	0.052	93	77.6	3.9
0.12	0.079	87	36.6	3.3
0.25	0.15	60	-	2.2
0.50	0.45	0	-	-
1.00	1.2	0	-	-

The NOEC for technical esfenvalerate to *Daphnia magna* over a 21-day period was reported to be 0.052 µg/L (mean measured concentration). The "Maximum Acceptable Toxicant Concentration" (MATC) was between 0.052 and 0.079 µg/L.

[Study 2]

Study reference:

J. W. Handley, I. G. Sewell, A. J. Bartlett (1991) An assessment of the effect of esfenvalerate (SAG 303) on the reproduction of *Daphnia magna*. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-01-0037

Detailed study summary and results:

In a chronic study *Daphnia magna* neonates were exposed in a semi-static system to esfenvalerate at nominal concentrations 0.00056, 0.0018, 0.0056, 0.018 and 0.056 μg/L for a duration of 21 days. Acetone and water control groups were also run concurrently. Assessments were made throughout the study for survival, growth and reproduction. The NOEC value for esfenvalerate to *Daphnia magna* over a 21-day period was reported to be 0.00056 μg/l which was based on the number of young produced per adult.

Test type:

Guideline OECD 202 Part II (1985); GLP compliant

Test substance:

Esfenvalerate; lot No. 7219; purity 97%

Materials and methods:

Groups of ten *Daphnia magna* less than 24 hours old were exposed to nominal concentrations of 0.00056, 0.0018, 0.0056, 0.018 and 0.056 μg/L esfenvalerate (total isomer content 97% purity) in a semi-static system at 21 ºC for 21 days. Acetone and water controls were also used. The study was conducted in glass flasks containing 400 mL of test solution. Each test concentration was replicated four times with a total of 40 *Daphnia magna* per concentration. Test solutions were changed three times *per* week over the exposure period.

Dissolved oxygen, temperature and pH were measured before and after each test material renewal. Assessments were made throughout the study for survival, growth and reproduction. The EC_{50} values were calculated using the method of Thompson, W. R. (1947)[(1)].

Results:

The variations verified in the dissolved oxygen, temperature and pH did not affect the conclusions of the study. The overall mean concentration of the solvent stock solutions (not the test media itself) were 99-110%of nominal values throughout the study. Although there was not clear confirmation of actual exposure concentrations in test media throughout the study, the results are presented in terms of the nominal concentrations.

Daphnia exposed to the highest concentration of 0.056 μg/L died within 96 hours. In groups exposed to 0.0056 and 0.018 μg/L a significant prolonged toxicity effect (25-55% mortality after 21 days) was also seen. There was no effect on the size and condition of surviving *Daphnia* over the 21-day period.

There were no significant differences in reproduction at days 14 and 21 between the control, solvent control and the groups exposed to esfenvalerate at concentrations of 0.00056, 0.0018 and 0.0056 μg/L.

The EC_{50} values for immobilisation over the 21 day period of the study were in the range 0.056 - 0.011 μg/L (after 21 days) and the 48-hour EC_{50} value was calculated to be 0.046 μg/L. The estimated EC_{50} value for reproduction at 14 days was between 0.0056 and 0.018 μg/L and the estimated EC_{50}

1 Thompson, W. R. (1947) *Bacterial Reviews* **11** p: 115 - 145

for reproduction after 21 days was estimated to be 0.018 μg/L. The number of unhatched eggs and dead young were low in the control and treatment groups surviving to maturation. However, the young produced by the group exposed to 0.018 μg/L were observed to be smaller than those produced by the controls and other test groups. The NOEC for esfenvalerate to *Daphnia magna* over a 21-day period was reported to be 0.0018 μg/L which was based on the parental mortality (see below).

Table 4.4.4-2: Chronic effects of esfenvalerate on *Daphnia magna*.

Nominal concentration (μg/L)	Survival of parents (%)	Reproduction (mean number of young)	
		Total	Per parent
Control	98	2118	54
Solvent control	100	2173	54
0.00056	100	2099	52
0.0018	90	1757	49
0.0056	75	1445	48
0.018	45	457	25
0.056	0	0	0

Based on these results it was concluded that exposure of *Daphnia magna* to esfenvalerate result in an immediate lethal effect at the test concentration of 0.056 μg/L. Prolonged mortality occurred in the adults at lower test concentrations, although since the 10% mortality at 0.0018 μg/L was within control validity criteria and not statistically significant, the NOEC for parent mortality was therefore set at 0.0018 μg/L. Statistically significant impairment of reproduction occurred at the test concentration of 0.018 μg/L and above, so the reproductive NOEC would be 0.0056 μg/L. Overall the lowest 21-day NOEC was a nominal 0.0018 μg a.s./L.

4.4.5 Chronic toxicity to algae or aquatic plants

No data are available for chronic toxicity to algae or aquatic plants. A study has been submitted (see Section 4.3.3) on the toxicity of esfenvalerate to green algae (*Scenedesmus subspicatus)*. This study was undertaken following the OECD 201 (1984) guideline, and the resulting NOEC value (growth rate and biomass) was 1.0 μg/L based on nominal concentrations.

4.5 Acute and/or chronic toxicity to other aquatic organisms

OECD TG 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment and OECD TG 219: Sediment-Water Chironomid Toxicity Using Spiked Water

Study reference:

A. E. Puttt (1997) [^{14}C]Esfenvalerate - The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: LLW-0085

Detailed study summary and results:

In a 28-day static spiked water study, non-biting midge larvae (*Chironomus riparius*) were exposed to esfenvalerate at nominal concentrations of 0, 20, 40, 80, 160 and 320 ng a.s./L. The solvent control, control and treatment groups comprised eight replicates; all contained 25 chironomid larvae. Mortality, time to emergence, sex and total number of emerged adults were assessed over the 28 day period.

The toxicological endpoints are based on nominal concentrations. There were no significant effects on the development rate over 28 days at the highest treatment concentration of 320 ng a.s./kg sediment d.w., although a significant decrease in mean emergence success was evident at concentrations of 320 ng a.s./L.

In a 28-day static water-spiked test with *Chironomus riparius*, the NOEC of esfenvalerate is 160 ng a.s./L based on emergence success. The EC_{50} is >320 ng a.s./L, based on development rate and emergence success.

Test type:

Guideline BBA "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995); GLP compliant

Test substance:

[^{14}C] Esfenvalerate; lot No. RIS994026; purity 98.9%

Materials and methods:

The study was conducted in a static, non-renewal system over a 28 day period with non-biting midge (*Chironomus riparius*), from in-house culture, 2-3 days old at test initiation. The non-biting midge larvae were exposed to esfenvalerate at nominal concentrations of 0, 20, 40, 80, 160 and 320 ng a.s./L. The solvent control (acetone), control and treatment groups comprised eight replicates, and each replicate contained 25 chironomid larvae. Mortality, time to emergence, sex and total number of emerged adults were assessed over the 28 day period. NOEC and EC_{50} for total number of emerged adults after 28 days and mean daily emergence rate. In addition to the test vessels containing the chironomids, four further replicate vessels were set up and were dosed with [^{14}C] esfenvalerate at the same concentrations. These latter replicates were used to establish the concentrations of esfenvalerate in the sediment-water system.

Larvae were housed in 1600 mL glass vessels, containing 170 ml wet artificial sediment (according to OECD 207; 10% sphagnum peat, 20% kaolin clay and 70% sand) and 1300 mL water. Environmental conditions were maintained as follows: temperature 20±0.5 °C; photoperiod: 16 h light: 8 h dark; light intensity 50-70 footcandles. The vessels underwent continuous gentle aeration and chironomids were fed a diet of ground flaked fish food prior and post-dosing. The physico-chemical parameters of the media were as follows: pH 7.1-8.8; oxygen content: 8.1-8.3 mg/L; total hardness: 90-110 mg/L (as $CaCO_3$); conductivity: 240 µS/cm; ammonia: not tested.

Analytical verification of test item concentrations in the radiolabelled replicates was conducted using liquid scintillation counter (LSC) on days 0, 7 and 28, post-treatment. In addition, the 320 ng a.s./L and the solvent control were subject to HPLC-RAM analysis, to confirm the amount of radio-labelled parent compound in the test vessels.

Shapiro-Wilks test for normality was used to establish the normal distribution of the data and Bartlett's test was used to determine the homogeneity of the data. Lowest observable effect concentration for midge emergence and development rate was established using the Williams t-test ($\alpha = 0.05$).

Results:

Analytical verification of test item concentrations in the overlying water, pore water and sediment was conducted for each concentration one hour, 7 days and 28 days post treatment.

Recoveries in the overlying water were in a range between 61.9% - 95% of the nominal concentrations at test initiation. At test termination, recoveries in the overlying water were in a range between 3% - 6% of the nominal. Recoveries in the sediment at nominal concentrations of 80 and 360 ng a.s./L were in a range between 20.8% and 16% of the nominal concentrations at test initiation, respectively. At test termination the detected concentrations ranged from 106% and 119% of the nominal values, respectively. Recoveries in the pore water at nominal concentrations of 80 and 360 ng

a.s./L were 4.8% and 1.7% of the nominal concentrations at test initiation, respectively. At test termination the detected concentrations ranged from 6.4 and 1.8% of the nominal values, respectively. Despite this the endpoints were based on initial nominal concentrations in the overlying water phase and mean measured concentrations were not determined.

Statistically significant differences in % emerged adults were apparent at concentrations of 320 ng a.s/L. However, no statistically significant differences in mean daily emergence rates were observed at any test item concentration when compared to the control (based on initial measurements).

Table 4.5-1: Effects of esfenvalerate on emergence success and development rate of *Chironomus riparius*.

Nominal concentration (ng /L)	Control	Solvent control (acetone)	20	40	80	160	320
% Emergence succes (SD)	82	87(8)	93(4)	96(6)	89(12)	90(5)	68(9)*
Development rate (SD)	0.0721	0.0716	0.0722	0.0750	0.068	0.0722	0.0675

SD: Standard deviation. *****Statistically significant differences compared to the control (Williams t-test, $\alpha = 0.05$).

Based on these results it was concluded that the NOEC of esfenvalerate is 160 ng a.s./L based on emergence success and initial nominal concentrations in the water phase. The nominal EC50 is >320 ng a.s./L, also based on development rate and emergence success.