

FENVALERATE

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Explanation

Fenvalerate is the International Organization for Standardization (ISO)–approved common name for (*RS*)- α -cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 51630-58-1. It is a broad-spectrum pyrethroid with neurotoxic effects on insect pests.

Fenvalerate (Figure 1) is a racemic mixture of four stereoisomers ([2*S*, α *S*], [2*S*, α *R*], [2*R*, α *S*] and [2*R*, α *R*]) (Figure 2) found in approximately equal proportions owing to the presence of two chiral centres. One of these four chiral isomers, esfenvalerate, is the [2*S*, α *S*] or A- α isomer and has been developed separately in the knowledge that it was the biologically active component of the fenvalerate racemic mixture. Fenvalerate is classified, according to its structure, as a type II pyrethroid.

Figure 1. Chemical structure of fenvalerate

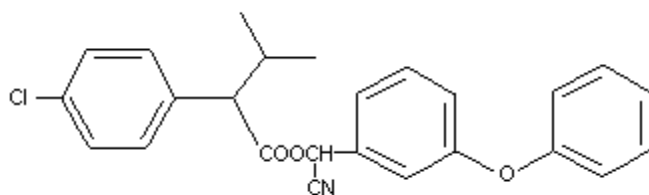
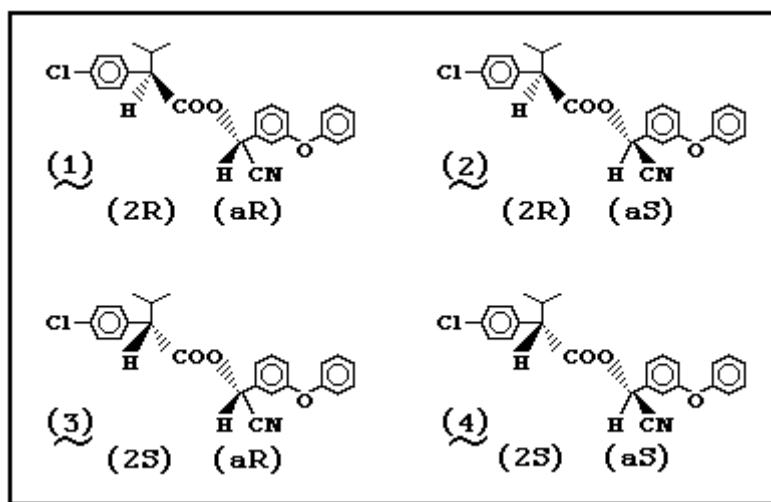


Figure 2. Chemical structure of the four stereoisomers of fenvalerate



Fenvalerate was reviewed by the Joint FAO/WHO Meeting on Pesticide Residues on four previous occasions. Temporary acceptable daily intakes (ADIs) of 0–0.06, 0–0.007 and 0–0.02 mg/kg body weight (bw) were established by the Meeting in 1979, 1981 and 1984, respectively, and an ADI of 0–0.02 mg/kg bw was established by the Meeting in 1986.

Fenvalerate is being reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

As the compound was not supported by a company that would provide toxicological studies for review, access to the toxicological studies for the current evaluation was provided by the United States Environmental Protection Agency (USEPA). The evaluation of fenvalerate was based on the previous reviews by JMPR (Annex 1, references 32, 33, 36, 37, 42, 43, 47 and 49), an International Programme on Chemical Safety evaluation (IPCS, 1990), the esfenvalerate review by JMPR (Annex 1, references 95 and 97), studies submitted to the USEPA and published studies from the open literature.

Most of the studies do not comply with good laboratory practice (GLP), as most of the data were generated before the implementation of GLP regulations. Overall, the Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Five male and five female ddY mice were administered [^{14}C -chlorophenyl]- or [^{14}C -phenoxybenzyl]fenvalerate (2.5 or 10 mg/kg bw), [^{14}C -chlorophenyl]- or [^{14}C -phenoxybenzyl]esfenvalerate (2.5 mg/kg bw) or [^{14}C -chlorophenyl]- or [^{14}C -phenoxybenzyl]esfenvalerate mixed with the three other unlabelled isomers. The chemicals were administered orally by gavage in corn oil. Urine and faeces were collected daily for 7 days. The animals were killed 7 days after dosing, and major tissues were excised. Excreta and tissues were analysed for radioactivity by LSC. In both sexes, 86–94% of the administered radioactivity was excreted within 1 day of dosing, and 94–102% was excreted within 7 days. Roughly equal quantities of radioactivity were excreted in the urine and faeces. The highest residue levels were detected in the fat tissues, with 0.118–0.484 μg equivalents per gram following a dose of 2.5 mg/kg bw and 1.16–1.579 μg equivalents per gram following a dose of 10 mg/kg bw (Kaneko et al., 1985).

In a 28-day dietary study, ddY mice were fed [^{14}C -chlorophenyl]esfenvalerate at a level of 25 parts per million (ppm) or [^{14}C -chlorophenyl]fenvalerate at a level of 25 or 100 ppm. The estimated daily intakes were 115–119 μg /mouse from the 25 ppm esfenvalerate diet and 112–118 μg /mouse and 424–477 μg /mouse from the 25 and 100 ppm fenvalerate diets, respectively. Groups of six male and six female mice were killed after feeding on the treated diets for 10, 19, 24 and 28 days and subsequently feeding on untreated diets for 4, 7, 21 and 28 days after termination of the 28-day treatment period. Concentrations of residues were highest in fat (about 7 μg esfenvalerate equivalents per gram tissue after 24–28 days on the 25 ppm esfenvalerate diet), about 7-fold lower in the adrenal gland, lymph node and skin, about 10-fold lower in liver and about 25-fold lower in blood. Levels of radioactivity approached a plateau after 24–28 days of feeding and subsequently declined when mice were returned to untreated diet. Total radioactivity concentrations in some tissues (adrenals, spleen and ovaries) were lower following feeding with esfenvalerate than with a similar dose level of fenvalerate. In other tissues, the levels of total radioactivity were similar. Total radioactivity was approximately 4 times higher in the tissues of animals fed fenvalerate at 100 ppm in the diet than in the tissues of animals fed fenvalerate at 25 ppm in their diet. There were no differences between the sexes (Isobe et al., 1985).

Groups of five male and five female ddY mice received 10 consecutive daily oral doses of [^{14}C -chlorophenyl]esfenvalerate at 2.5 mg/kg bw or [^{14}C -chlorophenyl]fenvalerate at 10 mg/kg bw. Excreta were collected daily for 7 days after the last dose. Most of the radioactivity (90% of that administered) was excreted within 1 day after the last dose. Excretion was 91–98% complete within 7 days, with equal amounts found in the urine and faeces. The study demonstrated that repeated dosing of either esfenvalerate or fenvalerate did not alter the excretion patterns with respect to dose, sex or species (Kaneko et al., 1985).

Mice and rats

A study was conducted in which ddY mice (four males and four females) and Sprague-Dawley rats (one male and one female) were given single oral doses of [^{14}C -carbonyl]-, [^{14}C -benzylic]- or [^{14}C -cyano]fenvalerate at 7.0 mg/kg bw or [^{14}C -chlorophenyl]-, [^{14}C -phenoxybenzyl]- or [^{14}C -cyano]esfenvalerate at 4.2 mg/kg bw. Each of the ^{14}C -labelled fenvalerates was also administered to two male and two female rats at the rate of 30 mg/kg bw. Urine and faeces were collected daily for 6–7 days, and carbon dioxide was collected from those mice and rats that received ^{14}C -cyano-labelled compound. The mice and rats were killed 6–7 days after dosing. Excreta and tissues were analysed for

metabolites by thin-layer chromatography (TLC), autoradiography and liquid scintillation counting (LSC).

The half-life for excretion in both rodent species was 0.5–0.6 day. Elimination of the ^{14}C -cyano-labelled fenvalerate was somewhat slower in both species, suggesting a different pattern of metabolism. Total recovery of the administered ^{14}C -cyano-labelled fenvalerate was achieved within 6 days following the acute administration. Tissue residues following acute administration were extremely low, with the highest concentrations being observed in fat, adrenal gland, skin, hair and intestines. High concentrations of the ^{14}C -cyano-labelled fenvalerate were noted in the hair and skin, which may account for the data showing that residues of this label were more slowly excreted from the body (Kaneko, Ohkawa & Miyamoto, 1979; Ohkawa et al., 1979).

Groups of four male and four female ddY mice and Sprague-Dawley rats were administered a single oral gavage dose of [^{14}C -carbonyl]fenvalerate and [^{14}C -benzylic]fenvalerate. The dose level was 6.7–7.8 mg/kg bw and 30 mg/kg bw for mice and rats, respectively. Urine and faeces were collected at several intervals for 6 or 7 days. The total recovery of the radioactivity 6 or 7 days after administration was 93–102% in mice and rats. The half-life of the radioactivity was 0.5–0.6 day in both species. In rats dosed with [^{14}C -chlorophenyl]- or [^{14}C -phenoxybenzyl]fenvalerate at 30 mg/kg bw, excretion in the urine and faeces was 86–97% complete in 6 days, with a half-life of 0.6–0.9 day. In rats given low doses of [^{14}C -cyano]fenvalerate and esfenvalerate, excretion of the radioactivity was 75–81% complete after 6–7 days, with a half-life of 1.7–2.0 days. In similarly treated mice, excretion was 88–89% complete by 6–7 days, with a half-life of 1–1.7 days. Exhaled $^{14}\text{CO}_2$ accounted for 6–14% of the administered radioactivity in both species. Tissue residues in mice and rats given low doses of [^{14}C -chlorophenyl]- and [^{14}C -phenoxybenzyl]esfenvalerate and [^{14}C -carbonyl]- and [^{14}C -benzylic]fenvalerate were very low 6–7 days after dosing. The highest levels were in fat, ranging from 0.45 to 0.89 mg equivalents per kilogram in mice and from 0.71 to 1.5 mg equivalents per kilogram in rats. When fenvalerate at 30 mg/kg bw was dosed to rats, residues in fat were 4.3–8.9 mg equivalents per kilogram. In blood, hair, liver and skin of both species, the levels were less than 0.36 mg equivalents per kilogram at the low dose level and 0.81 mg equivalents per kilogram at the high dose level. However, ^{14}C -cyano-labelled compounds produced higher residues in hair, skin and stomach contents (see Ohkawa et al., 1979, above) (Kaneko, Ohkawa & Miyamoto, 1981).

Rats

Single oral doses (20 $\mu\text{mol/kg}$ bw) or five daily doses (4 $\mu\text{mol/kg}$ bw per day) of [^{14}C -cyano]fenvalerate, [^{14}C -cyano][2S, α RS]-fenvalerate, [^{14}C -benzylic][2S, α RS]-fenvalerate, [^{14}C -carbonyl][2S, α RS]-fenvalerate, [^{14}C -carbonyl]2-(4-chlorophenyl)isovaleric acid (CPIA), KS[^{14}CN] or K[^{14}CN] suspended in 10% Tween 80 were administered to male Sprague-Dawley rats. Urine, faeces and expired carbon dioxide were collected daily for 6 and 14 days after administration of a single dose and for 6 days after the last of five daily doses. Tissue samples were obtained 6 and 14 days after dosing. Samples were analysed by TLC, autoradiography and LSC.

Similar excretion patterns were seen with single and repeated administration, except for KS[^{14}CN] and K[^{14}CN] (Tables 1 and 2). The administered radioactivity was almost completely recovered from the urine and faeces within 2 days of dosing with [^{14}C -carbonyl]- and [^{14}C -benzylic][2S, α RS]-fenvalerate (92.5% and 93.5%, respectively). None of the radioactivity administered as fenvalerate or as [2S, α RS]-fenvalerate was excreted as $^{14}\text{CO}_2$. Slower excretion was seen with KS[^{14}CN] and K[^{14}CN], of which small amounts were excreted as carbon dioxide in expired air (15.0% and 7.5%, respectively). Tissue residues resulting from dosing with ^{14}C -carbonyl- or ^{14}C -benzylic-labelled [2S, α RS]-fenvalerate were generally 0.3 mg equivalents per kilogram or less 6 days after a single dose and declined to 0.1 mg equivalents per kilogram by 14 days after dosing in adrenals, blood, brain, caecum, hair, heart, intestine, kidney, liver, lung, muscle, pancreas, sciatic nerve, skin, spinal cord, spleen, stomach and testis. The only exception was fat, which contained 2 mg equivalents per kilogram 6 days after dosing with [^{14}C -carbonyl][2S, α RS]-fenvalerate. These residues declined to 0.5 mg equivalents per kilogram 14 days after dosing.

Table 1. Percentage of administered radioactivity excreted 6 days after a single oral dose (20 $\mu\text{mol/kg bw}$) in rats

	% of administered radioactivity						K[^{14}CN]	KS[^{14}CN]
	[^{14}C -carbonyl]-[2S, α RS]-Fenvalerate	[^{14}C -benzylic]-[2S, α RS]-Fenvalerate	[^{14}C -cyano]-[2S, α RS]-Fenvalerate	[^{14}C -cyano]-Fenvalerate	[^{14}C -carbonyl]-CPIA			
Expired air	0	0	7.5	10	0	7.5		15.0
Urine	60.0	45.5	21.5	23.75	77.5	32.5		31.25
Faeces	32.5	48.0	37.5	41.25	20.0	22.5		12.50
Total	92.5	93.5	66.5	75	97.5	62.5		58.75

From Ohkawa et al. (1979)

CPIA, 2-(4-chlorophenyl)isovaleric acid

Table 2. Percentage of administered radioactivity excreted 6 days after five consecutive daily doses of [2S, α RS]-fenvalerate (4 $\mu\text{mol/kg bw}$ per day) in rats

	% of administered radioactivity		
	Carbonyl	Benzylic	Cyano
Expired air	0	0	7
Urine	47.5	58.75	25
Faeces	50.0	37.50	33.75
Total	97.5	96.25	63.75

From Ohkawa et al. (1979)

For [^{14}C -cyano]fenvalerate, [^{14}C -cyano][2S, α RS]-fenvalerate, K[^{14}CN] and KS[^{14}CN] administered as a single oral dose of 20 $\mu\text{mol/kg bw}$, the highest residue levels were found in hair and skin. Hair contained 36 mg [2S, α RS]-fenvalerate equivalents per kilogram, 41 mg KCN equivalents per kilogram and 119 mg KSCN equivalents per kilogram 6 days after dosing. At the same time point, skin contained 3 mg [2S, α RS]-fenvalerate equivalents per kilogram, 4.5 mg KCN equivalents per kilogram and 9 mg KSCN equivalents per kilogram. Fat and blood contained 1.2–1.4 mg [2S, α RS]-fenvalerate or fenvalerate equivalents per kilogram, and blood contained 1.4–1.7 mg KSCN or KCN equivalents per kilogram. A similar pattern was seen after repeated dosing.

Autoradiography showed that 6 and 24 hours after a single oral dose (20 $\mu\text{mol/kg bw}$) of [^{14}C -cyano][2S, α RS]-fenvalerate, radioactivity was distributed throughout the body, except to the brain and spinal cord. The highest concentrations (after the gastrointestinal tract) appeared to be in liver and lung, but this estimation is only as a result of visual inspection of the single autoradiograph presented 6 and 24 hours after dosing. At 144 hours after dosing, radioactivity was detected only in the hair, skin and stomach contents (the last, presumably, as a result of grooming). The distribution to the skin did not appear to show regional variation, which might have indicated that it was the result of contamination from urine. Six days after a single dose of [^{14}C -cyano][2S, α RS]-fenvalerate, TLC analysis of the stomach contents showed that essentially all of the radioactivity was due to thiocyanate at a concentration of 41 ppm. A similar pattern was seen with KS[^{14}CN] and K[^{14}CN]. Concentrations of [2S, α RS]-fenvalerate in blood and liver reached maximum levels (0.5 mg [2S, α RS]-fenvalerate equivalents per kilogram) 3 hours after a single dose of [^{14}C -cyano][2S, α RS]-fenvalerate and then rapidly declined to about 0.01 mg [2S, α RS]-fenvalerate equivalents per kilogram after 48 hours. There

appeared to be no significant differences in the excretion patterns of [2*S*, α *RS*]-fenvalerate and fenvalerate (Ohkawa et al., 1979).

Male and female rats fed fenvalerate (20 ppm) for 28 days and placed on control diets for an additional 28 days were examined for tissue residues and their depletion rates. Maximum residues were reached rapidly, within 3 weeks of dietary administration. Of the tissues measured, adipose tissue contained the highest residue. Trace amounts were observed in other tissues, including the brain, after 28 days of treatment. Dissipation of residues from all tissues following the cessation of treatment was rapid, although the dissipation was slower with adipose tissue than with other tissues. At 28 days after the cessation of administration of dietary fenvalerate, residues were still reported in adipose tissue, attesting to the slow clearance from this storage depot (Potter, 1976; Potter & Arnold, 1977).

In a separate study, fenvalerate was shown to dissipate from the adipose tissue of Sprague-Dawley rats, dosed orally with either [2*RS*, α *RS*]- or [2*S*, α *S*]-fenvalerate at 3 mg/kg bw, with a half-life of about 7 days in each case. The pyrethroid level in the brain of rats treated intraperitoneally with [2*S*, α *S*]-fenvalerate at 2.5 mg/kg bw dissipated with a half-life of about 2 days (Marei, Ruzo & Casida, 1982).

Five male and five female Sprague-Dawley rats were administered [^{14} C-chlorophenyl]- or [^{14} C-phenoxybenzyl]fenvalerate (2.5 or 10 mg/kg bw), [^{14} C-chlorophenyl]- or [^{14} C-phenoxybenzyl]esfenvalerate (2.5 mg/kg bw) or [^{14} C-chlorophenyl]- or [^{14} C-phenoxybenzyl]esfenvalerate mixed with the three other unlabelled isomers. The chemicals were administered orally by gavage in corn oil. Urine and faeces were collected daily for 7 days. The rats were killed 7 days after dosing, and major tissues were excised. Excreta and tissues were analysed for radioactivity by LSC. There were no significant differences between dose levels, sexes or quantities of metabolites in the excretion of ^{14} C-chlorophenyl- and ^{14} C-phenoxybenzyl-labelled material. Approximately 63–86% of the radioactivity was excreted 1 day after dosing, and 95–101% had been excreted by 7 days after dosing. Between 20% and 39% of the radioactivity was excreted in the urine, with the remainder in the faeces. Tissue levels were generally very low, with the highest residues found in fat (180–310 μ g equivalents per kilogram for the low-dose groups and 1320 μ g equivalents per kilogram for the high-dose group). Residue levels from the fenvalerate treatment (10 mg/kg bw) were generally 4 times higher than the residues from the esfenvalerate treatment (2.5 mg/kg bw) (Kaneko et al., 1985).

A placental transfer study was conducted in Sprague-Dawley rats. Groups of three pregnant rats were dosed orally with [^{14} C-chlorophenyl]esfenvalerate at 2.5 mg/kg bw per day or [^{14} C-chlorophenyl]fenvalerate at 10 mg/kg bw per day in corn oil. Dosing began on day 13 of gestation and consisted of either a single dose of ^{14} C-labelled compound or three consecutive daily doses of unlabelled compound followed by two consecutive daily doses of ^{14} C-labelled compound. The rats were killed 3, 6, 12, 24 or 48 hours after the final dose. The fetuses, amniotic fluid, placentas, maternal blood and ovaries were examined for radioactivity using LSC. Maximum levels of radioactivity were found within 12 hours after the last dose, after which they declined rapidly. Fenvalerate residues were generally 4 times higher than esfenvalerate residues, except in maternal blood, placenta, amniotic fluid and ovaries 24 hours after a single dose, where the difference was 18–31 times higher, and the ovaries 24 and 48 hours after multiple doses, where the levels were similar. Tissue levels were at their peak 3–6 hours after the last dose and then declined rapidly. The highest levels of residues from both pyrethroids were found in maternal blood and ovaries, and the lowest were found in the fetuses and amniotic fluid. The highest residue level found in the fetuses was 152 μ g esfenvalerate equivalents per kilogram, 6 hours after the last of five consecutive daily doses. Over the whole experiment, less than 0.07% of the applied radioactivity was found in the fetuses, indicating

that there was virtually no transfer of radioactivity from maternal blood to the fetuses. There was no evidence of accumulation of esfenvalerate in fetal tissues or amniotic fluid of rats (Shiba et al., 1985).

After administration of a single oral dose or five consecutive oral doses of [^{14}C]esfenvalerate or [^{14}C]fenvalerate labelled in the acid moiety to 13-day pregnant rats at a rate of 2.5 or 10 mg/kg bw per day, respectively, the maternal blood and placenta generally showed higher ^{14}C levels as compared with the fetus and amniotic fluid. Both compounds did not transfer readily from the maternal blood to the fetus, the amount of ^{14}C transferred being less than 0.07% of the dose. There were no substantial differences in the fetal ^{14}C level or the transfer ratio (^{14}C tissue level/ ^{14}C maternal blood level) between both labelled preparations. Overall, esfenvalerate and fenvalerate seemed to behave in the same manner as far as placental transfer was concerned (Shiba et al., 1990).

Dogs

The metabolism of fenvalerate was studied in dogs using [^{14}C -chlorophenyl]- and [^{14}C -phenoxybenzyl]fenvalerate. A single oral dose (1.7 mg/kg bw), dissolved in corn oil, was administered in gelatine capsules. Excreta and blood were collected daily for 3 days and analysed for radioactivity by LSC. Metabolites were analysed using TLC using four solvent systems. Elimination of radioactivity was rapid, primarily via the urine and faeces. Three days after dosing with [^{14}C -chlorophenyl]fenvalerate, 31.6% and 55.5% had been eliminated in the urine and faeces, respectively. Total recovery of radioactivity was higher in animals dosed with [^{14}C -chlorophenyl]fenvalerate than in those dosed with [^{14}C -phenoxybenzyl]fenvalerate. The half-lives were 1 day and 0.7 day for radioactivity administered as [^{14}C -chlorophenyl]- and [^{14}C -phenoxybenzyl]fenvalerate, respectively. Maximum levels of radioactivity in the blood (approximately 1 $\mu\text{g/ml}$) were obtained 2 hours after dosing. Radioactivity rapidly decreased in more than two phases. The blood levels 80 hours after dosing were less than 0.05 and 0.05–0.1 $\mu\text{g/ml}$ in animals dosed with [^{14}C -chlorophenyl]- and [^{14}C -phenoxybenzyl]fenvalerate, respectively. The level of the parent compound was below the limit of detection (0.01 $\mu\text{g/l}$) 48 hours after dosing (Kaneko et al., 1984).

1.2 Biotransformation

Investigation of the metabolites of [2*S*, α *RS*]-fenvalerate and fenvalerate produced by male Sprague-Dawley rats was carried out by one-dimensional TLC using two solvent systems with detection by autoradiography. Metabolites were either identified using co-chromatography with authentic standards or extracted from the gel and identified using infrared spectroscopy, mass spectrometry or nuclear magnetic resonance (NMR).

Fenvalerate undergoes several major metabolic reactions: cleavage of the ester linkage, hydroxylation in the acid and alcohol moieties and conversion of the cyano (CN) group to thiocyanate (SCN) and carbon dioxide. The resulting metabolite acids and phenols are subsequently conjugated with glucuronic acid, sulfuric acid or amino acids. More than 20 metabolites were identified. The major radioactive products in the faeces were unmetabolized fenvalerate and two ester metabolites (2'-OH-Fen and 4'-OH-Fen). No unmetabolized fenvalerate was found in the urine. The major urinary metabolites were CPIA, 3-phenoxybenzoic acid (PBacid), SCN^- and/or products of further oxidative and conjugation reactions. As it was determined that the excretion, tissue residues and metabolite excretion patterns between fenvalerate and esfenvalerate were similar, it was concluded that both compounds would be metabolized in a similar way. 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 carbon dioxide (Ohkawa et al., 1979).

In a study using male and female Sprague-Dawley rats and ddY mice dosed orally with ^{14}C -labelled acid, alcohol and cyano moieties of fenvalerate and the [2*S*, α *S*]-isomer (esfenvalerate), the

metabolites found were essentially the same in both species, with no differences between the isomers, doses or sexes. Differences between the species were observed in the nature and amounts of metabolites derived from the alcohol moiety. These were as follows:

- 4'-hydroxylation was greater in rats than in mice;
- PBacid was taurine conjugated in mice, but not in rats;
- sulfate conjugation of 3-(4'-hydroxyphenoxy)benzoic acid (4'-OH-PBacid) was greater in rats than in mice;
- conversion of the cyano group to SCN^- was greater in mice than in rats.

There was little difference between the species in the elimination of acid- and alcohol-labelled fenvalerate and esfenvalerate. Mice eliminated the cyano-labelled compounds more quickly and more completely than rats. Higher doses of acid- and alcohol-labelled compounds were excreted at a slower rate than lower doses, and the cyano labels were more slowly excreted than the acid and alcohol labels (Kaneko, Ohkawa & Miyamoto, 1981).

In a 28-day feeding study in mice, the major metabolites in the liver and kidney of animals fed [^{14}C -chlorophenyl]esfenvalerate were CPIA and the hydroxylated derivative of CPIA. These disappeared after the mice were fed untreated diets. These metabolites were also found in mice fed [^{14}C -chlorophenyl]fenvalerate. In addition, CPIA-cholesterol ester was found in mice fed [^{14}C -chlorophenyl]fenvalerate, but not in mice fed [^{14}C -chlorophenyl]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 that time (Isobe et al., 1985).

In a separate study, rats fed 20 ppm fenvalerate in the diet for 28 days were sacrificed, and residues in adipose tissue were examined. Based upon chromatographic and mass spectral analysis, the residues in fat were characterized as unchanged fenvalerate containing both diastereoisomers (Boyer, 1977).

In a placental transfer study with rats, metabolites were tentatively identified by co-chromatography with standards, using TLC and five solvent systems, visualized by autoradiography and ultraviolet (UV) fluorescence and quantified by LSC. The major metabolites, CPIA and 3-OH-CPIA in its free and lactone forms, and the parent compounds were found in the maternal blood and placenta and fetuses. A trace of the CPIA-cholesterol ester [cholesteryl (2*R*)-2-(4-chlorophenyl)isovalerate], derived from [^{14}C -chlorophenyl]fenvalerate, was found in the maternal blood and placenta, but not the fetuses. Consecutive treatment resulted in slightly higher 3-OH-CPIA levels in rats dosed with esfenvalerate than with fenvalerate and slightly higher CPIA levels after dosing with fenvalerate than after dosing with esfenvalerate (Shiba et al., 1985).

After administration of a single oral dose or five consecutive oral doses of [^{14}C]esfenvalerate or [^{14}C]fenvalerate labelled in the acid moiety to 13-day pregnant rats at a rate of 2.5 or 10 mg/kg bw per day, respectively, major ^{14}C compounds in the fetus, maternal blood and placenta were the parent compounds, CPIA and CPIA-hydroxylated derivatives, and there was no qualitative difference in metabolic fates between the two compounds, except that a trace amount of CPIA-cholesterol ester was detected in the maternal blood and placenta only with fenvalerate. CPIA-cholesterol ester did not seem to transfer from the maternal blood to the fetus (Shiba et al., 1990).

In a study with dogs given a single oral dose of ^{14}C -chlorophenyl- and ^{14}C -phenoxybenzyl-labelled fenvalerate, excreta were analysed for metabolites that were tentatively identified and quantified by TLC co-chromatography with standards, using six solvent systems, either with or

without enzymatic hydrolysis. Visualization was by UV fluorescence and autoradiography. The major metabolites 0–1 day after dosing are shown in Table 3.

Table 3. Excreted metabolites in dogs following a single oral dose of [^{14}C]fenvalerate (1.7 mg/kg bw)

		% of administered radioactivity			
		[^{14}C -Chlorophenyl]		[^{14}C -Phenoxybenzyl]	
		Urine	Faeces	Urine	Faeces
Fenvalerate		0.2	8.8	—	3.7
4'-OH-Fen		—	1.8	—	2.3
PBalc		—	—	—	1.3
4'-OH-PBalc	Free	—	—	—	3.3
	Glucuronide conjugate	—	—	0.6	—
PBacid	Free	—	—	3.1	1.4
	Glucuronide conjugate	—	—	—	0.4
	Glycine conjugate	—	—	8.4	—
4'-OH-PBacid	Free	—	—	2.4	3.5
	Sulfate conjugate	—	—	5.8	1.1
	Glucuronide conjugate	—	—	3.8	—
CPIA	Free	2.2	3.2	—	—
	Glucuronide conjugate	27.3	—	—	—
3-OH-CPIA	Free	2.9	—	—	—
	Glucuronide conjugate	4.9	—	—	—
3-OH-CPIA-lactone		—	1.9	—	—
2,3-OH-CPIA		3.4	—	—	—

From Kaneko et al. (1984)

CPIA, 2-(4-chlorophenyl)isovaleric acid; 2,3-OH-CPIA, 2-(4-chlorophenyl)-2,3-dihydroxymethyl-butenic acid; 3-OH-CPIA, 2-(4-chlorophenyl)-3-hydroxymethyl-butenic acid; 3-OH-CPIA-lactone, 2-(4-chlorophenyl)-3-methylbutane-4-olide; 4'-OH-Fen, α -cyano-3-(4'-hydroxyphenoxy)benzyl-2-(4-chlorophenyl) isovalerate; 4'-OH-PBacid, 3-(4'-hydroxyphenoxy)benzoic acid; 4'-OH-PBalc, 3-(4'-hydroxyphenoxy)benzyl alcohol; PBacid, 3-phenoxybenzoic acid; PBalc, 3-phenoxybenzyl alcohol

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

- 2'-hydroxylation of the alcohol moiety did not occur in dogs;
- 3-phenoxybenzyl alcohol (PBalc) and 3-(4'-hydroxyphenoxy)benzyl alcohol (4'-OH-PBalc) were detected in dogs only;
- 3-phenoxybenzoylglycine (glycine conjugate of PBacid) was the predominant conjugate of the alcohol moiety in dogs, but a minor one in rats;
- glucuronides of the acid moiety and its hydroxy derivatives were greater in dogs (Kaneko et al., 1984).

The excreta of the rats in an earlier comparative study were analysed for metabolites by TLC using five solvent systems, autoradiography and LSC, sometimes after enzyme treatment. The metabolites were tentatively identified by co-chromatography with unlabelled standards, which were visualized by UV fluorescence. There was no difference in metabolite excretion between the sexes or compounds. In faeces, 44.5–59.9% of the administered radioactivity from the ^{14}C -chlorophenyl- or ^{14}C -phenoxybenzyl-labelled materials was excreted as the parent compound, whereas 2.9–6.5% of the ^{14}C -chlorophenyl-labelled materials was excreted as the 2'-OH parent compound (2'-OH-Fen) and 4'-OH parent compound (4'-OH-Fen). The same amount of ^{14}C -phenoxybenzyl-labelled material was excreted as 4'-OH-Fen.

Other faecal metabolites were CPIA, its 3- and 2,3-hydroxy derivatives (3-OH-CPIA and 2,3-OH-CPIA) and 2-(4-chlorophenyl)-*cis*-2-butenedioic acid (Cl-BDacid) in their free forms from the acid moiety, and PBacid and 3-(4'-hydroxyphenoxy)benzoic acid (4'-OH-PBacid) in their free forms from the alcohol moiety. The major urinary metabolites from the acid moiety were CPIA-glucuronide, 3-OH-CPIA (free and lactone), 2,3-OH-CPIA-glucuronide and Cl-BDacid anhydride. The major urinary metabolite from the alcohol moiety was 4'-OH-PBacid sulfate (16.4–23.9% of the administered radioactivity). Other metabolites included PBacid (free and as glycine and glucuronide conjugates), 4'-OH-PBacid (free and as glucuronide) and 2'-OH-PBacid (free and as sulfate) (Kaneko et al., 1985).

The excreta of mice dosed with ^{14}C -acid- and ^{14}C -alcohol-labelled fenvalerate and esfenvalerate, as described above, were analysed for metabolites as above. There were no significant sex differences in metabolite excretion. The only difference between esfenvalerate and fenvalerate was that a trace amount of the cholesterol ester of CPIA was found in the faeces of mice treated with [^{14}C -chlorophenyl]fenvalerate. In faeces, 25.6–48.1% of the radioactivity from administration of ^{14}C -chlorophenyl- or ^{14}C -phenoxybenzyl-labelled materials was excreted as the parent, 1.4–4.4% was excreted as 4'-OH-Fen and 0.0–1.1% was excreted as 2'-OH-Fen. The other faecal metabolites were as found in the rats. The acid-labelled urinary metabolites mainly consisted of CPIA (free and glucuronide) and 2,3-OH-CPIA-glucuronide. The major alcohol-labelled urinary metabolites were the taurine conjugate of PBacid (7.9–17.3% of the administered radioactivity) and the free, sulfate and glucuronide forms of 4'-OH-PBacid (6.9–9.9% of the administered radioactivity). The minor metabolites were predominantly the same as those found in the rats (Kaneko et al., 1985).

The identification of the [2*R*, α *S*]-isomer of fenvalerate as the only source of CPIA-cholesterol ester was established. [^{14}C -chlorophenyl]Fenvalerate was administered to groups of two male Sprague-Dawley rats and four male ddY mice as a single oral dose of 2.5 mg/kg bw as the [2*S*, α *S*]-, [2*S*, α *R*]-, [2*R*, α *S*]- or [2*R*, α *R*]-isomers of fenvalerate. Excreta were collected for 6 days, and the radioactivity in the tissues and excreta was analysed by LSC. The residues of [2*S*, α *S*]-, [2*S*, α *R*]- and [2*R*, α *R*]-isomers were very low (100 μg equivalents per kilogram or less) in all tissues except fat (310–760 μg equivalents per kilogram) in both species. However, administration of the [2*R*, α *S*]-isomer resulted in higher residues in all tissues, particularly the adrenals, liver, mesenteric lymph nodes and spleen, although residues in fat were similar to those found as a result of administration of the other isomers. Thus, this lipophilic tissue residue does not occur to any appreciable extent in rats or mice dosed with esfenvalerate (Kaneko, Matsuo & Miyamoto, 1986).

To demonstrate this more clearly, groups of male ddY mice were fed diets containing [2*R*, α *S*]-, [2*S*, α *S*]- or [2*R*, α *R*]-isomer at 500 mg/kg bw for 1 or 2 weeks. Similar results were obtained, and further analysis by TLC showed that a lipophilic metabolite was predominant in all tissues of the mice treated with the [2*R*, α *S*]-isomer only. The liver of the [2*R*, α *R*]-treated mice contained trace amounts of the lipophilic metabolite. The lipophilic metabolite was identified by NMR or TLC co-chromatography, with authentic standards, using three different solvent systems, high-performance

liquid chromatography and mass spectrometry and was found to be the CPIA–cholesterol ester (Kaneko, Matsuo & Miyamoto, 1986).

An investigation of the mechanism of the formation of the CPIA–cholesterol ester from the [2*R*, α *S*]-isomer was conducted. Various tissues, including brain, kidney, liver, spleen, adrenals, intestines and lymph nodes, were excised from male Sprague-Dawley rats, ddY mice, Beagle dogs and Rhesus monkeys. The 9000 $\times$ *g* supernatant and pellet, microsomal fraction (100 000 $\times$ *g* pellet) and 100 000 $\times$ *g* supernatant were obtained by centrifugation. Plasma was obtained from blood by centrifugation. Incubation of each of the ¹⁴C-labelled isomers of fenvalerate with the 9000 $\times$ *g* supernatants revealed that the [2*S*, α *S*]-, [2*S*, α *R*]- and [2*R*, α *R*]-isomers produced the hydrolysis product, CPIA, only, whereas the [2*R*, α *S*]-isomer resulted in the formation of CPIA and the CPIA–cholesterol ester. The production of CPIA depended on the tissue. In mice, it was most rapidly formed from the [2*R*, α *S*]-isomer in the kidney, brain and spleen, from the [2*R*, α *S*]- and [2*R*, α *R*]-isomers in the liver and from the [2*S*, α *R*]- and [2*R*, α *R*]-isomers in plasma.

CPIA–cholesterol ester was formed only from the [2*R*, α *S*]-isomer in all tissues and species examined, apart from the mouse kidney, which produced only a trace amount from the [2*R*, α *R*]-isomer. There were some species differences in the enzymatic activities of various tissues, but in general, the greatest activities were obtained from mouse tissues. Mouse kidney, brain and spleen produced the most CPIA–cholesterol ester. Free CPIA was not a substrate for the formation of CPIA–cholesterol ester. The activity was destroyed by heat, inhibited by tetraethylpyrophosphate (TEPP) and not affected by *p*-chloromercuribenzoic acid. Incubation of the [2*R*, α *S*]-isomer with subcellular fractions of mouse brain, liver, kidney and spleen showed that most of the CPIA–cholesterol ester-forming activity was located in the microsomes (Miyamoto, Kaneko & Takamatsu, 1986).

The role of three known routes of cholesterol ester synthesis in the formation of CPIA–cholesterol ester was investigated. Mouse liver microsomes were incubated with oleic acid or CPIA with and without adenosine triphosphate, coenzyme A and TEPP. Although the CPIA–cholesterol ester was formed, this activity was inhibited by TEPP, whereas TEPP had no effect on the oleic acid–cholesterol ester formation. This indicated that acyl coenzyme A–cholesterol acyl transferase was not involved in the formation of the CPIA–cholesterol ester.

Mouse plasma and kidney microsomes were incubated with lecithin or [2*R*, α *S*]-fenvalerate. Lecithin–cholesterol ester was obtained from plasma but not kidney microsomes, and CPIA–cholesterol ester was obtained from kidney microsomes but not plasma. This showed that lecithin–cholesterol acyl transferase was not involved in the formation of the CPIA–cholesterol ester. Incubation of oleic acid, [2*R*, α *S*]-fenvalerate or CPIA with intestinal mucosal microsomes or intestinal mucosal 100 000 $\times$ *g* supernatant and cholesterol resulted in a cholesterol ester from oleic acid only. This showed that cholesterol esterase was not involved.

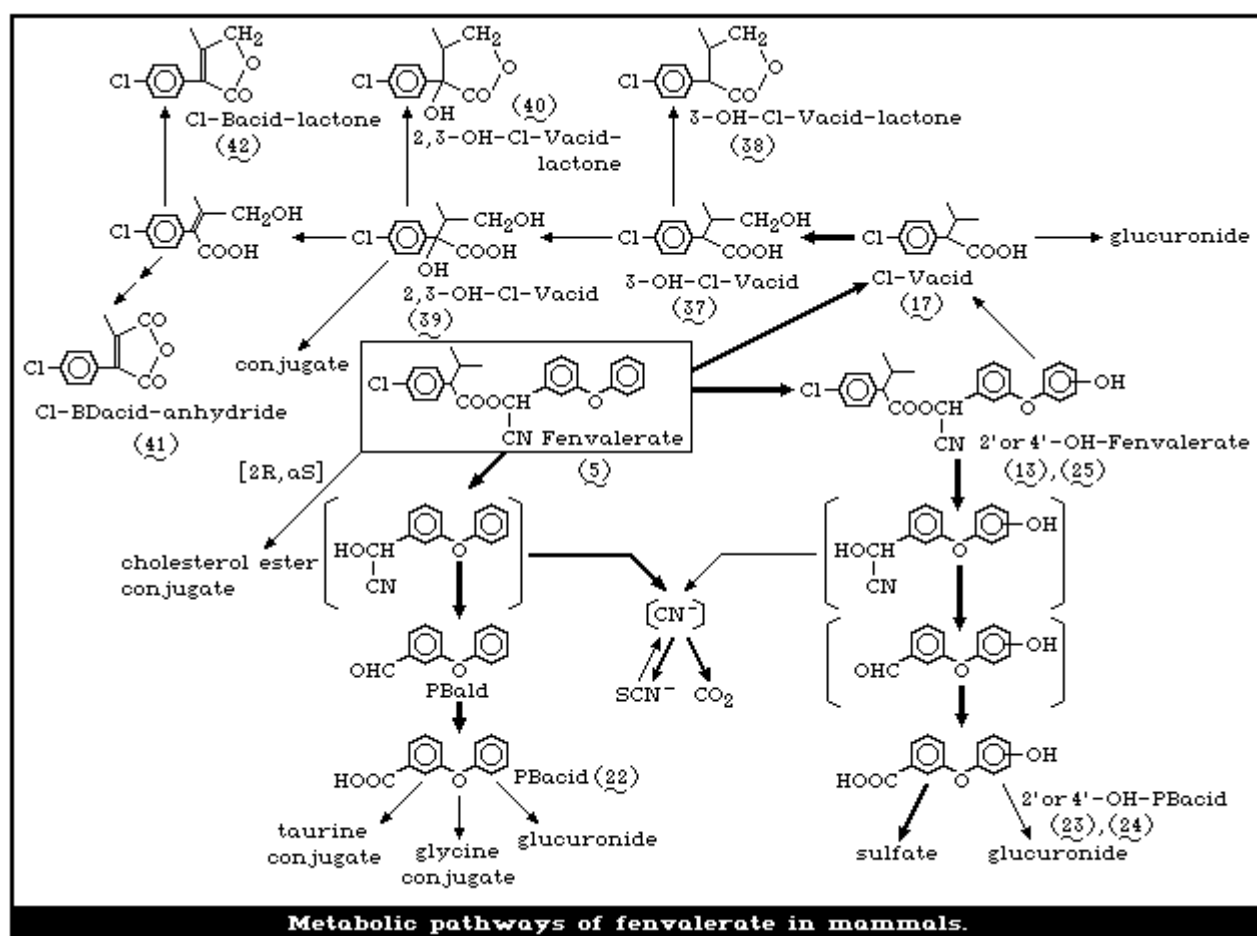
In order to determine the enzyme responsible for the formation of the CPIA–cholesterol ester, mouse kidney and liver microsomes were incubated with [2*R*, α *S*]-fenvalerate only or [2*R*, α *S*]-fenvalerate and methanol, ethanol, *n*-propanol or *n*-butanol. It was found that in the presence of the straight-chain alcohols, the amount of CPIA–cholesterol ester was reduced and the amount of CPIA–alkyl ester was increased with increasing chain length. In addition, incubation of mouse kidney microsomes with [2*R*, α *S*]-isomer and cholesterol or cholesterol oleate resulted in the production of CPIA–cholesterol ester from cholesterol only. Therefore, the CPIA–cholesterol ester is produced from free cholesterol, and alkyl alcohols also act as “receptors” for CPIA. It was inferred that the formation of the CPIA–cholesterol ester from [2*R*, α *S*]-fenvalerate was a transesterification reaction mediated by microsomal carboxylesterase(s). When mouse kidney microsomes were solubilized with digitonin, they retained the hydrolysis activity but did not produce the CPIA–cholesterol ester. Addition of liposomes to the solubilized enzyme restored the cholesterol ester-forming activity. These findings

suggest that, in microsomes, the carboxylesterase(s) and cholesterol are spatially arranged so that cholesterylolysis of the intermediary CPIA–enzyme complex is possible, resulting in the formation of CPIA–cholesterol ester (Miyamoto, Kaneko & Takamatsu, 1986; Kaneko et al., 1988).

Photolysis of fenvalerate in solvents by artificial light and as a thin film on glass or cotton by sunlight yields products resulting from ester cleavage. The major pathway in solution was identified as a photo-induced decarboxylation yielding an unusual product, not known to occur in mammals (Holmstead, Fullmer & Ruzo, 1978).

The metabolic pathways of fenvalerate in mammals are illustrated in Figure 3.

Figure 3. Metabolic pathways of fenvalerate in mammals



2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with fenvalerate are summarized in Table 4. All the studies were in compliance with European Union guidelines of the day and either in compliance with GLP or, where pre-1993, conducted and reported to an adequate standard.

Table 4. Acute toxicity of fenvalerate

Species	Sex	Route	Vehicle	Result (LD ₅₀ or LC ₅₀)	Reference
Mouse	M, F	Oral	DMSO	M: 200–300 mg/kg bw F: 100–200 mg/kg bw	Walker, Hend & Linnett (1975)
Mouse	NS	Oral	PEG/water	1202 mg/kg bw	Summitt & Albert (1977b)
Rat	NS	Oral	DMSO	451 mg/kg bw	Walker, Hend & Linnett (1975)
Rat	NS	Oral	PEG/water	> 3200 mg/kg bw	Summitt & Albert (1977a)
Chinese hamster	M, F	Oral	DMSO	M: 98 mg/kg bw F: 82 mg/kg bw	Walker, Hend & Linnett (1975)
Syrian hamster	NS	Oral	PEG/water	~760 mg/kg bw	Hart (1976a)
Dog	NS	Oral	PEG/water or corn oil	100–1000 mg/kg bw emetic	Hart (1976b)
Hen	NS	Oral	NS	> 1500 mg/kg bw	Milner & Butterworth (1977)
Rat	NS	Dermal	NS	5000 mg/kg bw	Okuno, Kadota & Miyamoto (1976)
Rabbit	NS	Dermal	Undiluted	1000–3200 mg/kg bw	Hine (1975)
Rat	M, F	Inhalation	Water	> 101 mg/m ³ (3 h)	Kohda, Kadota & Miyamoto (1976b)
Mouse	M, F	Inhalation	Water	> 101 mg/m ³ (3 h)	Kohda, Kadota & Miyamoto (1976b)
Mouse	M, F	Intraperitoneal	Corn oil	58–89 mg/kg bw	Kohda et al. (1979)
Mouse	NS	Intravenous	Glyceroformol	65 mg/kg bw	Albert & Summitt (1976)

DMSO, dimethyl sulfoxide; F, female; LC₅₀, median lethal concentration; LD₅₀, median lethal dose; M, male, NS, not specified; PEG, polyethylene glycol

Within 4 hours of dosing, all animals receiving acutely toxic levels were restless and developed tremors, piloerection, occasional diarrhoea and an abnormal gait. Following oral administration, animals recovered rapidly from acute clinical signs of poisoning and were asymptomatic within 3–4 days. Immediately after exposure, rats showed an abnormal gait, which is typical of pyrethroid intoxication. The animals walked with hindquarters held up and the hind legs more widely spaced than normal (splayed). Histological examination of the sciatic nerve and posterior tibial nerve, after poisoning and for 9 days over the course of recovery, showed axonal breaks, swelling and vacuolation accompanied by vacuolation and phagocytosis of myelin. The degree to which myelin was disrupted was dose dependent and was closely associated with the acute signs of toxicity (Butterworth & Carter, 1976).

No data are available on skin and eye irritation or skin sensitization. However, data are available on esfenvalerate, which is not irritating to the skin and minimally irritating to the unwashed eyes of rabbits. It was a skin sensitizer in guinea-pigs using the Magnusson and Kligman maximization test, but was not a sensitizer using the Buehler test method (Annex 1, references 95 and 97).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

In a 10-week published dose range-finding study, groups of 4–8 male and female C57B1/6 mice received fenvalerate (purity 99%) via gavage at 0, 20, 40, 80 or 160 mg/kg bw per day in arachis oil, 5 days/week for 10 weeks. All animals were necropsied and examined histopathologically.

At 160 mg/kg bw per day, four male and four female mice died the day after the treatment, and three male and three female mice died before the end of the experiment, probably due to gavage error at 80 mg/kg bw per day. All animals at 80 and 160 mg/kg bw per day were agitated for several hours after the treatment. No dose-related gross pathology was observed in any group. Treatment-related liver, spleen and lymph node microgranulomas were observed in mice surviving the 10-week treatment schedule. No such lesions were observed in control mice (Cabral & Galendo, 1990).

Rats

In a no-effect level determination study for neurotoxicity, male and female Sprague-Dawley rats were exposed to fenvalerate (purity 97.7%) in diets mixed with corn oil for 7 or 9 days for neuropathological examination. In the first study, 8 male and 8 female rats and 24 male and 24 female rats were treated at 0 or 3000 ppm (equivalent to 0 and 300 mg/kg bw per day), respectively. In another study, 10 rats of each sex were administered fenvalerate at 0, 500 or 1500 ppm (equivalent to 0, 50 and 150 mg/kg bw per day, respectively). During the whole feeding period, clinical signs and mortalities were checked daily. Feed consumption and body weight were recorded twice during the feeding period. The surviving rats were sacrificed on days 7–9 for neuropathological examinations.

Rats administered the highest dose of fenvalerate developed severe toxic symptoms, but the rats of other groups showed no toxic symptoms, except for those in the 1500 ppm group, which had slight hypersensitivity. Clinical signs of intoxication appeared within 24–48 hours, characterized by hypersensitivity, erratic jumping, tremor and hindlimb ataxia. Eight male and six female rats in the 3000 ppm group died within 8 days after treatment. Body weight and feed consumption were reduced at 1500 and 3000 ppm. Sciatic nerves of rats at the highest dose showed swelling and, in some rats, disintegration of the axons. Sex differences in these lesions were not found. The sciatic nerve changes were minute and sporadic at lower doses and occasionally found in control rats.

Thus, the histopathological no-effect level for fenvalerate was 1500 ppm (equivalent to 150 mg/kg bw per day), although the rats in this group showed slight symptoms. Therefore, the neurotoxic effects are considered to be found only in rats receiving lethal or near-lethal doses (Miyamoto, Okuno & Kadota, 1977).

Groups of six male and six female Carworth Farm E rats were fed diets containing 0 or 2000 ppm (equivalent to 0 and 200 mg/kg bw per day) of fenvalerate (WL 43775, purity 98.0%). A randomized block design was used. Daily observations were made on the general health and behaviour of each animal. After 8 days of feeding in the males and 10 days in the females, both treated and control animals were killed for pathological examination. Following gross pathological examination, sections of sciatic nerve were stained by the cresyl violet–luxol fast blue method for myelin and by the Gles and Marsland method for axons and then examined histologically.

In all male and female rats exposed to 2000 ppm fenvalerate, there were typical signs of ataxia, tremors and some hypersensitivity to noise indistinguishable from pyrethroid intoxication. Pathological examination of the 11 sciatic nerves of exposed rats (one sciatic nerve was lost) did not reveal neuropathological changes (Stevenson, Hend & Butterworth, 1976).

In a 90-day toxicity study, groups of Carworth Farm E rats (12 males and 12 females per group) were fed fenvalerate (98%) in the diet at a dose level of 0, 125, 500, 1000 or 2000 ppm

(equivalent to 0, 12.5, 50, 100 and 200 mg/kg bw per day, respectively) for 90 days. Mortality (11/12 males and 9/12 females) was observed at 2000 ppm. Body weight gain and feed consumption were decreased at 1000 and 2000 ppm. With the exception of an increase in blood urea nitrogen concentration at 1000 and 2000 ppm, haematological and clinical chemistry examinations at the conclusion of the study were normal. Gross and microscopic examinations of tissues and organs were performed at the conclusion of the study. Increases in liver to body weight and kidney to body weight ratios were observed at 500 ppm and above. These gross changes were not accompanied by observable microscopic changes on histopathological examination. Sciatic nerves, examined at the conclusion of the study, showed no myelin or axonal degeneration.

The NOAEL was 125 ppm (equivalent to 12.5 mg/kg bw per day), based on increases in relative liver and kidney weights seen at 500 ppm (equivalent to 50 mg/kg bw per day) (Hend & Butterworth, 1975).

Hamsters

In a short-term dietary toxicity study, 12 male and 12 female Golden Syrian hamsters were fed diet containing fenvalerate (SD-43775, technical grade, purity was not specified) at 0, 1, 10, 100 or 1000 ppm (equivalent to 0, 0.1, 1, 10 and 100 mg/kg bw per day, respectively) for 28 days.

No dose-related effects on body weight or feed consumption were noted. At postmortem examination, no gross pathology was apparent, but decreases in spleen weight (males at 1000 ppm, females at all dose levels) and adrenal weights (males at 100 and 1000 ppm, females at all but the lowest dose level) and increases in liver weight (high-dose females only) were noted (Hart, 1975a).

In a dose range-finding study, groups of 4–8 male and female Syrian Golden hamsters received fenvalerate (purity 99%) via gavage at 0, 20, 40, 80 or 160 mg/kg bw per day in arachis oil, 5 days/week for 10 weeks. All animals were necropsied and examined histopathologically.

All hamsters survived to the end of the study. Slight hepatocyte hypertrophy was observed in those hamsters receiving fenvalerate at 80 and 160 mg/kg bw per day. No microgranulomatous changes were observed in any organs (Cabral & Galendo, 1990).

Dogs

In a short-term toxicity study, groups of young adult Beagle dogs (four males and four females per group) were fed fenvalerate (SD 43775, purity 98.0%) in the diet at a dose level of 0, 0.05, 0.25, 1.25 or 12.5 mg/kg bw per day for 90 days.

There were no abnormalities over the course of the study. Body weight gain, feed consumption and behaviour were normal. Results of clinical laboratory examinations performed 3 times during the course of the study showed no effects of fenvalerate administration in the diet. At the conclusion of the study, data from gross and microscopic examinations of a variety of tissues and organs substantiated the clinical data, again showing no effects of dietary fenvalerate administration. Daily administration at a dose of 12.5 mg/kg bw per day for a period of 90 days produced no detectable evidence of toxicological effects.

The NOAEL was 12.5 mg/kg bw per day, the highest dose tested (Hart, 1975b).

In a 6-month toxicity study, male and female Beagle dogs (six of each sex per group) were fed diets containing fenvalerate (purity 91%) at 0, 250, 500 or 1000 ppm (equivalent to 0, 18.8, 37.5 and 75.0 mg/kg bw per day, respectively).

Prominent clinical signs related to treatment were emesis, head shaking, biting of the extremities and tremors. The mean body weights of female dogs fed fenvalerate at 1000 ppm were significantly lower than those of controls. Red blood cell counts and haematocrit and haemoglobin

values in both male and female dogs fed the highest dose were significantly lower than those of controls. Serum cholesterol and alkaline phosphatase levels were also increased, mostly in the group fed 1000 ppm. Hepatic multifocal microgranulomas observed during microscopic examination increased in incidence and severity in a dose-dependent manner and were considered to be related to treatment. Histiocytic cell infiltrates in the mesenteric lymph nodes of some female dogs fed 500 or 1000 ppm and of male dogs fed 1000 ppm were the only other treatment-related effects observed microscopically.

The NOAEL was 250 ppm (equivalent to 18.8 mg/kg bw per day), based on microscopic findings in the mesenteric lymph nodes and histiocytic cell infiltrates in females seen at 500 ppm (equivalent to 37.5 mg/kg bw per day) (Piccirillo, 1981; Parker et al., 1984).

(b) *Dermal application*

Rats

In a dermal neurotoxicity study, male Sprague-Dawley rats were exposed to fenvalerate (purity 97.7%) via dermal application. Four rats were treated dermally with a single application of fenvalerate and NRDC-149 (cypermethrin) at 5000 mg/kg bw. Another group of 10 males was treated dermally with fenvalerate and NRDC-149 at 2500 and 5000 mg/kg bw per day for five consecutive repeated applications. The single-application rats were killed on day 5, and the repeated-exposure groups were killed on day 7.

Toxic symptoms, such as hypersensitivity, tremor and hindlimb ataxia, were observed in all rats of each treated group, except that hindlimb ataxia was not seen with a single application of 5000 mg/kg bw of fenvalerate. Some animals in each repeated-dose group except NRDC-149 at 2500 mg/kg bw died during the observation period. Lesions such as swelling and/or disintegration of axon in sciatic nerves of all rats treated with fenvalerate and NRDC-149 were found histopathologically. The changes were related to total dosage of the test compounds. The degree of lesion in fenvalerate-treated groups was less than that in NRDC-149-treated groups. The above results suggest that the dermal application of lethal or near-lethal doses of fenvalerate and NRDC-149 caused neurotoxic effects in male rats (Okuno & Kohda, 1976).

Rabbits

In a 21-day dermal toxicity study, groups of rabbits (7–8 males per group) were administered fenvalerate dermally at a dose level of 0, 100 or 400 mg/kg bw per day for 6 hours/day (14 exposures were performed over a 22-day period).

Mortality was observed at the high dose level, accompanied by severe weight loss, clinical signs of poisoning and gross dermal effects. Rabbits tolerated the 100 mg/kg bw per day dose with minor local dermatological effects (Hine, 1975).

In a 21-day dermal toxicity study, fenvalerate (DPX-Y4306-90, purity 95.4%) was applied to the shaved skin of five New Zealand White rabbits of each sex per dose at a dose level of 0, 100, 300, or 1000 mg/kg bw per day, 6 hours/day, during a 21-day (male) or 22-day (female) period consecutively under occlusive dressing. A control group (five of each sex) was similarly treated with deionized water. After an exposure period of approximately 6 hours, the bandages were removed and the test substance was removed by washing. The test substance was not soluble or miscible in water; therefore, the test site was first washed with dilute (50–70%) acetone, followed by an Ivory[®] soap and water solution to remove excess test substance. The test site of each rabbit was then gently rinsed with lukewarm water, and the skin was patted dry. Control animals received the same washing technique as treated animals. At this time, the animals were observed for dermal irritation and clinical signs of toxicity. Dermal irritation was scored according to the Draize scale. All surviving male and female rabbits were killed by design for gross and microscopic pathological examinations following blood collection on test days 22 and 23, respectively.

There were no compound-related effects on mortality, clinical signs, body weight, feed consumption, feed efficiency, haematology, clinical chemistry, organ weights, or gross and histological pathology. There were treatment-related increased incidences of dermal irritation, which included the following: moderate erythema in 2/8, 2/10, 4/8 and 4/10 animals; mild oedema in 0/8, 1/10, 3/8 and 3/10 animals; superficial necrosis in 0/8, 0/10, 3/8 and 4/10 animals; and scar tissue in 0/8, 0/10, 3/8 and 2/10 animals in the control, low-dose, mid-dose and high-dose groups, respectively.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (MacKenzie, 1992).

(c) *Exposure by inhalation*

In a 4-week inhalation toxicity study, groups of Sprague-Dawley rats and ICR mice (10 males and 10 females of each species per group) were administered fenvalerate by inhalation exposure 4 hours daily for 4 weeks at a concentration of 0 or 20 mg/m³. Histopathological examinations were performed on various tissues/organs.

No treatment-related findings were observed in a comparison between treated groups of rats and mice and corresponding controls (Ito, 1976b).

In a second 4-week inhalation toxicity study, groups of Sprague-Dawley rats and ICR mice (10 males and 10 females of each species per group) were administered fenvalerate by inhalation exposure 3 hours daily for 4 weeks at a concentration of 0, 2, 7 or 20 mg/m³. Animals were exposed to a small, fully respirable particulate (1–2 µm) during the course of the study.

Mortality was not noted over the course of the study, but animals at the high dose level showed acute toxic signs of poisoning. No treatment-related effects on body weight, haematology or clinical chemistry parameters were observed. Gross and microscopic examinations of tissues and organs at the conclusion of the study showed no changes that were related to the administration of fenvalerate. The sciatic nerve was not examined in this study (Kohda et al., 1976c).

2.3 *Long-term studies of toxicity and carcinogenicity*

Mice

In a chronic toxicity and carcinogenicity study, groups of ddY mice (35–47 of each sex per group) were administered fenvalerate in the diet for 78 weeks at a dose level of 0, 100, 300, 1000 or 3000 ppm (equivalent to 0, 5, 15, 50 and 150 mg/kg bw per day, respectively). Body weights were recorded periodically. At 3 months and terminal sacrifice, haematological and biochemical changes were determined, and a gross macroscopic examination of tissues and organs was carried out.

In the early stages of the study, mortality was evident at 1000 and 3000 ppm. Body weight was depressed at 3000 ppm throughout the study and at 1000 ppm during the first 3 months. Hypersensitivity, excitability and other behavioural changes were observed in the 1st month of feeding at 1000 and 3000 ppm. A variety of haematological parameters were affected during the first 3 months of the study, predominantly at the high dose level, but no haematological changes were observed at the end of the study. Several biochemical changes were observed, including a decrease in alkaline phosphatase, an increase in blood urea nitrogen, an increase in leucine aminopeptidase (LAP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities and a decrease in cholesterol and glucose occurring at 300 (sporadically), 1000 and 3000 ppm, with several of these parameters being affected at 100 ppm (e.g. glucose decrease in females). Biochemical changes noted at the 3-month sacrifice interval were not seen at the conclusion of the study (increased blood urea nitrogen, decreased glucose, etc.). There were gross changes in several organ weights and in organ to body weight ratios, predominantly in the liver in both male and female mice exposed to 3000 ppm. Other organ weight changes were also observed in the kidney, heart, spleen, pituitary and ovary at the highest dose level. Microscopic examination suggested

changes in the liver, mesenteric lymph nodes and kidney at the two highest dose levels. Multiple small necrotic foci in the liver and changes in the epithelial cells of the proximal convoluted tubules were noted, apparently related to the presence of fenvalerate in the diet. There were no indications in this study of tumorigenicity or carcinogenicity as a result of fenvalerate administration.

The NOAEL was 300 ppm (equivalent to 15 mg/kg bw per day), based on reduced body weights, clinical signs of hyperactivity, mortality and clinical chemistry changes seen at 1000 ppm (equivalent to 50 mg/kg bw per day). The NOAEL for carcinogenicity in mice was 3000 ppm (equivalent to 150 mg/kg bw per day), the highest dose tested (Suzuki, Kadota & Miyamoto, 1976; Ito, 1976a, 1978; Suzuki et al., 1977b).

In a second carcinogenicity study, groups of 50 male and 50 female B6C3F1 mice were fed fenvalerate (SD-43775, purity 98%) at a dietary concentration of 0, 10, 50, 250 or 1250 ppm (equivalent to 0, 1.5, 7.5, 37.5 and 187.5 mg/kg bw per day) for 2 years. Two groups of control mice, 50 of each sex per group, received basal diet only.

Mortality was significantly increased in male and female mice in the 1250 ppm treatment group. Mortality ranged from 19% to 56% and was largely unaffected by treatment. Mortality in the 1250 ppm group was 56% for males and 54% for females, these proportions being significantly greater than in the controls, but mortality also reached 54% for males in the 10 ppm group (Table 5). There were no compound-related signs of toxicity.

Table 5. Mortality in a 2-year carcinogenicity study in mice

Dietary concentration (ppm)	Males (dead or moribund)	%	Females (dead or moribund)	%
0	29/97	29.9	39/98	39.8
10	26/48	54.2	10/49	20.4
50	12/50	24.0	15/50	30.0
250	16/48	33.3	9/48	18.8
1250	27/48	56.3	27/50	54.0
Totals	110/291	37.8	100/295	33.9

From Johnston (1979)

Mean body weights and body weight gains of males and females fed 1250 ppm were significantly lower throughout the study, and those of females fed 250 ppm were also slightly, but significantly, lower from week 60 onwards. There were no significant changes in feed consumption or haematological parameters. Clinical biochemical changes were found in both sexes fed 1250 ppm, at which the AST level was increased and serum albumin levels were decreased. The observation of palpable masses did not show any dose-related trend, but it is clear that males had consistently more masses than females. At necropsy, there were no treatment-related macroscopic observations. For some organs, mean organ weights and organ to body weight ratios were statistically significantly different from controls. However, these changes were not consistent in the treatment groups (i.e. not dose related). Histopathology revealed no increases in neoplastic lesions. There was an increased incidence of microgranulomatous changes due to the treatment. In female mice, this lesion was present in mesenteric lymph nodes, liver and spleen at dose levels of 250 and 1250 ppm. In male mice, 1250 ppm fenvalerate induced multifocal granulomata in mesenteric lymph nodes, other visceral and peripheral lymph nodes, liver and spleen. Less severe granulomatous lesions were present in mesenteric lymph nodes of male mice administered 50 or 250 ppm fenvalerate.

In conclusion, the NOAEL was 10 ppm (equivalent to 1.5 mg/kg bw per day), based on the incidence of microgranulomatous changes in the spleen, liver and lymphatic tissue of males fed 50

ppm fenvalerate (equivalent to 7.5 mg/kg bw per day) (Johnston, 1979; McCullough & Gellatly, 1979; Parker et al., 1983).

In a third carcinogenicity study, groups of 50 male and 50 female ddY mice were fed fenvalerate (S5602 technical, purity 91.4%) at a dietary concentration of 0, 10, 30, 100 or 300 ppm for 87 (females) or 91 (males) weeks. Achieved mean compound intakes were 0, 1.24, 3.48, 12.3 and 36.0 mg/kg bw per day in males and 0, 1.42, 4.29, 13.6 and 40.8 mg/kg bw per day in females at 0, 10, 30, 100 and 300 ppm, respectively. Additional groups of 10 male and 10 female ddY mice were fed the same concentrations and were sacrificed at 12 months. This study is supplementary to the previously described study by Suzuki et al. (1977b) and was intended to establish a no-effect level.

No treatment-related effects, including mortality, body weight, feed consumption, water intake, ophthalmological examination and urine analysis, were detected by clinical observations. Slight reductions of erythrocyte counts in the 100 and 300 ppm groups of males and slightly lower haemoglobin concentration in the 300 ppm female group were observed. Blood glucose concentration was slightly lower in females fed 100 and 300 ppm, and ALT activities were slightly increased in females fed 300 ppm. There were no treatment-related changes in macroscopic findings at necropsy. No treatment-related effects were detected in organ weights or organ to body weight ratios, except that a slightly higher adrenal to body weight ratio was obtained in the 300 ppm female group at month 12. Histopathological examination revealed a dose-related increase in histiocytic infiltration (liver and lymph nodes) and the incidence of granulomatous changes in lymph nodes and liver of both sexes fed 100 and 300 ppm and in spleen of both sexes fed 300 ppm. There was no evidence of carcinogenicity to ddY mice.

The NOAEL for fenvalerate in ddY mice was concluded to be 30 ppm (equal to 3.48 mg/kg bw per day in males and 4.29 mg/kg bw per day in females), based on the slight decrease in erythrocyte counts, increased histiocytic infiltration (liver and lymph nodes) and granulomatous changes in the liver and lymph nodes in animals fed 100 ppm (equal to 12.3 and 13.6 mg/kg bw per day in males and females, respectively) (Arai et al., 1981b,c).

In a published carcinogenicity study, inbred C57Bl/6 mice (50 of each sex per dose) were given fenvalerate (purity 99%) by gavage at 0, 40 or 80 mg/kg bw per day, 5 days/week, for 120 weeks. Arachis oil was used as the vehicle to administer the fenvalerate. Two groups of mice (50 of each sex) were included as controls, one untreated controls and the other vehicle-treated controls.

Survival was decreased, especially among females receiving the high dose (Table 6).

Exposure to fenvalerate resulted in a slight increase in the incidence of liver cell tumours over that in controls only in male mice receiving the high dose. The incidence of liver tumours in high-dose males was 21% compared with 13% and 8% for untreated and vehicle-treated controls, respectively. The increased incidence of liver tumours in male mice at the high dose was not significant by Fisher's exact test, but was significant by the trend test ($P < 0.05$). No significant difference in the incidence of other types of tumours was observed in treated groups when compared with controls. Fenvalerate-induced microgranulomas occurred concomitantly in the liver, spleen and lymph nodes of male and female mice, but their overall incidence did not increase with dose. Considering the two sexes together, the incidence of these lesions was 35% in male mice receiving the low dose and 36.5% in the group receiving the high dose.

In a separate experiment, groups of female SJL/ola mice were administered two different samples of fenvalerate (purity 92% and 99%) once per week for 12 weeks. In animals that received 92% pure compound, the latent period for induction of lymphomas was shortened and their incidence increased, when compared with the group receiving 99% pure fenvalerate and with controls.

Table 6. Survival in mice administered fenvalerate for 120 weeks

Treatment	No. of each sex	No. of survivors							% survival
		10 weeks	20 weeks	40 weeks	60 weeks	80 weeks	100 weeks	120 weeks	
Controls									
Untreated	50 F	49	48	47	45	42	32	20	40
	50 M	50	50	50	48	48	47	32	64
Vehicle-treated	50 F	49	49	49	48	47	44	22	44
	50 M	49	48	48	46	42	38	28	56
Fenvalerate									
40 mg/kg bw per day	50 F	50	50	48	45	44	37	25	50
	50 M	50	50	48	48	46	42	29	58
80 mg/kg bw per day	50 F	57	56	51	44	40	29	17	34
	50 M	58	58	55	54	51	43	29	58

From Cabral & Galendo (1990)

F, females; M, males

The study authors concluded that the results of this study do not provide evidence for carcinogenicity of fenvalerate in C57Bl/6 mice (Cabral & Galendo, 1990).

Rats

Groups of rats (15 male and 15 female Wistar rats per group) were fed fenvalerate at a concentration of 0, 50, 150, 500 or 1500 ppm (equivalent to 0, 5.0, 15.0, 50 and 150 mg/kg bw per day) for 15 months in the diet.

There was no mortality in the study that was attributable to fenvalerate. Growth, feed consumption and behavioural changes were significantly affected at the highest dose level. Hypersensitivity was observed at the early stages of the experiment, disappearing within 3 months. Clinical examinations, performed at either 1 year (urine analysis) or the conclusion of the study (haematology, blood biochemistry and gross and microscopic pathology), showed significant abnormal values in a variety of parameters at the 1500 ppm dose level. No ophthalmological effects were noted. Haemoglobin concentration was depressed in males at the highest dose level and in females at 150 ppm and above. Blood biochemistry was significantly altered at 1500 ppm with respect to several parameters (blood urea nitrogen in both males and females; protein and cholinesterase in females). Gross and microscopic examination of tissues and organs, including specific sections of sciatic nerve and trigeminal ganglia and nerve, showed no dose-related effects. Generalized inflammatory and degenerative changes were seen in both control and treated animals. Tumour incidences were low and not related to the presence of fenvalerate. There was no suggestion of a carcinogenic potential observed in the study (Suzuki et al., 1977a). This study was not considered to be adequate for a carcinogenicity assessment of fenvalerate in rats, as inadequate numbers of animals were used per dose group and the duration of treatment was less than 2 years.

In a carcinogenicity study, groups of Sprague-Dawley rats (93 males and 93 females per group, 183 of each sex were used as the controls, and an additional 22 of each sex were used as separate control and high-dose groups) were fed fenvalerate (purity 98%) in the diet at a dose level of 0, 1, 5, 25, 250 or 500 ppm (equivalent to 0, 0.05, 0.25, 1.25, 12.5 and 25 mg/kg bw per day). After 3 and 6 months (all groups) and 12 and 18 months (0, 1, 5, 25 and 250 ppm groups) of treatment, rats (10 of each sex per group, except the control group, 20 of each sex) were sampled for haematology and blood and urine chemistry, after which they were killed and examined for histopathology.

There was no effect of the treatment with fenvalerate on mortality, clinical signs, incidence of palpable masses (includes all palpable masses and not necessarily tumours), feed consumption, urine analysis data, haematological parameters, coagulation, clinical chemistry, serum protein or organ weight compared with the untreated controls. Some values of these parameters on some occasions were significantly different from the control values, but these alterations were not consistent and therefore are not considered treatment related. Body weight gain in females fed 500 ppm was slightly decreased during most of the treatment period (6 months). The 500 ppm group and a separate control group were sacrificed at 26 weeks, whereas the other animals were maintained for 2 years. There were no significant effects on feed consumption, growth or behaviour at 250 ppm. Haematology, clinical chemistry tests and urine analyses, performed at various time intervals over the course of the study on at least 10 animals of each sex at each dose level, showed no dose-related effects. At the conclusion of the study, organ weights and organ to body weight ratios were normal. Gross and microscopic examinations of tissues and organs did not differ significantly from controls. Benign and malignant lesions occurred at random throughout all groups examined at the end of the study and in those animals that died during the course of the study. There were no lesions attributable to fenvalerate. A specific pathological examination of the sciatic nerve of animals fed 250 ppm was performed. The sciatic nerve was not found to have been significantly affected by fenvalerate. This study was considered adequate for a carcinogenicity assessment of fenvalerate in rats.

The NOAEL was 250 ppm (equivalent to 12.5 mg/kg bw per day), based on a reduction in body weight gain in females fed 500 ppm (equivalent to 25 mg/kg bw per day) for a period of 6 months (Gordon & Weir, 1978).

A supplementary study was performed to complement the study by Gordon & Weir (1978) described above. A group of 50 male and 50 female Sprague-Dawley rats was fed fenvalerate (SD-43775 technical, 98% purity) at a dietary concentration of 1000 ppm (equivalent to 50 mg/kg bw per day) for 2 years. A concurrent group of 50 male and 50 female rats received basal diet only.

Six male rats of the treatment group developed transitory weakness of the hindlimbs during weeks 3 and 4 of the study. Five of the treated males developed skin lesions, typified by localized hair loss and oozing, slowly healing sores. Although the etiology of these lesions is unknown, these were judged unrelated to treatment with the test material. Mortality and feed consumption were not affected by compound administration. There was a decrease in body weight gain from week 16 in treated males and from week 44 in treated females to the end of the study. No compound-related changes were seen in the parameters of clinical chemistry, haematology or urine analysis. Organ weights of the treated group were similar to control values. As body weight gain was significantly decreased in the treated rats, the organ to body weight ratios were significantly greater ($P < 0.05$) in the treated males for brain, liver, heart, spleen and testes and in the treated females for brain, kidneys, liver, spleen and heart, when compared with control values.

There were no differences between the treated and control groups in the incidence of mammary tumours. Malignant neoplasms of mesenchymal origin (sarcomas) were observed in the subcutis and dermis of 5 of 51 male rats (2 terminal, 3 intercurrent) fed 1000 ppm compared with 0/50 in the controls. One of these lesions occurred in each of the cervical, hindlimb, thoracic, perianal and axillary regions. The characteristics of these lesions varied in different portions of the individual tumours, and there were differences among the neoplasms. They were, however, predominantly collagenous and generally contained frequent mitoses. Because the cells of origin could not be determined, they were simply designated as spindle cell sarcomas. The term spindle cell sarcoma encompasses a number of malignant mesenchymal neoplasms that are difficult and sometimes impossible to diagnose definitively and include fibrosarcoma, neurofibrosarcoma, malignant fibrous histiocytoma, liposarcoma and osteosarcoma. A spindle cell sarcoma also occurred in 1 of 49 female rats fed 1000 ppm, compared with 0/50 controls. A review of five studies of 2 years' duration conducted with Sprague-Dawley rats at the same laboratory revealed that sarcomas of the subcutis occurred in control group males with the following frequencies: 2/102, 2/50, 2/50, 0/64 and 0/83. A

review by Shell of unpublished literature revealed that in a 2-year study with Sprague-Dawley CD rats, spindle cell sarcomas were found in 3 of 48 male control rats (Piccirillo & Voelker, 1978). The historical incidence of spindle cell sarcomas ranged widely from 0% to 6%, indicating the variable spontaneous incidence of this type of lesion. Thus, although there are doubts regarding the relationship of these tumours to fenvalerate treatment, the possibility cannot be entirely excluded (Gordon & Weir, 1979). This study was considered to be inadequate for carcinogenicity assessment, as only one dose was tested; however, it provides valuable information for the overall evaluation of the carcinogenicity of fenvalerate.

In the study by Gordon & Weir (1979), different types of tumour might be diagnosed as the same type (spindle cell sarcoma) owing to the inappropriate method of tumour classification. Therefore, the tumours must be properly diagnosed on the basis of careful observation. To obtain the proper findings, the slides of the subcutaneous sarcomas sent from Litton Bionetics were examined histopathologically by a single pathologist (Ito, 1981). Upon re-examination, the tumours were not diagnosed as a group of spindle cell sarcomas, but as 1) a malignant schwannoma or perhaps a leiomyosarcoma, 2) a malignant mesenchymoma, 3) a fibrosarcoma and 4) two malignant amelanotic melanomas. Thus, the incidence of each sarcoma became very low (either 1 or 2 in 51 rats). There has been no attempt to reconcile these different diagnoses, which detracts from a ready acceptance of the re-evaluation. However, even in the original evaluation, there was no accumulation of sarcomas in female rats (and there is no reason to expect a sex difference in this respect). Furthermore, there was no evidence for an increase in these tumours in the Arai et al. (1980) study (see below) in which Wistar/SLC rats were administered fenvalerate at dietary concentrations up to 1500 ppm (equivalent to 75 mg/kg bw per day), which is 50% higher than in the current experiment. Thus, the observation of spindle cell sarcomas was not reproducible.

In a separate carcinogenicity study, groups of 80 male and 80 female Wistar/SLC rats were fed fenvalerate (S5602 technical, purity 93.4%) at a dietary concentration of 0, 50, 150, 500 or 1500 ppm (equivalent to 0, 2.5, 7.5, 25 and 75 mg/kg bw per day, respectively) for 104 (males) or 119 (females) weeks. Clinical signs were observed daily; body weight and feed consumption and water consumption were measured weekly; and ophthalmological examinations, urine analysis, haematology, blood chemistry, gross examinations, organ weights and histopathology were performed at termination.

No compound-related changes were observed in mortality, clinical signs, water intake, blood chemistry, haematology, urine analysis, eye examination or organ weights. Although the apparent dose-related decrease in mortality was observed in weeks 64 and 68, owing to respiratory disease, which spread more rapidly in the control than in the treated rats, final mortality of the treated groups was comparable to that of the control group. Body weight gains of males from the 500 and 1500 ppm groups and females from the 1500 ppm group were probably affected, although it was difficult to evaluate the male data exactly, owing to higher initial body weight of control males. The histopathological examination revealed giant cell infiltration in the spleen and liver at 1500 ppm and in lymph nodes and adrenals at 500 and 1500 ppm. Reticuloendothelial cell proliferation in the mesenteric lymph nodes was observed at the two highest doses. The neoplastic changes—namely, leukaemia, pituitary adenoma and pheochromocytoma in adrenals—occurred in all the groups and in both sexes with similar incidence in treated and control groups and so were not related to the treatment. In females, uterine and mammary tumours were observed in all groups, including controls, with similar incidence and were not considered substance related.

The incidences of Leydig cell tumours were significantly increased in several groups, the overall values being as follows: 0 ppm, 21/75; 50 ppm, 36/78 ($P < 0.05$); 150 ppm, 27/79; 500 ppm, 56/76 ($P < 0.01$); and 1500 ppm, 53/77 ($P < 0.01$). In addition to these differences in Leydig cell tumour incidence, testicular atrophy and/or Leydig cell hyperplasia were seen in many males of all groups. The incidence of testicular atrophy was slightly higher in treated groups than in the controls, and the incidence of Leydig cell hyperplasia was highest in the controls: 0 ppm, 19/75; 50 ppm,

12/78; 150 ppm, 7/79; 500 ppm, 10/76; and 1500 ppm, 3/77. The distinction between Leydig cell hyperplasia and Leydig cell adenoma is solely on the basis of size. As summarized in the esfenvalerate review by JMPR (Annex 1, references 95 and 97), according to some systems (e.g. the United States National Toxicology Program, as described by Boorman, Hamlin & Eustis, 1987), an adenoma is diagnosed if an area of hyperplasia is greater than a seminiferous tubule, whereas according to other systems (e.g. McConnell et al., 1992), an adenoma is diagnosed if it is equal to or greater than three seminiferous tubules. While such high incidences are common in Fischer 344 rats, this is not normally the case in Wistar rats. Indeed, a highly variable incidence of testicular tumours (11–100%) was observed at the time of the study in control Wistar/SLC rats from the same supplier over a 6-year period. Genetic analysis of Wistar/SLC rats and comparison with analysis of other strains in Japan indicate that two Wistar strains, one of which is Wistar/SLC, appear to be very similar to Fischer rats. The occurrence of Leydig cell adenomas is strongly age dependent. In the Wistar/SLC strain of rats, data from two reports from relevant time periods give the following total incidences: Experiment I (1974)—13 months, 10.0%; 19 months, 16.7%; 25 months, 100.0%; Experiment II (1974)—14 months, 14.0%; 20 months, 100.0%; 26 months, 98.7%. Although the final mortality percentages were generally similar across the groups in the current experiment (0 ppm, 79%; 50 ppm, 75%; 150 ppm, 72%; 500 ppm, 64%; 1500 ppm, 73%), there were higher numbers of rats at risk at 84 weeks (i.e. about the time that the rapid increase in incidence occurs) in the 500 and 1500 ppm groups, the mortality percentages at this time being as follows: 0 ppm, 65%; 50 ppm, 59%; 150 ppm, 55%; 500 ppm, 31%; 1500 ppm, 43%. This factor could have contributed to the between-group variations in adenoma incidence. It is also to be noted that in two studies described above, in which SD rats were fed diets containing fenvalerate at a concentration of 250 ppm (Gordon & Weir, 1978) or 1000 ppm (Gordon & Weir, 1979) for up to 2 years, there were no increases in Leydig cell tumours. It is concluded, therefore, that there may have been an accelerated growth rate of the Leydig cell proliferating lesions in some dose groups, but these cannot be interpreted as an increased emergence of tumours. It is concluded that fenvalerate is not carcinogenic. This study was considered to be adequate for the carcinogenicity assessment of fenvalerate in rats.

The NOAEL for systemic toxicity in this study was 150 ppm (equivalent to 7.5 mg/kg bw per day), based on reduced body weight gains and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day). There was no evidence of carcinogenic response, and the dietary no-effect level of fenvalerate was found to be 150 ppm (equivalent to 7.5 mg/kg bw per day) (Arai et al., 1980, 1981a).

2.4 Genotoxicity

The results of various mutagenicity studies (in vivo and in vitro) on fenvalerate are summarized in Table 7.

Table 7. Mutagenicity studies with fenvalerate

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Bacterial reverse mutation (Ames test) and rec assay	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535 and TA1538 and <i>Bacillus subtilis</i> (H17 and M45)	1, 10, 100 and 1000 µg/plate (with and without S9 mix) or 10, 100, 1000 and 10 000 µg/disc (without S9 mix)	95	Negative	Suzuki & Miyamoto (1976)
Bacterial reverse mutation (Ames test)	<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537 and TA1538	Up to 1 mg/plate (with and without S9 mix)	95	Negative	Suzuki & Miyamoto (1977); Suzuki, Kishida & Miyamoto (1979)

Table 7 (continued)

End-point	Test object	Concentration	Purity (%)	Results	Reference
Bacterial reverse mutation (Ames test)	<i>S. typhimurium</i> strains TA97, TA98, TA100 and TA1535	0, 10, 50, 100, 500, 1000, 2500 and 5000 µg/plate (with and without S9 mix)	97.6	Negative	Reynolds (1991a)
Fluctuation test and mammalian clastogenicity	<i>S. typhimurium</i> strains TA98 and TA100 and Chinese hamster (V79) cells	0, 1, 3 and 10 µg/ml and 0, 4, 20 and 40 µg/ml (with and without S9 mix)	99	Negative	Pluijmen et al. (1984)
Unscheduled DNA synthesis	Primary rat hepatocytes	0, 0.5, 5, 10, 50, 100, 500 and 1000 µg/ml	97.6	Negative	Bentley (1991)
In vivo					
Standard host-mediated assay	Mice	0, 60 and 125 mg/kg bw	95	Negative	Suzuki & Miyamoto (1976)
Standard host-mediated assay	Mice	0, 25 or 50 mg/kg bw	96	Negative	Brooks (1976)
Dominant lethal assay	Mice; male	0, 25, 50 and 100 mg/kg bw	96	Equivocal ^a	Dean (1975)
Bone marrow chromosomal aberrations	Chinese hamsters	0, 12.5 and 25 mg/kg bw	NS	Negative	Dean & Senner (1975)
Bone marrow micronucleus assay	Mice	0, 20, 38 and 75 mg/kg bw	97.6	Negative	Reynolds (1991b)
Bone marrow micronucleus assay	Mice	0, 100, 150 and 200 mg/kg bw, intraperitoneal	NS	Positive	Pati & Bhunya (1989)
Sex-linked recessive assay	<i>Drosophila melanogaster</i>	Adult 20 mg/l, larvae up to 50 mg/l or adult intraperitoneal 20 µg/ml	NS	Negative	Batiste-Alentorn et al. (1987)
Chromosomal aberration and SCE	Mice	0, 5, 10 and 20 mg/kg bw intraperitoneally	98.4	Weak clastogen; potent inducer of SCEs	Giri et al. (2002)
Micronucleus test in bone marrow cells	Mice	0, 2.5, 5, 10 and 20 mg/kg bw intraperitoneally	98.4	Positive	Giri, Giri & Sharma (2011)
		10 mg/kg bw	98.4	Negative	
Bone marrow micronucleus assay	Mice	0, 32.50, 75 and 150 mg/kg bw (single oral dose)	NS	Positive	Ghosh, Sharma & Talukder (1992)
Chromosomal aberrations test	Norway rats	Repeated gavage dose, 21 days	NS	Positive	Chatterjee, Talukder & Sharma (1982)

DNA, deoxyribonucleic acid; NS, not specified; S9, 9000 × *g* supernatant fraction of rat liver homogenate; SCE, sister chromatid exchange

^a Significant reduction in viable fetuses, a significant increase in early fetal deaths occurring in females mated during the 4th week to males dosed at the highest level.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a three-generation reproductive toxicity study, groups of Sprague-Dawley rats (11 male and 22 female rats per group) were administered fenvalerate (SD-43775, purity 98.0%) in the diet at a dose level of 0, 1, 5, 25 or 250 ppm (equivalent to 0, 0.07, 0.3, 1.7 and 16.7 mg/kg bw per day, respectively). The animals were fed for 9 weeks prior to mating and initiation of a standard three-generation (two litters per generation) reproduction study. Fertility, viability, gestation and lactating indices were calculated for each treatment group and compared with control values. All parental animals were necropsied. Ten male and 10 female weanlings from the F_{3b} litter were examined histologically at the conclusion of the study. Brain, heart, liver and kidney weights from F_{3b} litters were recorded. Several organs and tissues were examined microscopically.

The observations on the parental rats (F₀) reflected no effect related to treatment, although there was an unusually high frequency of skin lesions in treated and control rats. Necropsy findings on F₀ rats did not suggest any effect of the chemical, although the frequency of pulmonary tract lesions suggested that chronic respiratory disease was present in the rats without regard to treatment. In addition, enlarged adrenals were observed in most females. A review of historical data revealed that this was normal; considering the additional stress of pregnancy, they may have even been larger in comparison with the males. No remarkable effects were noted in F₀ parents at necropsy. No effect on reproductive parameters was seen in F_{1a} or F_{1b} litters, except for an apparent decreased fertility in females at 1 ppm. However, this effect showed no dose-response relationship in the higher treatment groups during the F₁ generation. The general appearance and behaviour of the parent rats of the second generation were judged to reflect no compound-related effect. The necropsy of the F_{1b} parents demonstrated a frequency of kidney changes (mottled, pale appearance) suggestive of a compound-related response (Table 8); there was no effect on reproductive parameters in F_{2a} or F_{2b} litters except for an apparent decrease in female fertility in the low-level (1 ppm) test group. As a dose-response relationship was not involved, this reduced reproductive capacity was not judged to be compound induced.

Table 8. Gross kidney changes in a multigeneration reproduction study in rats

Dietary concentration (ppm)	Gross kidney changes in F _{1b} and F _{2b} rats			
	Males (F _{1b})	Females (F _{1b})	Males (F _{2b})	Females (F _{2b})
0	3	3	5	0
1	7	5	8	5
5	5	4	5	4
25	6	7	5	2
250	8	7	10	2

From Beliles, Makris & Weir (1978)

The mean body weights of the parents of the third-generation F_{2b} adults revealed a significant reduction at the high level (250 ppm) when control and treated groups were compared. The necropsy of F_{2b} parents revealed gross kidney changes similar to changes noted in the F_{1b} parents. However, the distribution with regard to dose was not judged to be consistent with a compound-related change. There was no effect on reproductive parameters in the F_{3a} or F_{3b} litters except for an apparent decrease in female fertility in the low-level (1 ppm) test group. As a dose-response relationship was not involved, this reduced reproductive capacity was not judged to be compound induced. Histopathological examination of the F_{3b} weanlings was unremarkable.

With the exception of weight reduction in the third-generation parents, there was no effect of fenvalerate on any parameter measured in the study. The NOAEL for parental systemic toxicity was 25 ppm (equivalent to 1.7 mg/kg bw per day), based on reduced mean body weights seen at 250 ppm (equivalent to 16.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 250 ppm (equivalent to 16.7 mg/kg bw per day), the highest dose tested (Stein, 1977; Beliles, Makris & Weir, 1978).

(b) *Developmental toxicity*

Mice

In a teratogenicity and reproduction evaluation study, groups of pregnant ICR mice (32–33 mice per group) were administered fenvalerate (S5602, purity 97.6%) at a dose level of 0, 5, 15 or 50 mg/kg bw per day on gestation days 6 through 15. On day 18, groups of 20–21 mice were sacrificed, and fetuses were removed and examined for somatic and skeletal abnormalities. About 12 females were allowed to deliver naturally, and the young were maintained for 3 weeks to weaning to evaluate postnatal deficits. In addition to the teratogenicity study, two male and two female weanlings from each dam were maintained for 8 weeks and mated to note any effects on their reproductive potential.

Toxic signs of poisoning were noted in maternal mice at the high dose level. Irregular respiration, hypersensitivity, tremors and salivation were noted after administration of the compound (first 30–60 minutes after dosing only). However, the symptoms were not seen the following morning and were not potentiated by consecutive treatment. There was no significant mortality over the course of the study, and no effects were noted on any of the animals as a result of continuous administration of fenvalerate. In fetal examinations, there were no somatic or skeletal changes noted in internal or external tissue evaluations. The mean fetal body weights were decreased at 5 mg/kg bw per day, but there was no dose–response relationship. The animals maintained in an abbreviated reproduction study showed no differences from control values in their ability to reproduce. There were no changes in the reproduction indices evaluated with any animals examined. Under the conditions of this bioassay, fenvalerate was shown to have no teratogenic potential in mice.

The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on the clinical signs of toxicity seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested (Kohda, Kadota & Miyamoto, 1976a).

Rabbits

In a developmental toxicity study, groups of pregnant Dutch rabbits (group size varied from 20 to 31 rabbits) were administered fenvalerate (WL 43775, purity 97.0%) at 0, 12.5, 25 or 50 mg/kg bw per day from day 6 to day 18 of gestation. On day 28, the rabbits were sacrificed, and standard teratogenicity assessments were made with respect to early or late fetal death, viability and standard somatic and skeletal teratogenic potential.

Reduced body weight of dams at the highest dose level was observed. There were no significant differences from control values in any of the other parameters measured in the study. Under the conditions of the study, fenvalerate did not induce a teratogenic effect in rabbits.

The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight of dams seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested (Van der Pauw et al., 1975).

2.6 *Special studies*

(a) *Neurotoxicity*

Hens

Groups of six hens were administered fenvalerate (purity 96.0%) orally in dimethyl sulfoxide (DMSO) at a dose level of 0 or 1000 mg/kg bw per day for 5 days. A positive control of tri-*ortho*-cresyl phosphate (TOCP; 0.5 ml/kg bw) was also included in the study. After 3 weeks, the fenvalerate and

negative control animals were retreated using the same dosage regimen. At the conclusion of an additional 3-week observation period, animals were sacrificed, and histological examinations were performed on the central and peripheral nervous systems.

All animals receiving TOCP developed readily defined signs of delayed neurotoxicity, and histological lesions were seen in the sciatic nerve and spinal cord. Clinical signs and histopathological lesions were not noted in controls or fenvalerate-treated hens (Milner & Butterworth, 1977).

Mice and rats

In a special acute toxicity study, B6C3F1 mice and Sprague-Dawley rats showed the characteristic signs of intoxication following single oral doses of fenvalerate ranging from 56 to 320 mg/kg bw and from 133 to 1000 mg/kg bw, respectively. Neurological signs, such as splayed gait, tremors, ataxia and hindlimb incoordination, were observed at doses of 100 mg/kg bw or more (mice) and 133 mg/kg bw or more (rats) within 1–8 hours after dosing. These signs had disappeared in most animals within 72 hours. Slight peripheral nerve fibre damage was detected in surviving mice and rats sacrificed 10 days after dosing. The incidence and severity were dose related at doses above 56 and 180 mg/kg bw; however, even at lethal doses, there was no evidence of nerve lesions in some animals. Thus, two distinct neurological effects were observed: 1) a reversible ataxia and 2) incoordination plus a neuropathological effect manifested as sparse axonal damage in peripheral nerves (Parker et al., 1985).

Rats

A series of studies was performed to evaluate the neurotoxic potential (to induce axonal and myelin disruption) of a group of synthetic pyrethroid esters and of natural pyrethrum. Acute oral administration of fenvalerate, cypermethrin, resmethrin, permethrin and natural pyrethrum to rats at very high dose levels resulted in severe clinical signs of poisoning and mortality within 24 hours. Histopathological lesions were observed in the sciatic nerve with all compounds tested. At lower levels, fenvalerate (200 mg/kg bw), cypermethrin (100 mg/kg bw), permethrin (200 mg/kg bw) and pyrethrum (3500 mg/kg bw) did not show clinical signs of poisoning or histopathological lesions (Okuno, Kadota & Miyamoto, 1977a,b).

A histopathological examination was performed on the sciatic nerve and posterior tibial nerve of rats that had been exposed to acutely toxic levels of fenvalerate. After poisoning, and for 9 days during the course of recovery, axonal breaks, swelling and vacuolation, accompanied by phagocytosis of myelin, were seen. The degree to which myelin was disrupted was dose dependent and was closely associated with the acute signs of toxicity (Butterworth & Carter, 1976).

Groups of six male and six female rats were fed fenvalerate in the diet at a concentration of 0 or 2000 ppm for 8–10 days, after which the sciatic nerve was examined for histological signs of degeneration. All animals exposed to fenvalerate showed typical signs of acute intoxication, including ataxia, tremors and hyperexcitability. Histological examinations at the end of the feeding interval did not reveal any adverse effects of fenvalerate on the sciatic nerve (Hend & Butterworth, 1976).

In a study to evaluate the reversibility of the lesions induced in the sciatic nerve, rats were administered fenvalerate (purity 97.7%) in the diet at a dose level of 0 or 3000 ppm for 10 days. Mortality was evident, as 60% of the animals died within the course of the dietary treatment. Surviving animals were fed normal control diets and examined for 12 weeks following completion of the feeding study. Animals were sacrificed every 3 weeks and examined histologically for sciatic nerve disruption. Sciatic nerves of rats on control diets sacrificed at 3 weeks continued to show swelling and disintegration of axons. At 6 weeks, there were no histological lesions. These results were also observed at the 9- and 12-week intervals, suggesting reversibility of the histopathological lesions observed following high-dose treatment with fenvalerate (Okuno, Kadota & Miyamoto, 1977b).

In a special study, fenvalerate was administered orally to Wistar or Carworth Farm E rats either as single doses or in the diet. When given in large quantities as a single dose of 250, 500, 800, or 1000 mg/kg bw, which were sufficient to kill some of the treated animals, fenvalerate produced sporadic Wallerian degeneration in the sciatic nerve. The neuropathy was never severe and was not seen in animals given the compound in sublethal doses. In feeding studies (for 5 weeks at 1000 mg/kg diet and for 3 months at 2000 mg/kg diet), no lesions were seen in the peripheral nerve, brain or spinal cord, and there was no evidence of cumulative neurotoxicity (Butterworth & Hend, 1976).

Groups of rats (4–6 of each sex per group) were administered fenvalerate orally at dose levels ranging from 0 to 400 mg/kg bw per day for 7 consecutive days. Mortality and clinical signs of acute poisoning were seen only at the highest dose level. A significant neurological deficit was demonstrated using an inclined plane test (expressed as the angle at which the animals cannot maintain their hold on an inclining plane). In addition to functional deficits, increases in β -glucuronidase and β -galactosidase activities in the posterior tibial nerve and trigeminal ganglia were observed. Functional motor deficits appeared to coincide with the administration of fenvalerate, reaching maximum effects between days 5 and 7 of treatment. Stimulation of lysosomal enzyme activity appears to coincide with neurological deficits and with sciatic nerve degeneration noted with acute (high level) intoxication (Dewar, Moffett & Sitton, 1977).

Hamsters

Groups of hamsters (five males and five females per group) were administered fenvalerate at a dose level of 0, 5, 10, 20 or 40 mg/kg bw per day for 5 consecutive days. In three separate experiments, dose-related increases in β -glucuronidase and β -galactosidase activities of peripheral nerves were observed. Minimal functional deficits were also noted during the treatment period and for 2 weeks following treatment. These data appear to be consistent and to be associated with axonal degeneration in the peripheral nerve (Dewar et al., 1978).

(b) Comparative neurotoxicity potential between fenvalerate and esfenvalerate

Mice

In a comparative study of esfenvalerate and fenvalerate, groups of B6C3F1 mice (12 of each sex per group) were fed diets containing 0, 50, 150 or 500 ppm esfenvalerate (lot no. PKG-85036, total purity of isomers 94.5%, of which 87.2% was the A α isomer, esfenvalerate) for 3 months. An additional group of mice (12 of each sex) was fed 2000 ppm fenvalerate (lot no. 41028, total purity of isomers 95.5%, isomeric ratios A α :A β :B α :B β = 24.2:25.4:26.3:24.1), also for 3 months. Mean achieved concentrations in male mice were 0, 10.5, 30.5, 106 and 422 mg/kg bw per day at the dietary concentrations of 0, 50, 150, 500 and 2000 ppm, respectively. Mean achieved concentrations in female mice were 0, 12.6, 36.8, 113 and 462 mg/kg bw per day at the dietary concentrations of 0, 50, 150, 500 and 2000 ppm, respectively.

There were no deaths during the study. Clinical signs of toxicity were seen only in animals fed diets containing esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. These were typical type II pyrethroid neurological responses, which were seen from the beginning and throughout the feeding period (i.e. salivation, hypersensitivity, fibrillation, tremor, convulsion, hunched posture and unsteady gait), as well as gross lesions, such as alopecia, skin scabs and sores. They were more severe in the mornings. Decreased body weight gain was seen in both sexes fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. There were no consistent changes in feed consumption, and water intake was reduced only initially in the animals fed 500 ppm esfenvalerate and 2000 ppm fenvalerate. Urinary pH was decreased, whereas urinary protein, ketone, bilirubin, urobilinogen and specific gravity were increased, in animals fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. In the same dose groups, haematological changes were found, which included decreased erythrocyte counts, haemoglobin concentration, packed cell volumes and/or mean cell volumes. Decreased total protein, glucose, phospholipid, cholesterol and triglyceride levels, with increased blood urea nitrogen, ALT, AST and LDH activities, were also observed in animals fed esfenvalerate at 500 ppm and/or fenvalerate at 2000 ppm. Males fed esfenvalerate at 150 ppm also showed decreased glucose and

triglyceride concentrations. The major difference in blood biochemistry between esfenvalerate and fenvalerate was the LAP activity, which was decreased in animals fed esfenvalerate and increased in animals fed fenvalerate.

Skin lesions, thought to be due to scratching, were increased, along with, in some cases, enlargement of regional lymph nodes, at the high dose for both compounds. The salivary gland weights were increased in animals fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. Compound-related histopathological changes were seen in the liver, spleen, lymph nodes, thymus, skin, kidney and stomach. There was a decrease in fat deposits in the liver and kidneys that was probably related to the decreased blood lipid levels. The animals dosed with fenvalerate also showed an increased incidence of microgranulomas and giant cell infiltration in the liver, spleen and lymph nodes. Very slight ulcerative changes in the glandular stomach were observed in four males receiving 500 ppm esfenvalerate, but not in females at the same dose, males and females at lower dose levels or any of the fenvalerate-treated mice. Examination of nervous tissue (brain, spinal cord and sciatic nerve) revealed no treatment-related tissue changes.

In summary, with the exception of the minor gastric ulcerations with esfenvalerate, there were no significant toxicological differences between mice fed esfenvalerate at 500 ppm and those fed fenvalerate at 2000 ppm. Esfenvalerate produced decreased LAP in the blood. Fenvalerate, but not esfenvalerate, at 500 ppm produced microgranulomatous changes in the liver, spleen and lymph nodes and increased LAP in the blood.

The NOAEL for esfenvalerate in mice in this study was 50 ppm in males (equal to 10.5 mg/kg bw per day), based on blood biochemical changes in male mice fed 150 ppm (equal to 30.5 mg/kg bw per day), and 150 ppm in females (equal to 36.8 mg/kg bw per day), based on blood biochemistry and overt toxic signs in female mice fed 500 ppm (equal to 113 mg/kg bw per day) (Koyama, 1985).

Rats

A comparative study of the neurotoxic potential of esfenvalerate (lot no. PKG-85036, purity 94.5%, 87.2% of which was A α isomer, esfenvalerate) and fenvalerate (lot no. 41028, purity 95.5%, 24.2% of which was A α isomer, esfenvalerate) was conducted in rats (8 of each sex per group except for the highest-dose groups, in which there were 16 of each sex). The compounds were administered in corn oil at a single oral dose of either esfenvalerate at 0, 5, 20 or 90 mg/kg bw or fenvalerate at 0, 20, 80 or 360 mg/kg bw, followed by observation for 2 weeks. Clinical observations, body weight measurements and functional testing using an inclined plane (slip angle test) were conducted during the observation period. At the end of this period, the rats were perfused with 10% buffered formalin, and the brain, spinal cord and sciatic/tibial nerves were examined histologically.

Eight animals, two males and one female receiving esfenvalerate at 90 mg/kg bw and one male and four females receiving fenvalerate at 360 mg/kg bw, were found dead within 24 hours after dosing. The toxic signs occurred within 2 hours of dosing and were the typical type II pyrethroid symptoms (i.e. muscular fibrillation, ataxia, tremors, salivation, limb paralysis, irregular respiration, hypersensitivity to sound, hunched posture and urinary incontinence). All neurological symptoms disappeared within 2 days of dosing. Muscular fibrillation, ataxia, salivation and/or hunched posture were seen in a few rats receiving esfenvalerate at 20 mg/kg bw and fenvalerate at 80 mg/kg bw. No toxic signs related to compound treatment were observed in any rat dosed with esfenvalerate at 5 mg/kg bw or fenvalerate at 20 mg/kg bw. Body weight depression was observed in males and females receiving fenvalerate at 80 and 360 mg/kg bw. The slip angle test did not reveal any functional deficit in the treated groups. There were no treatment-related gross changes at necropsy. Slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves were recognized in many males and females treated with esfenvalerate at 90 mg/kg bw and fenvalerate at 360 mg/kg bw on histopathology. The incidences of these neural changes in the low- and mid-dose groups were comparable with those in controls. There were no remarkable differences in the incidence and severity of nerve damage between the high-dose groups of esfenvalerate and fenvalerate. The fact

that these effects were not seen at the middle dose, although overt signs of toxicity were seen in both mid- and high-dose groups, suggests that the toxic signs are not related to peripheral nerve damage, but are more likely to be of pharmacological origin (Okuno, 1985).

(c) *Antidotal studies*

In an antidotal study, phenobarbital, pentobarbital and diphenylhydantoin were found to be effective in relieving the acute signs of intoxication caused by fenvalerate in rats. Phenobarbital (50 mg/kg bw, intraperitoneal) prevented tremor, diphenylhydantoin (100 mg/kg bw, intraperitoneal) reduced the toxic reaction and pentobarbital (35 mg/kg bw, intraperitoneal) removed the tremor reaction completely within 30 minutes. The combination of diphenylhydantoin with either of the barbiturates was effective in reducing the onset and severity of tremors, whereas a variety of other agents (delta-tubocurarine, atropine, meprobamate, diazepam, biperidin and trimethadione) were ineffective (Matsubara et al., 1977).

Fenvalerate administered to dogs at acute dosage rates sufficient to induce toxic signs of poisoning showed no consistent cardiovascular effects as measured by electrocardiogram. Respiratory stimulation was noted at high levels and was not reduced by anaesthetic supplements such as urethane, chloralose and pentobarbital (Kirkland & Albert, 1977).

The therapeutic potency of intraperitoneally administered methocarbamol was examined as an antidote against the acute oral intoxication of rats by a lethal dose of fenvalerate. Methocarbamol was initially administered at a dose of 400 mg/kg bw, followed by repeated doses of 200 mg/kg bw when tremors or hypersensitivity to sound were observed. Methocarbamol markedly decreased the mortality from 80%, which would be caused by an administration of 850 mg fenvalerate per kilogram body weight, to 0% and was effective in alleviating motor symptoms such as fibrillation, tremors, hyperexcitability, clonic seizures and choreoathetotic movements. A subcutaneous administration of atropine sulfate (25 mg/kg bw) was also effective in reducing the salivation produced by fenvalerate (Hiromori et al., 1986).

Effective treatments against fenvalerate-mediated effects have been investigated by quantifying behavioural skin sensory responses such as licking, scratching or biting of the treated sites by fenvalerate-treated guinea-pigs. Preparations containing vitamin E, corn oil or the local anaesthetic benzocaine were most effective (Malley et al., 1984).

(d) *Pesticide interactions/synergistic effects*

Intraperitoneal administration of *O*-ethyl-*O*-(4-nitrophenyl)phenylphosphonothioate or *S,S,S*-tributyl phosphorotrithioate to mice at 25 mg/kg bw increased the intraperitoneal toxicity of fenvalerate (administered 1 hour later) by more than 25-fold; the median lethal dose (LD₅₀) decreased from greater than 1000 mg/kg bw to 37 or 42 mg/kg bw. This study suggests that mammalian esterases highly sensitive to inhibition by certain organophosphorus compounds may play a critical role in fenvalerate detoxication. This kind of synergism among pesticides would be detrimental in increasing the toxicity of certain pyrethroids to mammals (Gaughan, Engel & Casida, 1980).

Esterases play a major role in the detoxification of many pyrethroid insecticides (Abernathy & Casida, 1973; Jao & Casida, 1974a; Soderlund & Casida, 1977a). Some inhibitors of "pyrethroid esterases" serve as synergists for pyrethroid toxicity to insects and mammals (Abernathy et al., 1973; Jao & Casida, 1974b; Soderlund & Casida, 1977b). Currently, the major use of pyrethroids is in cotton protection, where the crops may also be treated with esterase inhibitors (e.g. organophosphorus and methyl carbamate compounds).

Fenvalerate, when fed to adult rats for 14 days, did not induce hepatic microsomal enzyme, as measured by the comparative rates of deethylation of an organophosphate pesticide (chlorfenvinphos) (Creedy & Potter, 1976). Dietary levels of 1000 ppm did not induce the oxidative *O*-dealkylation that has been shown to be reflective of microsomal enzyme induction in rat liver.

The evaluation of several insecticides used on cotton for possible effects in altering the metabolism, toxicity and persistence of pyrethroids has been reported (Gaughan, Engel & Casida,

1980). Profenofos, *O*-ethyl-*O*-(4-nitrophenyl)phenylphosphonothioate and *S,S,S*-tributylphosphorotrithioate administered intraperitoneally to mice at 25 mg/kg bw strongly inhibited the liver microsomal esterases that hydrolyse fenvalerate and increased the intraperitoneal toxicity of fenvalerate by more than 25-fold.

(e) *Behaviour*

Guinea-pigs responded to dermal applications of fenvalerate by scratching the treated sites of the skin. This characteristic response was essentially over within 3–4 hours. When the powerful skin irritant oil of mustard was applied to fenvalerate-treated sites of skin 4–72 hours after the fenvalerate treatment, the behavioural skin sensory response was restimulated. Oil of mustard alone did not produce skin sensory stimulation. These results indicate that pyrethroid treatment causes a transient sensitivity to stimulation produced by chemical irritants (Malley et al., 1984).

To develop an animal model for studying skin sensory stimulation, Dunkin-Hartley guinea-pigs were treated with pyrethroid solutions on one side and control substances on the other side of their shaved backs. The animals responded by licking, scratching or biting the test sites, and activity was quantified by counting the number of times the animals responded. This behavioural activity reached a maximum 1–4 hours after treatment. A chemical irritant (oil of mustard) was able to restimulate the behavioural activity when applied within 24 hours after pyrethroid application. Skin sensory stimulation produced by cyano-containing pyrethroids, including fenvalerate, was significantly greater than that produced by non-cyano-containing pyrethroids. This behavioural model provides a quantitative means of evaluating pyrethroid non-erythematous skin sensory stimulation (Cagen et al., 1984).

(f) *Endocrinological effects*

The potential for fenvalerate and esfenvalerate to cause effects on the male endocrine system, primarily related to testicular tumorigenesis, was investigated. Groups of male Wistar/SLC rats were fed diets containing fenvalerate (lot no. 41103G, purity 92.9%) at a concentration of 0, 50, 150, 500 or 1500 ppm and esfenvalerate (lot no. 60610G, purity 86.0%) at a concentration of 375 ppm for 26 weeks. This mechanistic study included a component in which female rats were housed in close proximity (but without contact) to subgroups of the males from the low- and high-dose fenvalerate groups and the esfenvalerate group. Blood samples were taken at 4-week intervals.

No rats died during the course of the study. Lower body weights and decreased feed consumption relative to controls were observed in the 375 ppm esfenvalerate group and the 1500 ppm fenvalerate group. Neither serum luteinizing hormone (LH) nor serum testosterone concentrations were affected to any biologically significant extent; those statistically significant changes that were observed lacked either a dose–response relationship or temporal consistency. Marginal increases in relative (but not absolute) organ weights observed in the phase of the study where the males were housed separately from the females were as follows: testes (+5%), pituitary (+15%) and liver (+9%) for the 1500 ppm fenvalerate group; and epididymides (+4%), seminal vesicles (+9%) and liver (+4%) for the 375 ppm esfenvalerate group. There were no gross pathological or histopathological changes that were considered to be related to treatment. Housing males and females in close proximity without physical contact produced no effects in this study relative to males-only housing (Yamada, 1999).

In a published study, behavioural (open-field, stereotyped and sexual behaviours), physical (sexual maturation, body and organ weights) and endocrine (testosterone levels) parameters were evaluated in male offspring following maternal exposure during the prenatal and postnatal periods. In this study, pregnant Wistar rats received intraperitoneal injections of fenvalerate (purity not reported) at 10.0 mg/kg bw per day on gestation day 18, postnatal day (PND) 1 (during the first 10 minutes after delivery, before the dam started nursing) and daily on PNDs 2–5. Male offspring were evaluated daily for testis descent. On PND 120, a subset of male offspring was evaluated for sexual behaviour

towards untreated ovariectomized females sexually activated with exogenous estradiol and progesterone. Stereotypical behaviour and motor activity in an open field were also assessed in the male offspring. Testosterone and organ weights of testis, seminal vesicles and ductus deferens were measured in a subset of male offspring sacrificed on PND 120.

The results of the study indicate that the treated male offspring had significantly ($P < 0.05$) reduced plasma testosterone levels (2.00 ± 0.29 ng/ml) compared with control male offspring (3.16 ± 0.24 ng/ml), significantly ($P < 0.05$) lower prostate weights (0.076 ± 0.006 g) when compared with control male offspring (0.103 ± 0.006 g) and significantly ($P < 0.05$) lower seminal vesicle weights (0.212 ± 0.020 g) when compared with control male offspring (0.299 ± 0.019 g). In sexual behaviour, treated male offspring had a significant increase in the total number of mounts until ejaculation (22.6 ± 2.77) compared with control male offspring (15.8 ± 1.1) and a significant decrease in the number of ejaculations during a 40-minute testing period (2.0 ± 0.3) when compared with control male offspring (3.0 ± 0.2). In the open-field study, male offspring had decreased periods of immobility compared with offspring from control dams. The study authors concluded that “these results indicate that perinatal exposure to fenvalerate during the critical periods of male brain sexual differentiation has long-term effects on the reproductive physiology and behavior of male rats” (Moniz et al., 1999).

In another study, behavioural (open-field, stereotyped and sexual behaviours), physical (sexual maturation, body and uterine weights) and neuroendocrine (estrous cycle and gonadal hormone levels) parameters were evaluated in female offspring following maternal exposure during the prenatal and postnatal periods. Pregnant Wistar rats received intraperitoneal injections of fenvalerate (purity not reported) at 10.0 mg/kg bw per day on gestation day 18, PND 1 (during the first 10 minutes after delivery, before the dam started nursing) and daily on PNDs 2–5. Female offspring were evaluated daily for vaginal opening. Beginning on PND 120, the estrous cycle of female offspring was assessed over a period of 15 days. On PND 120, a subset of female offspring was ovariectomized, stimulated with 17β -estradiol and progesterone, and evaluated for sexual behaviour towards experienced males. Stereotypical behaviour and motor activity in an open field were also assessed in female offspring. Progesterone and *p*-estradiol were measured in a subset of female offspring on PND 125.

The study results show that 1) sexual maturation was delayed, although body weight was unchanged until adulthood; 2) there was a reduction in sexual behaviour; 3) the estrous cycle was abnormal, and the uterine weight at different phases of the estrous cycle was modified; and 4) gonadal hormone levels in the plasma, stereotypy and open-field behaviours were not affected. The study authors attributed these results to an anti-estrogenic effect of perinatal exposure to fenvalerate during the critical periods of female brain sexual organization (Moniz et al., 2005).

In a study by Kaul et al. (1996), male ITRC bred Wistar rats received fenvalerate (purity 98.9%) in corn oil via intraperitoneal injections at 0, 100 or 200 mg/kg bw per day for a period of 45 days; the vehicle control group received corn oil (20 rats per group). Thyroid hormone (triiodothyronine [T_3] and thyroxine [T_4]) levels in blood were measured by radioimmunoassay. Intraperitoneal administration of fenvalerate in male rats for 45 days at a dose of 100 or 200 mg/kg bw per day induced hyperexcitability, tremors and paralysis. Tremors were observed after 7 days and gradually reached maxima on day 45. The symptoms were more marked in rats treated with 200 mg/kg bw per day. Fenvalerate provoked significant elevation of circulatory thyroid hormones, namely T_3 and T_4 . When compared with controls, there was a statistically significant ($P < 0.001$) and dose-dependent increase in both hormone levels at both doses, as shown in Tables 9 and 10. Significant increases in total calcium as well as protein-bound calcium in whole brain and hypothalamus were recorded.

Table 9. Effect of fenvalerate on serum T_3

Dose (mg/kg bw per day)	Serum T_3 level $\pm$ standard deviation (ng/ml)		
	15 days	30 days	45 days
0 (control)	0.41 $\pm$ 0.004	0.42 $\pm$ 0.003	0.40 $\pm$ 0.003
100	0.63 $\pm$ 0.009	0.92 $\pm$ 0.007	0.95 $\pm$ 0.004
200	1.00 $\pm$ 0.004	1.30 $\pm$ 0.006	1.51 $\pm$ 0.04

From Kaul et al. (1996)

Table 10. Effect of fenvalerate on serum T_4

Dose (mg/kg bw per day)	Serum T_4 level $\pm$ standard deviation (μ g/dl)		
	15 days	30 days	45 days
0 (control)	43.01 $\pm$ 0.70	42.75 $\pm$ 0.95	42.00 $\pm$ 0.003
100	52.25 $\pm$ 0.86	60.50 $\pm$ 0.65	62.50 $\pm$ 0.004
200	60.00 $\pm$ 2.16	84.00 $\pm$ 1.83	93.00 $\pm$ 0.04

From Kaul et al. (1996)

The study authors concluded that the elevation of circulatory thyroid hormones as well as the active calcium pool could together be responsible for impairment of motor activity by altering various neuronal processes (Kaul et al., 1996).

In a published study, the effects of fenvalerate on female reproduction in rats whose mothers were exposed during gestation and lactation were evaluated. Pregnant Wistar rats were exposed to fenvalerate (40 mg/kg bw) or corn oil (vehicle) orally from gestation day 12 until the end of lactation.

Results showed decreases in ovarian weight, number of preantral follicles and number of corpora lutea at PND 75 and an increase in the resorption number when the fertility test was performed at PND 80. The study authors concluded that fenvalerate may impair the reproductive development of female offspring, manifested as reduced fecundity and ovulation number, resulting from the decrease in the number of corpora lutea (Guerra, de Toledo & Kempinas, 2011).

In a published study by Zhang et al. (2009), the effects of maternal fenvalerate (purity not reported) exposure on testicular development and spermatogenesis in ICR mice was evaluated. Pregnant mice received fenvalerate at 60 mg/kg bw per day in corn oil via gavage daily from PND 0 to weaning (PND 21); the vehicle control group received corn oil only. On PND 21, plasma testosterone was measured, and testes were weighed and subjected to histopathological examinations. On PND 80, another set of pups was sacrificed, plasma testosterone levels and testes weights were measured, epididymal sperm were examined and testicular histopathology was conducted. To assess the impact of maternal exposure to fenvalerate on the fertility of male offspring, a third set of male pups was maintained to PND 80 and subsequently co-housed with untreated females.

Lactational fenvalerate exposure markedly decreased the absolute and relative weights of testes and increased the number of apoptotic cells in testes of pups at weaning. Histological examinations showed abnormal seminiferous tubules with large vacuoles or complete spermatogenic failure in testes of fenvalerate-treated mice at weaning. Additional experiments showed that lactational fenvalerate exposure markedly reduced messenger ribonucleic acid (mRNA) and protein levels of testicular P450scc, a testosterone synthesis enzyme. Consistent with down-regulation of testicular P450scc, the levels of serum and testicular testosterone at weaning were significantly

decreased in pups whose mothers were exposed to fenvalerate during lactation. Although the expression of testicular P450scc and serum and testicular testosterone levels in adulthood were restored to control levels, the decreased weights of testes and histological changes were irreversible. Importantly, the percentage of mature seminiferous tubules (stages VII and VIII) and the number of spermatozoa were obviously decreased in adult male mice whose mothers were exposed to fenvalerate during lactation. Taken together, these results suggest that lactational fenvalerate exposure permanently impairs testicular development and spermatogenesis (Zhang et al., 2009).

In a separate study, male mice were administered fenvalerate (60 mg/kg bw) by gavage daily from PND 35 to PND 63. Results showed that sperm count was significantly decreased in fenvalerate-treated mice. In addition, fenvalerate markedly decreased the layers of spermatogenic cells, disturbed the array of spermatogenic cells and increased the number of apoptotic cells in testes. The adverse effects of fenvalerate on male reproduction seemed to be associated with a decrease in serum and testicular testosterone levels. Although pubertal and early adult exposure to fenvalerate had little effect on the number of Leydig cells in testes, mRNA and protein levels of testicular biosynthetic enzymes, including P450 (17 α) and P450scc, were significantly down-regulated in fenvalerate-treated mice.

The study authors concluded that pubertal and early-adult fenvalerate exposures induce a decrease in sperm counts in adulthood. The impairment of spermatogenesis is due to decreased testicular testosterone synthesis (Zhang et al., 2010a).

In a separate study, pregnant mice were administered fenvalerate (30 mg/kg bw) by gavage daily from gestation days 13 to 18. The weights of testes and epididymides were significantly decreased in mice whose mothers were exposed to fenvalerate during pregnancy. In addition, marked decreases in the number of mature seminiferous tubules (stages VII and VIII) were observed in the testes of adult male offspring. Fenvalerate also reduced the number of epididymal spermatozoa in adult male offspring. Serum testosterone level was decreased, and mRNA and protein levels of P450(17 α) were significantly decreased in fetal testes.

The study authors concluded that the prenatal fenvalerate exposure irreversibly impairs testicular development and spermatogenesis, at least into early adulthood (Zhang et al., 2010b).

In a published study by Giray et al. (2010), effects of fenvalerate on thyroid hormone parameters in both healthy and selenium- and/or iodine-deficient rats were evaluated. In this study, 3-week-old male Wistar rats were administered fenvalerate (purity 92%) intraperitoneally. Eight groups of eight rats each were treated under various conditions. The treatment with fenvalerate had no effect on the T₄ levels of healthy controls, but caused significant increases in the T₄ levels of both iodine-deficient and selenium- plus iodine-deficient rats and a significant decrease in selenium-deficient rats. Fenvalerate treatment resulted in dramatic increases in the T₃ levels of all groups. Moreover, it caused an insignificant decrease in thyroid stimulating hormone in healthy controls, no effect in selenium-deficient rats and a significant elevation in iodine-deficient and selenium- plus iodine-deficient rats.

The study authors concluded that fenvalerate has the potential to significantly change thyroid hormone parameters in both normal and deficiency states, and the consequences of its modifying effect on thyroid status might be of critical importance, particularly in sensitive individuals and patients with thyroid dysfunction (Giray et al., 2010).

Fenvalerate was evaluated for its binding affinity with androgen receptor- and estrogen receptor-mediated mechanisms using short-term in vivo assays (Hershberger and uterotrophic). A 5-day Hershberger assay using castrated male rats measures the androgenic and antiandrogenic ability of the test substance. This test evaluates interaction of the test chemicals with the androgen receptor

present in several accessory glands or tissues (the ventral prostate, dorsolateral prostate, seminal vesicles with coagulating glands, and levator ani plus bulbocavernosus muscles). Fenvalerate (20, 40 or 80 mg/kg bw per day) was administered by oral gavage for 5 days to castrated male Crj:CD(SD)IGS rats (1 week after the castration, 11 weeks of age) with or without co-administration of testosterone propionate at 0.25 mg/kg bw per day (subcutaneous injection on the dorsal surface). Fenvalerate did not show any androgenic or antiandrogenic effects.

Potential effects of fenvalerate mediated through the estrogen receptor were evaluated by means of a 3-day uterotrophic assay using ovariectomized Crj:CD(SD)IGS rats (2 weeks after the ovariectomy, 8 weeks of age). No increase in weight of uterus (wet or blotted) was observed following oral exposure to fenvalerate (20, 40 or 80 mg/kg bw per day).

It was concluded by the study authors that, based on the results of these two reliable *in vivo* assays, fenvalerate did not exhibit any potential to cause adverse antiandrogenic or estrogenic effects at dose levels below those causing excessive systemic toxicity (Kunimatsu et al., 2002).

In a published study, the effects of fenvalerate on the reproductive system in rats were evaluated. Fenvalerate was administered orally at 0, 2, 4, 12 or 60 mg/kg bw to two groups of adult male SD rats. One group was sacrificed after 15 days of dosing, and the second group was sacrificed after 30 days of dosing. The levels of serum follicle stimulating hormone (FSH), LH, testosterone and testis homogenate testosterone were determined by radioimmunoassay. In addition, enzymes such as acid phosphatases and gamma-glutamyl transpeptidase (GGT) were examined, and sperm head counts were measured to explain the changes in daily sperm production.

In 15 days, serum FSH levels markedly increased in rats exposed to fenvalerate at or below 12 mg/kg bw ($P < 0.01$), and serum levels of LH were increased in the 12 mg/kg bw group ($P < 0.01$). In addition, testosterone levels in testis homogenates decreased after treatment with doses of 12 mg/kg bw or higher compared with the control group ($P < 0.01$). In 30 days, serum contents of FSH were significantly elevated at doses of 12 mg/kg bw or higher ($P < 0.01$), and homogenate levels of testosterone were diminished in the low-dose groups (2 and 4 mg/kg bw) ($P < 0.05$). Activity of acid phosphatases increased in the 12 and 60 mg/kg bw groups after 15 days ($P < 0.05$) and remained decreased in the high-dose group (60 mg/kg bw) after 30 days ($P < 0.05$), but the GGT level decreased dose dependently in the testis ($P < 0.05$). Fenvalerate caused a dose-dependent reduction in sperm head counts and daily sperm production, which were markedly reduced at doses of 12 mg/kg bw and above.

The study authors suggested that fenvalerate has obvious reproductive toxicity in male rats and can change the serum and testis homogenate levels of sex hormones or activity of testosterone, which may be correlated with the impairment of Sertoli cell and spermatogenic epithelium (Hu et al., 2002).

In a published study, the potential estrogenic activity of fenvalerate was evaluated in Wistar rats by examining reproductive and fertility capabilities. Adult male animals were treated for 30 days with fenvalerate at 20 or 40 mg/kg bw per day or corn oil (vehicle) by oral gavage. Further, a possible estrogenic activity of fenvalerate (0.4, 1, 4, 8 or 40 mg/kg bw) was tested after a 3-day treatment of immature female rats using the uterotrophic assay.

Exposure to the higher dose of fenvalerate was toxic to testis and epididymis, as shown by a decrease in the absolute weights and sperm counts in both organs. Although the sperm counts were reduced, fertility and sexual behaviour were similar in control rats and rats treated with 40 mg/kg bw. Fenvalerate did not exert estrogenic activity *in vivo* at the tested doses. Data suggest that fenvalerate treatment in this study failed to compromise fertility, possibly due to enhanced reproductive capacity in rodents compared with humans (Arena et al., 2008).

In a published study, fenvalerate was examined to evaluate whether pubertal fenvalerate exposure altered behavioural development. ICR mice were orally administered either vehicle or fenvalerate (purity not specified; 7.5 or 30 mg/kg bw per day) from PND 28 to PND 56. Learning and memory were assessed by the Morris water maze. Aggressive performance was evaluated by the aggressive behaviour test. Anxiety-related activities were detected by three tests: open-field, plus-maze and black–white alley. Sensorimotor function was analysed using beam walking and tightrope.

Results showed that the impairment of spatial learning and memory was more severe in fenvalerate-exposed female mice than in male mice. In addition, pubertal fenvalerate exposure inhibited aggressive behaviour in males. Moreover, pubertal fenvalerate exposure increased anxiety activities in females. The study authors concluded that, altogether, these results suggest that pubertal fenvalerate exposure impairs spatial cognition and behavioural development in a sex-dependent manner (Meng et al., 2011).

In a published study, pregnant rats were exposed to fenvalerate at 40 or 80 mg/kg bw per day or corn oil (vehicle, control) by oral gavage from gestation day 12 to lactation day 21 to evaluate reproductive, developmental and immunotoxic effects in male offspring. Immune and reproductive developmental effects were assessed in male offspring at PNDs 40 (peripuberty), 60 (post-puberty) and 90 (sexual maturity).

Treatment with the higher dose (80 mg/kg bw per day) resulted in convulsive behaviour, hyperexcitability and mortality in 45% of the dams. Fenvalerate was detected in the fetus due to placental transfer, as well as in pups due to breast milk ingestion, persisting in male offspring until PND 40, even though pesticide treatment was terminated on PND 20. However, fenvalerate did not produce marked alterations in age of testicular descent to the scrotum and preputial separation, parameters indicative of puberty initiation. In contrast, at puberty, there was a reduction in testicular weight and sperm production in male offspring of maternal treated rats. At adulthood, sperm counts and fertility did not differ between control and treated groups. Testosterone levels were not changed at any time during reproductive development. Similarly, no apparent exposure-related effects were detected in the histological structures of the lymphohaematopoietic system.

The study authors concluded that fenvalerate, in this experimental model, interfered with initial development of the male reproductive system, but that these effects on sperm production or fertility did not persist into adulthood (Nassr et al., 2010).

(g) *Microgranulomatous lesions*

A feeding study was conducted to examine the reversibility of the granulomatous changes in mouse liver, spleen and mesenteric lymph nodes, in which ddY strain mice were kept on a diet containing 0, 1000 or 3000 ppm fenvalerate for 6 weeks, followed by rearing on the control diet. Histopathological examination was conducted periodically. The granulomatous changes in liver, lymph nodes and spleen, which had been observed immediately and/or shortly after termination of the administration of fenvalerate, were lower in incidence as well as severity after 6 months of the recovery phase. Although these granulomatous changes were still observed at 12 months of the recovery phase, the incidence and severity were clearly reduced. Therefore, it was concluded that the granulomatous changes are not progressive and are reversible in nature (Ito et al., 1981).

In studies by Okuno et al. (1986a), male ddY mice were fed diets containing the [2S,αS]-, [2S,αRS]-, [2R,αS]- and [2R,αR]-isomers of fenvalerate at a dietary dose level of 0, 500 or 1000 ppm for 52 weeks, 500, 1000 or 2000 ppm for 52 weeks, 0, 125 or 1000 ppm for 13 weeks and 125 or 1000 ppm for 13 weeks. Microgranulomatous changes were observed in the mice treated with the [2R,αS]-isomer after 1, 2 or 3 months. In contrast, the changes did not occur in mice treated with the [2R,αR]-isomer under the same conditions. Neither [2S,αS]- nor [2S,αRS]-isomers caused microgranulomatous changes at 500 or 1000 ppm after 1 year. To clarify the causative agent of granuloma formation, the cholesterol ester of CPIA, a lipophilic conjugate from the [2R,αS]-isomer of fenvalerate, was injected intravenously into ddY mice. Microgranulomatous changes were observed in the liver of mice treated

with the [2R]-, [2S]-, or [2RS]-CPIA–cholesterol ester 1 week after a single treatment of 1, 10 or 100 mg/kg bw, as well as in the liver of mice treated with a single dose of 10 or 30 mg/kg bw of the [2R]-CPIA–cholesterol ester and kept up to 26 weeks afterwards. Histochemical examination and microscopic autoradiography of the liver demonstrated the presence of tritium, derived from ^3H -labelled [2R]-CPIA and cholesterol in giant cells and Kupffer cells. Another histochemical examination showed the presence of cholesterol ester in the liver of mice treated with the [2R, α S]-isomer. These results support the hypothesis that the CPIA–cholesterol ester is the causative agent of the microgranulomatous changes induced by fenvalerate.

In further studies by Okuno et al. (1986b), male and female ddY mice were fed diets containing technical fenvalerate (0, 10, 30, 100 or 300 ppm in the diet for 20 months or 0, 100, 300, 1000 or 3000 ppm in the diet for 17–18 months). Microgranulomatous changes were observed in the lymph nodes, liver and spleen, the NOAEL for such changes being 30 ppm. To examine the reversibility of these changes, ddY mice (males and females) were fed a diet containing technical fenvalerate at a dose level of 1000 or 3000 ppm for 6 weeks, followed by a control diet for up to 12 months. The size and number of the microgranulomatous changes were reduced with time. These changes were typical of foreign body granulomas and did not have the appearance of granulomas formed in response to an immunological stimulus. In order to further elucidate the fate of the granulomatous changes, mice were similarly treated with 10 or 30 ppm of the [2R]-CPIA–cholesterol ester and sacrificed at 1, 4 or 8 weeks after injection. Microgranulomatous changes, including microgranulomas and giant cell infiltrations, were seen in each case. Giant cells were seen clearly at the later stages, whereas microgranulomas were more evident at the early stages. The presence of the microgranulomas 8 weeks after injection is suggestive of foreign body microgranulomas.

To exclude hypersensitivity as the cause of the microgranulomatous changes previously observed, groups of five female BALB/cA/nu/nu/SLC nude mice were fed dietary racemic fenvalerate at 1000 or 3000 ppm in the diet for 4 weeks. Histopathological changes typical of the fenvalerate-induced microgranulomas were observed in all of the treated mice. This study indicates that granulomatous changes induced by fenvalerate are not mediated by hypersensitivity reactions (Miyamoto et al., 1984).

More recently, a dose-related incidence of hepatic microgranulomas was found in groups of six male and six female Beagle dogs fed fenvalerate at 0, 250, 500 or 1000 ppm in the diet for 6 months. Histiocytic cell infiltration of mesenteric lymph nodes also occurred in female dogs fed 500 or 1000 ppm and in males fed 1000 ppm. Multinucleated cells were occasionally seen. The reversibility of these effects was not studied (Parker et al., 1984).

2.7 *Studies on metabolites and degradates*

The major photodegradation product of fenvalerate, SD 54597, decarboxyfenvalerate [2-(3-phenoxyphenyl)-3-(4-chlorophenyl)-4-methylpentanenitrile], is less acutely toxic to mammals than fenvalerate itself. It is also a plant metabolite. The results of acute toxicity studies on decarboxyfenvalerate are summarized in Table 11.

Groups of Fischer 344 rats (30 of each sex per group) were fed diets containing decarboxyfenvalerate (lot no. not stated, purity 98%) at a concentration of 0, 30, 100, 300, 3000 or 10 000 ppm for up to 13 weeks. Mortality was not affected by treatment, but body weight gains were significantly reduced in males of the 10 000 ppm group throughout the study and in females during the first 7 weeks. Body weight gains were also significantly reduced in males of the 3000 ppm group during the first 7 weeks. White blood cell counts were significantly reduced at weeks 7 and 13 in both sexes of the 10 000 ppm group and in females of the 3000 ppm group ($P < 0.05$, Dunnett's test). Reductions in white blood cell counts in males of the 100 and 300 ppm groups at 13 weeks were not dose related. Mean corpuscular volume was significantly reduced at 7 and 13 weeks in both sexes of the 3000 and 10 000 ppm groups ($P < 0.05$, Dunnett's test), except for females of the 3000 ppm group at 7 weeks. Some deviations in blood chemistry values were noted, but these were generally inconsistent or lacking sufficient magnitude to be of biological importance. Absolute and relative (to

body weight) liver weights were increased in both sexes of the 300 ppm group and higher, except for males of the 300 ppm group at 7 weeks. Consistent, significant increases were also seen in absolute or relative kidney weights in both sexes of the 10 000 and 3000 ppm groups. Significant microscopic changes were limited to centrilobular hepatocellular hypertrophy in both sexes of the 10 000 ppm group, minimal focal areas of hepatocellular necrosis in some rats of the 10 000 and 3000 ppm groups and an increased incidence, but not severity, of glomerulonephrosis in both sexes of the 10 000 ppm group at 13 weeks.

Table 11. Acute toxicity of decarboxyfenvallerate (SD 54597, plant metabolite)

Species	Strain	Sex	Route	Vehicle	Result	Reference
Rat	Sprague-Dawley	M, F	Oral	Corn oil	> 5000 mg/kg bw	Cannelongo (1983a)
Rabbit	New Zealand White	M, F	Dermal	Deionized water	> 2000 mg/kg bw	Cannelongo (1983b)
	New Zealand White	M, F	Dermal	Undiluted	Not a skin irritant	Cannelongo (1983c)
	New Zealand White	M, F	Eye	Undiluted	Not an eye irritant	Cannelongo (1983d)
Guinea-pig Buehler test	Dunkin-Hartley	M, F	Skin	Undiluted	Not a skin sensitizer	Parker (1983)

F, female; M, male

The NOAEL for this study was 300 ppm (equivalent to 30 mg/kg bw per day), based on reduced body weight gains in males during the first 7 weeks, a decrease in white blood cell count in females, reduced mean corpuscular volume in both sexes, increases in liver and kidney weights, and hepatocellular necrosis in both sexes at 1000 ppm (equivalent to 100 mg/kg bw per day) (Parker et al., 1986).

In a teratology study, decarboxyfenvallerate (purity 98%) was dissolved in corn oil and administered by gastric intubation to groups of 30 sperm-positive COBS CD (SD) BR albino rats at a dose of 0, 30, 300 or 3000 mg/kg bw per day on gestation days 6–15. Control dams received corn oil only.

Few clinical signs were observed in any treatment group throughout gestation, and no statistically significant differences were noted in maternal body weights, body weight gains, numbers of corpora lutea, implantations or resorptions, pregnancy rates or implantation rates among all groups. However, the maternal carcass weights at sacrifice (maternal body weight minus the weight of the gravid uterus) were statistically significantly decreased and the maternal relative liver weights were increased in the high-dose group.

No fenvalerate-related adverse effects were observed in the pups in terms of reduction in mean fetal weights or increased incidences of malformations, anatomical variations or resorptions. The malformations and variations observed in this study included those that are commonly seen among rats, such as short ribs, misaligned sternbrae, hydronephrosis and hydroureter. Only two severely malformed fetuses were observed: one was a control with cleft palate, cleft lip, absence of the tongue and a short lower jaw; the other was an animal in the low-dose group that exhibited umbilical hernia, short limbs and fused digits.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased body weights and increased relative liver weights seen at 3000 mg/kg bw per day. The developmental toxicity NOAEL was 3000 mg/kg bw per day, the highest dose tested (Lu, 1983).

Acute intraperitoneal toxicity studies were conducted on fenvalerate metabolites in mice. Mice were treated with test compound at various dose ranges using corn oil as the vehicle (except PBacid, which was dissolved in DMSO). Treated mice were observed for 14 days. Gross pathological examination was conducted at termination. The results of the acute toxicity tests are summarized in Table 12.

Table 12. Acute intraperitoneal toxicity of fenvalerate metabolites in mice

Chemical	Purity (%)	LD ₅₀ (mg/kg bw)	
		Males	Females
Fenvalerate	97.6	88.5	85
CPIA	> 99	351	350
PBalc	> 99	371	424
4'-OH-PBalc	> 99	750–1000	750–1000
2'-OH-PBalc	> 99	876	778
PBacid	97.3	154	169
4'-OH-PBacid	99	783	745
2'-OH-PBacid	99	859	912
3-Phenoxybenzaldehyde	99	415	416
Sodium thiocyanate	99	604	578

From Kohda et al. (1979)

CPIA, 2-(4-chlorophenyl)isovaleric acid; LD₅₀, median lethal dose; 2'-OH-PBacid, 3-(2'-hydroxyphenoxy)benzoic acid; 4'-OH-PBacid, 3-(4'-hydroxyphenoxy)benzoic acid; 2'-OH-PBalc, 3-(2'-hydroxyphenoxy)benzyl alcohol; 4'-OH-PBalc, 3-(4'-hydroxyphenoxy)benzyl alcohol; PBacid, 3-phenoxybenzoic acid; PBalc, 3-phenoxybenzyl alcohol

The mutagenic potential of two fenvalerate metabolites in bacteria has been investigated. Neither COOH-fenvalerate (lot nos CTS94001 and A1-1M, purities 96.1% and 91.3%, respectively) nor CONH₂-fenvalerate (lot no. CTS94002, purity 98.4%) induced mutations in *Salmonella typhimurium* strain TA98, TA100, TA1535 or TA1537 or in *Escherichia coli* WP2uvrA in the presence or absence of an exogenous metabolic system (Yamada & Kitamoto, 1995a,b).

It can be concluded that the minor metabolites of fenvalerate are all less toxic than the parent compounds.

3. Observations in humans

3.1 Occupational studies

In a Swedish study, occupational exposure to three products containing synthetic pyrethroids used in forestry to protect conifer seedlings against the large brown pine weevil (*Hylobius abietis*) was studied. Fenvalerate was used in one product and permethrin in two products containing a mixture of *trans/cis* isomers in the proportions 60/40 and 75/25, respectively. A high frequency of symptoms was reported in connection with the use of fenvalerate, which led to a withdrawal of the product for use in forestry. Some symptoms were also reported in groups exposed to permethrin. For

example, in interviews carried out with 139 planters, irritative symptoms of the skin and upper respiratory tract were reported in 73% of users for fenvalerate, 63% for permethrin (*trans/cis* 75/25) and 33% for permethrin (*trans/cis* 60/40), respectively (Kolmodin-Hedman, Swensson & Akerblom, 1981, 1982).

Among 23 workers exposed to synthetic pyrethroids, including fenvalerate, 19 experienced one or more episodes of abnormal facial sensation developing between 30 minutes and 3 hours after exposure and persisting for 30 minutes to 8 hours. None of the subjects had experienced symptoms on any other part of the body. The sensations were described as “tingling”, “burning”, “like coming from the cold” and “nettle rash”. However, there were no abnormal neurological signs, and electrophysiological studies showed normal responses in the arms and legs. It was concluded that the facial symptoms were probably due to spontaneous firing of sensory nerve fibres or sensory nerve endings whose threshold has been transiently lowered by the exposure to a pyrethroid. The localization of symptoms, their time of onset and duration probably depend on the local concentration of pyrethroid, which is influenced by factors affecting absorption through the skin locally (Le Quesne, Maxwell & Butterworth, 1980).

A field study of 16 workers engaged in the agricultural application of fenvalerate associated exposure with cutaneous symptomatology. Paraesthesia usually developed at exposed body sites after several hours, and the symptoms then progressed from a mild itch to a stinging sensation and peaked with numbness. Sweating, exposure to sun or heat or the application of water to the exposed site aggravated these symptoms. Normal sensation returned with 24 hours of cessation of exposure (Tucker & Flannigan, 1983).

No clinical case of pyrethroid poisoning had been reported until outbreaks of acute deltamethrin and fenvalerate poisoning occurred among cotton growers in China in 1982. Having been told (in error) that pyrethroids were non-toxic, the farmers handled the pyrethroid insecticides without taking any precautions. After repeated spraying in the cotton fields, the mild cases presented with severe headaches, dizziness, fatigue, nausea and anorexia, with transient changes in the electroencephalogram, while a severe case developed muscular fasciculation, repetitive discharges in the electromyogram and frequent convulsions. However, all were found by follow-up studies to have completely recovered, and the prognosis of acute pyrethroid poisoning proved to be correct (He, 1987).

More recently, the same author reviewed 573 cases of acute pyrethroid poisoning reported in the Chinese medical literature during 1983–1988. Among these were 196 cases of acute fenvalerate poisoning, 63 of which were occupational, due to inappropriate handling, and 133 accidental, mostly due to ingestion. Two died of convulsions. All others recovered with symptomatic and supportive treatment within 1–6 days. A comprehensive review of clinical manifestations was included (He et al., 1989).

3.2 Mode of action

The toxicity of fenvalerate and other pyrethroids has been reviewed by Aldridge (1990), Vijverberg & Van den Bercken (1990) and Appel & Gericke (1993). In all species tested, high doses of pyrethroids induced toxic signs that are characteristic of a strong excitatory action on the nervous system (Vijverberg & Oortgiessen, 1988). The principal action of pyrethroids on the peripheral nervous system is to induce pronounced repetitive activity. In particular, sensory organs produce trains of nerve impulses instead of single impulses, both *in vitro* and *in vivo*. Depending on the particular pyrethroid, sensory nerves, motor nerve endings and muscle fibres may also show repetitive activity. Another possible effect is depolarization of membranes, leading to increased release of neurotransmitters or even blockage of excitation.

The pyrethroids can be divided into two classes by the pattern of toxic signs shown in rats after the administration of sublethal and/or lethal doses. The early synthetic pyrethroids induced signs of intoxication in rats very similar to those of the natural pyrethrins. Initial tremors, aggressive behaviour and extreme sensitivity to sensory stimuli were followed by prostration, with whole-body tremors preceding death. This sequence, later designated T syndrome, is characteristic of non-cyano

pyrethroids and may be incomplete, depending on the molecular structure and on the route of application and dose of the pyrethroid. Most of the α -cyano-3-phenoxybenzyl pyrethroids evoke a distinct sequence of symptoms, called the CS syndrome. Initial pawing and burrowing behaviour is rapidly followed by profuse salivation and whole-body tremors, progressing to sinuous writhing (choreoathetosis), which gradually becomes more intense. In the final stage, clonic seizures may occur. As in the case of non-cyano pyrethroids, symptoms may be evoked or enhanced by sensory stimuli.

Vijverberg & Van den Bercken (1990) summarized the evidence for the hypothesis that the main biological activity of pyrethroids is mediated by effects on sodium channels. The stereoselective pyrethroids fix themselves onto the sodium channel and cause them to stay open much longer than normal, resulting in prolongation of the transient current associated with membrane depolarization. In frog myelinated nerve fibres, this prolongation can range from 6 milliseconds with phenothrin to 1770 milliseconds with deltamethrin. The time constant is a characteristic of each structure, and those causing the T syndrome and those causing the CS syndrome in rats can generally be distinguished on this basis. Investigations into the site of action of pyrethroids within the nervous system have not resulted in a clear distinction between central and peripheral effects. Several published results suggest that in rats, the lethal site of pyrethroids is located peripherally, possibly in the cardiovascular or respiratory system. These effects can be attributed to modifications of presynaptic and postsynaptic sodium channels. Postsynaptic neurotransmitter responses are unaffected by concentrations of pyrethroids that cause marked sodium channel modification. At high concentrations, insecticidal as well as non-insecticidal pyrethroid isomers cause a nonspecific suppressive effect on the postsynaptic neurotransmitter response.

3.3 *Clinical studies*

Fenvalerate was administered dermally to the skin of the arm or face of adult men and women at dose levels of approximately 20–40 mg. Control applications were carried out on the same individuals, and subjective evaluations were performed with respect to dermal irritation. There was no erythema or other visible skin effects, and an evaluation of the subjective responses suggested no significant differences between the fenvalerate-treated and control portions of the body (Hine, 1976).

A double-blind study utilizing 29 male volunteers was performed to test the skin reaction of formulated fenvalerate. The emulsifiable concentrate formulation was diluted with water and administered to one side of the face, on the cheek, with a control formulation applied to the opposite cheek. There were no signs of dermatitis noted at 24 hours following administration. Subjective analyses of irritation or skin sensation were performed with each individual. Under the conditions of the study, the formulation of fenvalerate did not produce abnormal skin sensations. There were no indications that any of the symptoms noted (which included tingling, burning, stinging, itching, swelling, numbness or heat) was associated with fenvalerate (Brown & Slomka, 1979).

A double-blind study was performed to compare human discrimination of technical fenvalerate, the heavy-ends fraction of distilled fenvalerate, and ethyl alcohol (vehicle) applied to the lower edge of each earlobe of 36 adult (both male and female) volunteers on three separate occasions. Both forms of fenvalerate caused a statistically significant increase in paraesthesia, compared with the vehicle alone. The onset of the cutaneous sensations occurred 1 hour after application, peaked at 3–6 hours and lasted approximately 24 hours. Numbness, itching, burning, tingling and warmth were the most frequently reported sensations. The difference between the effects of the two fractions of fenvalerate was not statistically significant (Knox, Tucker & Flannigan, 1984).

A clinical study was conducted to evaluate the difference in the degree of paraesthesia induced by a number of pyrethroids. Applications of 0.05 ml fenvalerate formulated to field strength (0.13 mg/cm²) were made to a 4 cm² area of earlobe on five occasions, the opposite earlobe receiving distilled water. Participant evaluation after each application continued for 48 hours and involved description of the cutaneous sensations. Each participant was treated after each application with one of the remaining compounds. Fenvalerate (like the other pyrethroids) induced skin sensations. The

paraesthesia developed with a latency period of approximately 30 minutes, peaked by 8 hours and deteriorated as early as 24 hours. The local application of dl- α -tocopheryl acetate markedly inhibited the occurrence of skin sensations (Flannigan et al., 1985a,b; Malley et al., 1985).

3.4 Summary

In summary, fenvalerate has been found to induce skin sensations in some of the workers who handle this insecticide. Clinical studies showed that the skin sensations develop with a latency period of approximately 30 minutes, peak by 8 hours and deteriorate after 24 hours. Numbness, itching, tingling and burning are symptoms frequently reported. α -Tocopheryl acetate has been found to inhibit the occurrence of these skin sensations.

Comments

Biochemical aspects

Metabolism studies have been conducted in rats, mice and dogs using ^{14}C -labelled esfenvalerate and fenvalerate. Fenvalerate was rapidly absorbed in these mammals, widely distributed to organs and tissues and rapidly metabolized. Excretion of an isomeric mixture of fenvalerate and esfenvalerate was very rapid in rats and mice, with 63–94% of the administered label being excreted within 1 day after oral dosing. Approximately equal quantities of radioactivity were eliminated in the urine and faeces. Tissue residue concentrations were generally very low, with residue levels being lower in mice than in other species. Fenvalerate and its esters concentrate in adipose tissue, adrenal gland, intestinal mucosa, skin and hair. The cyano moiety remains in the body (particularly in skin and hair) longer than other components. A placental transfer study of fenvalerate and esfenvalerate in rats indicated that there was virtually no transfer of radioactivity from maternal blood to the fetus and no evidence of accumulation in the fetus or amniotic fluid. In dogs administered fenvalerate orally, the total recovery of radioactivity in excreta was less than that in mice or rats, but the elimination half-life was similar to those found in the rodents (0.5–0.6 day).

Fenvalerate undergoes several major metabolic reactions, including cleavage of the ester linkage, hydroxylation in the acid and alcohol moieties and conversion of the cyano group to thiocyanate and carbon dioxide. The pattern of hydroxylation was different in rats and dogs, and the glycine conjugate, 3-phenoxybenzoylglycine, was the major conjugate of the alcohol moiety in dogs, whereas it was a minor one in rats. The proportions of glucuronides formed at the acid moiety and their hydroxy derivatives were also greater in dogs. There were no major sex differences in the metabolism of fenvalerate.

In a 28-day feeding study in mice, the major metabolites in the liver and kidney of animals fed [^{14}C -chlorophenyl]esfenvalerate and [^{14}C -chlorophenyl]fenvalerate were CPIA and the hydroxylated derivative of CPIA. These disappeared after administration of untreated diets. In addition, CPIA-cholesterol ester was found in mice fed [^{14}C -chlorophenyl]fenvalerate, but not in mice fed [^{14}C -chlorophenyl]esfenvalerate.

Photolytic degradation on plants can produce a decarboxylated fenvalerate not known to occur in mammals.

Toxicological data

Clinical signs, such as choreoathetosis (coarse tremors progressing to sinuous writhing), sedation, salivation, dyspnoea and/or clonic seizures and sometimes body tremors and prostration, were observed in acute studies. These signs, which are typical of a type II pyrethroid, have been observed in various mammalian species tested with either esfenvalerate or fenvalerate and are characteristic of a strong excitatory action on the nervous system.

The oral LD_{50} in rats was greater than or equal to 451 mg/kg bw (vehicle dependent), whereas in mice, it was greater than or equal to 100 mg/kg bw (vehicle dependent). The dermal LD_{50} in rats was 5000 mg/kg bw. The inhalation LC_{50} in rats was greater than 101 mg/m³ (3-hour exposure). No

data are available on skin and eye irritation or skin sensitization. However, data are available on esfenvalerate, which is not irritating to the skin and minimally irritating to the unwashed eyes of rabbits. It was a skin sensitizer in guinea-pigs using the Magnusson and Kligman maximization test, but was not a sensitizer using the Buehler test method.

In a 90-day dietary toxicity study of fenvalerate in rats, the NOAEL was 125 ppm (equivalent to 12.5 mg/kg bw per day), based on increased relative kidney and liver weights at 500 ppm (equivalent to 50 mg/kg bw per day). Although survival was reduced at 2000 ppm (equivalent to 200 mg/kg bw per day), the highest dose tested in this study, no degeneration of the sciatic nerve was observed.

The overall NOAEL in dogs fed fenvalerate for 3 or 6 months was 250 ppm (equivalent to 18.8 mg/kg bw per day), based on hepatic multifocal microgranulomas and histiocytic infiltration of the mesenteric lymph nodes in females at 500 ppm (equivalent to 37.5 mg/kg bw per day).

Three long-term dietary studies of fenvalerate toxicity and carcinogenicity in mice have been reported. In the first study, the NOAEL was 300 ppm (equivalent to 15 mg/kg bw per day), based on reduced body weight gain, clinical signs of hyperactivity, mortality, clinical chemistry changes and microscopic changes in liver, kidney and mesenteric lymph nodes observed at 1000 ppm (equivalent to 50 mg/kg bw per day). In the second study, the NOAEL was 10 ppm (equivalent to 1.5 mg/kg bw per day), based on an increased incidence of microgranulomatous changes in mesenteric lymph nodes and other visceral and peripheral lymph nodes observed at 50 ppm (equivalent to 7.5 mg/kg bw per day). In the third study, the NOAEL was 30 ppm (equal to 3.48 mg/kg bw per day), based on slightly decreased erythrocyte counts, decreased serum glucose concentrations and increased histiocytic infiltration (liver and lymph nodes) and granulomatous changes in the liver and lymph nodes at 100 ppm (equal to 12.3 mg/kg bw per day). The overall NOAEL for long-term dietary exposure of mice to fenvalerate was 30 ppm (equal to 3.48 mg/kg bw per day), based on histopathology in various organs, but most consistently in lymph nodes, at 50 ppm (equivalent to 7.5 mg/kg bw per day).

There was no evidence for carcinogenicity from any of these studies in mice at fenvalerate concentrations up to 3000 ppm (equivalent to 150 mg/kg bw per day).

In a published oral gavage study of carcinogenicity of fenvalerate in mice, no evidence for carcinogenicity was observed at the highest dose of 80 mg/kg bw per day.

Four chronic toxicity and carcinogenicity studies of fenvalerate have been performed in rats, but two of the studies are inadequate for the assessment of carcinogenic potential.

Groups of Sprague-Dawley rats were administered diets containing fenvalerate at either 0 or 1000 ppm (equivalent to 50 mg/kg bw per day) for 2 years. No group difference in the incidence of any specific tumours was reported.

In a second 2-year study in rats (Wistar/SLC strain), there were observations of body weight gain depressions and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day).

The overall NOAEL for long-term toxicity of fenvalerate in rats was 150 ppm (equivalent to 7.5 mg/kg bw per day), on the basis of body weight gain reduction in males and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day). In males, Leydig cell atrophy, Leydig cell hyperplasia and Leydig cell adenomas were significantly increased in some groups, but there was no clear dose-response relationship. Similar to the Fischer 344 strain rats, Leydig cell adenomas are particularly common and variable in the Wistar/SLC strain (which is not to be confused with the Wistar strain, in which incidences of these tumours are low). Consequently, variation in the incidence of this tumour type cannot be used with any confidence in carcinogenicity evaluation. There was no evidence of carcinogenicity in rats at doses up to 1500 ppm (equivalent to 75 mg/kg bw per day), the highest dose tested.

The Meeting concluded that fenvalerate was not carcinogenic in mice or rats.

Fenvalerate was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity emerged from the in vitro assays, but inconsistent results were obtained in the male mouse dominant lethal assays and the in vivo cytogenetic assays.

Available data allowed the Meeting to conclude that fenvalerate is unlikely to be a DNA reactive compound, but no firm conclusion could be reached on its in vivo clastogenicity.

On the basis of the absence of carcinogenicity in mice and rats and the absence of DNA reactivity, the Meeting concluded that fenvalerate is unlikely to pose a carcinogenic risk to humans at expected dietary levels.

In a three-generation reproduction study in rats, the NOAEL for parental toxicity was 25 ppm (equivalent to 1.7 mg/kg bw per day), based on reduced mean body weights seen at 250 ppm (equivalent to 16.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 250 ppm (equivalent to 16.7 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in mice, the NOAEL for maternal toxicity was 15 mg/kg bw per day, based on irregular respiration, hypersensitivity, tremors and salivation after administration of the compound (first 30–60 minutes after dosing only) seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight of dams seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested.

The Meeting concluded that fenvalerate is not teratogenic in mice or rabbits.

A study of the neurotoxic potential of esfenvalerate and fenvalerate in corn oil was conducted in rats following a single oral gavage dose. The NOAELs were 5 and 20 mg/kg bw for esfenvalerate and fenvalerate, respectively, based on the toxic signs typical of type II pyrethroids. Signs were observed within 2 hours of dosing at 20 and 90 mg/kg bw for esfenvalerate and fenvalerate, respectively.

No histopathological lesions in the sciatic nerve were observed in rats following a single-dose administration of fenvalerate at 200 mg/kg bw. In a separate study, rats were administered fenvalerate orally at dose levels ranging from 0 to 400 mg/kg bw per day for 7 consecutive days. A significant neurological deficit was demonstrated using an inclined plane test (expressed as the angle at which the animals cannot maintain their hold on an inclining plane). In addition to functional deficits, increases in the activity of the lysosomal enzymes β -glucuronidase and β -galactosidase in the posterior tibial nerve and trigeminal ganglia were observed.

Fenvalerate did not cause delayed neuropathy in hens at 1000 mg/kg bw per day for 5 days.

Several published studies are available that evaluate the effects of fenvalerate on sexual maturation, stereotyped and sexual behaviour, hormonal measurements, sperm measurements and changes in various organ weights and histopathology in mice and rats. These studies were conducted following single or multiple oral administrations of fenvalerate. Generally, reproductive parameters and neurobehavioural parameters were affected at about 20 mg/kg bw per day via oral doses.

The acute intraperitoneal toxicity study results indicate that fenvalerate metabolites were less toxic than the parent compound fenvalerate.

The major photodegradation product of fenvalerate, decarboxyfenvalerate, or 2-(3-phenoxyphenyl)-3-(4-chlorophenyl)-4-methylpentanenitrile, was evaluated for its acute toxicity; results indicated that it was less toxic than the parent compound. In a 90-day toxicity study in rats with decarboxyfenvalerate, the NOAEL was 300 ppm (equivalent to 30 mg/kg bw per day), based on reduced body weight gains in males during the first 7 weeks, a decrease in white blood cell count in females, reduced mean corpuscular volume in both sexes, increases in liver and kidney weights, and hepatocellular necrosis at 1000 ppm (equivalent to 100 mg/kg bw per day). In a developmental toxicity study with decarboxyfenvalerate in rats, the NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreases in body weights and increases in relative liver weights seen at 3000

mg/kg bw per day. The developmental toxicity NOAEL was 3000 mg/kg bw per day, the highest dose tested.

Observations in humans indicate that fenvalerate causes the transient facial sensations that appear to be common to pyrethroids, particularly those possessing a cyano group.

The Meeting concluded that the existing database on fenvalerate was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.02 mg/kg bw on the basis of a parental systemic toxicity NOAEL of 1.7 mg/kg bw per day observed in the three-generation reproduction study in rats, based on reduced mean body weights seen at 16.7 mg/kg bw per day and using a safety factor of 100. This ADI was supported by the NOAEL of 3.5 mg/kg bw per day observed in the long-term toxicity and carcinogenicity studies in mice, based on the slight decrease in erythrocyte counts, increased histiocytes and granulomatous changes in the liver and lymph nodes (mesenteric, visceral and peripheral) at 7.5 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.2 mg/kg bw on the basis of the NOAEL of 20 mg/kg bw observed in the single oral dose neurotoxicity study in rats, based on clinical signs of toxicity (muscular fibrillation, ataxia, salivation and/or hunched posture) seen at 90 mg/kg bw and using a safety factor of 100. This ARfD was supported by the developmental toxicity study in mice in which the NOAEL was 15 mg/kg bw per day, based on irregular respiration, hypersensitivity, tremors and salivation after administration of the compound (first 30–60 minutes after dosing) seen at 50 mg/kg bw per day.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month to 2-year studies of toxicity and carcinogenicity ^{a,b}	Toxicity	30 ppm, equal to 3.5 mg/kg bw per day	50 ppm, equivalent to 7.5 mg/kg bw per day
		Carcinogenicity	3000 ppm, equivalent to 150 mg/kg bw per day ^c	—
	Developmental toxicity study ^d	Maternal toxicity	15 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day ^c	—
Rat	Two-year studies of toxicity and carcinogenicity ^{a,b}	Toxicity	150 ppm, equivalent to 7.5 mg/kg bw per day	500 ppm, equivalent to 25 mg/kg bw per day
		Carcinogenicity	1500 ppm, equivalent to 75 mg/kg bw per day ^c	—
	Three-generation study of reproductive toxicity ^a	Reproductive toxicity	250 ppm, equivalent to 16.7 mg/kg bw per day ^c	—
		Parental toxicity	25 ppm, equivalent to 1.7 mg/kg bw per day	250 ppm, equivalent to 16.7 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
		Offspring toxicity	250 ppm, equivalent to 16.7 mg/kg bw per day ^c	—
	Neurotoxicity study (single dose) ^d	Neurotoxicity	20 mg/kg bw	90 mg/kg bw
Rabbit	Developmental toxicity study ^d	Maternal toxicity	25 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day ^c	—
Dog	Thirteen-week and 6-month studies of toxicity ^{a,b}	Toxicity	250 ppm, equal to 18.8 mg/kg bw per day	500 ppm, equal to 35.5 mg/kg bw per day

^a Dietary administration.

^b Two or more studies combined.

^c Highest dose tested.

^d Gavage administration.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.2 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to fenvalerate

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Rapid and complete
Dermal absorption	Not available
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete (half-life 0.5–0.6 day)
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Parent compound and decarboxylated fenvalerate

Acute toxicity

Rat, LD ₅₀ , oral	≥ 451 mg/kg bw (vehicle dependent)
Rat, LD ₅₀ , dermal	5000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 101 mg/m ³ (3 h)
Rabbit, dermal irritation	Not available
Rabbit, ocular irritation	Not available
Dermal sensitization	Not available

<i>Short-term studies of toxicity</i>	
Target/critical effect	Nervous system, clinical signs
Lowest relevant oral NOAEL	12.5 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	7 mg/m ³ (rat)
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Multiple target organs
Lowest relevant NOAEL	3.5 mg/kg bw day (mouse)
Carcinogenicity	Not carcinogenic in mice or rats
<i>Genotoxicity</i>	
	Not DNA reactive, inconsistent results in in vivo cytogenetic assay
<i>Reproductive toxicity</i>	
Target/critical effect	None
Lowest relevant reproductive NOAEL	16.7 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	1.7 mg/kg bw per day
Lowest relevant offspring NOAEL	16.7 mg/kg bw per day (highest dose tested)
<i>Developmental toxicity</i>	
Target/critical effect	None
Lowest relevant maternal NOAEL	15 mg/kg bw per day (mouse)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (mouse and rabbit)
<i>Neurotoxicity</i>	
Target/critical effect	Clinical signs typical of type II pyrethroids
Acute neurotoxicity NOAEL	20 mg/kg bw (rat)
Subchronic neurotoxicity	No data
<i>Other toxicological studies</i>	
Studies on metabolites	Metabolites/degradation products less toxic than parent
Immunotoxicity	No data
<i>Medical data</i>	
	Transient facial sensations in humans

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Three-generation reproduction study (rat)	100
ARfD	0.2 mg/kg bw	Single-dose study (rat)	100

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