Section 4

EVALUATION OF THE MAMMALIAN TOXICOLOGY AND METABOLISM TOXICOKINETICS

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ABBREVIATIONS

ng	Nanogram	**nM**	Nanomolar
µg	Microgram	**mM**	Millimolar
mg	Milligram	**sec**	Second
kg	Kilogram	**min**	Minute
mL	Millilitre	**h**	Hour
L	Litre	**m**	Metre
GI	Gastrointestinal	**SC**	Subcutaneous
IM	Intramuscular	**LH**	Luteinising hormone
IP	Intraperitoneal	**mg/kg bw/day**	mg/kg bodyweight/day
IV	Intravenous	**ppb**	Parts per billion
PO	Oral	**ppm**	Parts per million

ADI	Acceptable Daily Intake
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
ALT	Alanine aminotransferase (SGPT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatinine phosphokinase
DDM	4,4'-Diaminodiphenylmethane
DMSO	Dimethyl sulfoxide
EUP	End Use Product
GLP	Good Laboratory Practice
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LOEL	Lowest Observed Effect Level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MRL	Maximum Residue Limit
NOEL	No Observable Effect Level
NTE	Neuropathy target esterase
OP	Organophosphorus pesticide
2-PAM	Pyridine-2-aldoxime methiodide
P-2-S	2-pyridine-aldoxime methyl methanesulfonate
TGAC	Technical Grade Active Constituent

ACPH	Advisory Committee on Pesticides and Health
NHMRC	National Health and Medical Research Council
NDPSC	National Drugs and Poisons Scheduling Committee

SUMMARY

Background

Chlorfenvinphos (CFVP) is an organophosphate insecticide. It was introduced in 1963 by the Shell Development Co. under the code number 'SD 7859' and the trade name 'Birlane'. It was also produced by Ciba AG under the code number 'C 8949' and the trade mark 'Sapecron' and by Allied Chemical Corp. as 'GC 4072'. It is no longer produced by these last 2 companies or their successors. It has been in use in Australia since 1968, with current uses to control external parasites in large animals. Structurally related compounds include tetrachlorvinphos, dicrotophos and bromofenvinphos.

Chlorfenvinphos was allocated a Schedule 7 poisons classification by the Poisons Scheduling Committee (PSC) in 1968, and maximum residue limits (MRL) in crops were set by the Pesticides and Agricultural Chemicals Committee from July 1969. The current Australian ADI is 0.002 mg/kg bw/day, based on a NOEL of 0.15 mg/kg bw/day for plasma ChE inhibition seen in a rat dietary study and using a 100-fold safety factor.

Kinetics and Metabolism

The toxicokinetics and metabolism of chlorfenvinphos was investigated in a series of investigations in Porton rats and Beagle dogs (Hutson & Hathway, 1967). Rats were given chlorfenvinphos by stomach tube and the blood chlorfenvinphos concentration assayed. Female rats had higher blood levels than males, with the maximum level reached later after dosing.

- Radiolabelled chlorfenvinphos was administered to female rats, and the brain ChE activity and radioactivity was analysed. Chlorfenvinphos concentrations (estimated from radioactivity) increased rapidly, with maximal levels 25 min after dosing while ChE inhibition increased more slowly, and was not marked until 50 min after dosing.

- A female dog was dosed with chlorfenvinphos and the portal and peripheral chlorfenvinphos concentrations assayed. The portal levels were much greater than the peripheral concentrations.

- A male dog was dosed with radiolabelled chlorfenvinphos, and the radioactivity and concentration of chlorfenvinphos in the peripheral circulation determined. The radioactivity level was approximately 30 times the chlorfenvinphos concentration, indicating that the dog metabolised chlorfenvinphos rapidly.

- Rat and dog brain homogenates were incubated with chlorfenvinphos for 30 min. The concentration of chlorfenvinphos required to reduce the hydrolysis of acetylcholine was determined, with rat brain ChE being 10-times more sensitive to inhibition by chlorfenvinphos than dog brain ChE.

- When radiolabelled chlorfenvinphos was incubated with dog or rat plasma, the dog plasma hydrolysed 5% of the insecticide in 6 h, while rat plasma hydrolysed 4% in 16 h, indicating that dog plasma was more efficient than rat plasma at metabolising chlorfenvinphos.

- Radiolabelled chlorfenvinphos was mixed with dog or rat plasma, and dog and rat erythrocytes were added. Erythrocytes were also added to bovine serum and labeled chlorfenvinphos. The ratio of radioactivity in erythrocytes to plasma was approximately the same for both rat and dog erythrocytes when incubated with artificial serum. Dog erythrocytes incubated in dog plasma had a much lower ratio than rat erythrocytes incubated in rat plasma, indicating that dog plasma may have bound chlorfenvinphos to a greater extent. This was supported by a trial in which chlorfenvinphos in dog or rat plasma was dialysed against horse serum. Only 15% of the chlorfenvinphos in the dog serum diffused out, whereas 25% of the chlorfenvinphos in the rat serum diffused, indicating that the dog serum bound chlorfenvinphos more strongly.

- The greater tolerance of the dog to chlorfenvinphos in comparison to the rat appears to be a combination of increased metabolism in the liver, an increased binding by plasma protein and a decreased sensitivity of the brain to chlorfenvinphos.

^{14}C-Radiolabelled chlorfenvinphos was administered by gavage to Porton rats which were then maintained in a metabolism cage for 4 days following treatment. Over the 4-day period, approximately 86% of the administered dose was excreted in the urine, with 67.5% excreted by this route on the first day. Approximately 11% of the administered dose was excreted in the faeces, with the majority excreted on the second day after dosing (6.7%). A small amount was excreted as expired gases. Beagle dogs were dosed with gelatin capsules containing 0.3 mg/kg bw radiolabelled chlorfenvinphos and maintained in metabolism cages. Over 4 days, approximately 89% of the administered radioactive dose was excreted in the urine, with 86% excreted on the first day. Only 4.5% of the administered dose was excreted in the faeces, with the majority of this (4.1%) excreted on the first day. A female dog given a large dose of unlabelled chlorfenvinphos (5.5 g) following a radioactive dose showed an increase in faecal excretion, with 35% of the administered dose excreted, mainly as unchanged chlorfenvinphos. Urine from a number of orally dosed dogs and rats was analysed for metabolites. O-Desethyl chlorfenvinphos formed 78% of excreted metabolites in dogs and 37% of excreted metabolites in rats. The metabolite forming the greatest percentage (47%) in rats was [1-(2,4' dichlorophenyl) ethyl -D-glucopyranosid]uronic acid. Other metabolites seen in both rats and dogs were 2,4-dichloromandelic acid and 2,4-dichlorophenylethanediol glucuronide. Dogs had a more rapid urinary excretion of chlorfenvinphos than rats. They also had an increased faecal excretion when challenged with very large doses (Hutson, Akintonwa & Hathway, 1967).

Chlorfenvinphos metabolism was investigated in Carworth Farm E rats following treatment with dieldrin for 12 days. The LD50 for chlorfenvinphos alone in control rats was 15.4 mg/kg bw, while that in rats receiving dieldrin and chlorfenvinphos was 157 mg/kg bw. Rats given a low dose (2.5 mg/kg bw) of chlorfenvinphos did not show major differences in urinary excretion related to pretreatment with dieldrin, although the excretion in the first 12 h and the total excretion over the observation period was increased. When rats were given a high dose (13.2 mg/kg bw), the differences were more marked, with only 29% of the administered dose excreted in the urine over 72 h in the controls and 74% excreted in the dieldrin pretreated rats. In the rats receiving dieldrin and a high dose of chlorfenvinphos, the proportion of de-ethylchlorfenvinphos increased. Dieldrin appeared to decrease the toxicity of chlorfenvinphos by increasing the liver metabolism of large

doses, resulting in higher levels of de-ethylchlorfenvinphos which was excreted in the urine (Hutson & Wright, 1980).

Chlorfenvinphos was administered by gavage to male New Zealand White rabbits for 5 days at 12 mg/kg bw/day. Chlorfenvinphos concentrations in body fat were assessed on days 1 and 3 after cessation of treatment. On the last treatment day, chlorfenvinphos levels were between 1.17 and 2.51 ppm. On the next day, levels were 0.07 to 1.02 ppm, while on the 3rd day after cessation of treatment they were between 0.07 and 0.28 ppm. The biological half-life of chlorfenvinphos in the rabbit was 1 day (Brown et al, 1964).

Radiolabelled chlorfenvinphos was administered orally to a male human volunteer, and the radioactivity excreted in the urine was measured. In the first 4 h 30 min after dosing, 72% of the administered dose was excreted. Over the 26-h observation period, 94% of the administered dose was excreted in the urine. Desethyl chlorfenvinphos made up 23.8% of the urinary material. 2,4-dichloromandelic acid made up 23.9% of the metabolites. In the subject used in this study, chlorfenvinphos was rapidly metabolised and excreted (Hutson, 1969 and Donninger, Hutson & Pickering, 1972, compared with results from other species.

A Friesian cow was dosed with 233 mg radiolabelled chlorfenvinphos by IM injection (approximately 0.6 mg/kg bw) and maintained in a metabolism cage. Excretion of radioactive material in the milk was measured for 8 milkings after dosing. In the first milking, 0.13% of the administered dose was excreted in the milk, with levels dropping to 0.04% by the second milking and lower levels after this. Initially 75% of the radioactive material was unchanged chlorfenvinphos, with metabolites similar to those seen in the rat and dog (Hutson, Hoadley & Donninger, 1969).

Chlorfenvinphos induced porphyria in primary liver cell cultures of chicken embryos, both with and without previous treatment of the cells with a drug enzyme inducing compound. The porphyrinogenic potential of chlorfenvinphos was markedly increased by treatment with an enzyme-inducing substance (Koeman, Debets & Strik, 1980).

Chlorfenvinphos was incubated with plasma and washed erythrocytes from guinea pigs, NZW rabbits, Beagle dogs, Hooded Lister rats, CF1 mice and a human volunteer. ChE activity in each of the samples was determined, and the concentration required to produce a 50% inhibition of ChE activity in blood was determined. The species, in decreasing order of sensitivity were: mice, rabbits, rats, humans and guinea pigs. Chlorfenvinphos was also incubated with liver slices from mice, rats and dogs, and ChE inhibition measured over a 30 min period. Chlorfenvinphos concentration was also determined. Over the incubation period, the concentration of chlorfenvinphos decreased by 38% in the rat, 30% in the mouse and 11% in the dog. ChE inhibition in the liver of the rat decreased over the period of metabolism, while in the mouse the inhibition peaked at 30 min. In the dog, there was an initial drop in inhibition, followed by a later rise; the increase in ChE inhibition associated with a drop in chlorfenvinphos concentration seen in dogs was not explained (Brown, 1964).

Radiolabelled chlorfenvinphos was incubated with liver homogenates from male rats, mice, rabbits and a dog. The only major metabolite present in each species was de-ethyl chlorfenvinphos, which was produced with relative reaction rates of: rats, 1; mice, 8; rabbit, 24; and dog, 88. This is comparable to the oral LD50 in these species (10 mg/kg bw, 100 mg/kg bw, 500 mg/kg bw and >12000 mg/kg bw respectively. A male rabbit was dosed with radiolabelled chlorfenvinphos by gavage and maintained in a metabolism cage. The rabbit excreted 65% of the administered dose in

the first 2 days after dosing, with the major metabolite the de-ethylation product. A homogenate of rabbit liver was prepared, and it was determined that the de-ethylase enzyme was associated with the microsomal fraction of the liver, but required co-factors in the supernatant for activity. The enzyme, while most active at physiological pH tolerated a wider range of temperatures (Hutson, 1970).

Chlorfenvinphos was applied to sheep either in a dip or in a spray race at the recommended application rate. Sheep were then examined for tissue residues 3, 7, 14 or 21 days after treatment. The highest mean chlorfenvinphos levels of 0.043 ppm were seen in omental fat 3 days after treatment with a 0.1% dip treatment. After 21 days, the mean levels in omental fat were 0.005 ppm, while in organs the levels were below the limit of detection (0.003 ppm) (Robinson, Malone & Bush, 1966).

Mitochondria were prepared from rat cerebral hemispheres, and the effect of chlorfenvinphos on mitochondrial respiration was determined. State 3 respiration was decreased by chlorfenvinphos in a concentration-dependent fashion. Cytochrome oxidase activity was not consistently altered by chlorfenvinphos, however the ADP/O ratio was decreased in a dose related manner. Succinate dehydrogenase activity was slightly decreased by increasing chlorfenvinphos concentrations (Sitkiewicz et al, 1978).

Liver homogenates of Wistar rats, New Zealand White rabbits and human accident victims were prepared, and microsomal fractions isolated. Radiolabelled chlorfenvinphos was incubated with the microsomal proteins, and the de-ethyl chlorfenvinphos metabolite was isolated and quantified. The rate of de-ethylation in rabbits was 6 - 7 times that in rats, while in humans the rate was around 4 times that of rats (Hutson & Logan, 1986).

Male Fischer 344 rats given chlorfenvinphos by gavage showed clinical signs consistent with organophosphorus poisoning. The LD50 in this strain was 34.6 mg/kg bw. When rats were pretreated with 15 mg/kg bw chlorfenvinphos, the LD50 when measured 8 h later was slightly increased, and at 24 h later was three times the previously determined LD50. Brain ChE activity was maximally inhibited 4 h after a challenge dose, with 75% inhibition, which recovered over the next 48 h to approximately 35% inhibition. Pretreatment with chlorfenvinphos produced lower ChE activity at the time of administering the challenge dose, however the overall inhibition after the challenge dose was less than in rats which had not been pretreated. Plasma ChE activity was not significantly changed by pretreatment with chlorfenvinphos. The oral LD50 of diazinon was decreased by pretreatment with chlorfenvinphos, while the IV LD50 of oxotremorine was increased (Ikeda et al, 1990).

Rats were orally dosed either with chlorfenvinphos or phenobarbital 24 h before euthanasia. Liver and kidney homogenates were prepared immediately after euthanasia, and the supernatant and microsomal fraction isolated. The liver fractions were incubated with chlorfenvinphos. Liver metabolism was negligible without the addition of an NADPH-generating system. Metabolism in rats pretreated with chlorfenvinphos was approximately twice that of controls, while rats pretreated with phenobarbital metabolised at approximately 5-times the control rate. Little chlorfenvinphos metabolism occurred in the kidneys. Cytochrome P450 content in the liver was increased approximately 30% by chlorfenvinphos pretreatment and around 80% in phenobarbital pretreated rats. Chlorfenvinphos pretreatment did not change the phenobarbital sleeping times of rats (Ikeda et al, 1991).

Rats were treated with chlorfenvinphos 24 h followed by either a second chlorfenvinphos dose, or tissue sampling to determine chlorfenvinphos levels. Following the challenge dose, samples of blood, brain and liver were removed. ChE activity in the brain, and chlorfenvinphos concentration in the brain liver and plasma were determined. The unbound fraction of chlorfenvinphos in plasma and liver was also determined. Chlorfenvinphos pretreatment increased the IV LD50 from 6.51 to 9.15 mg/kg bw, and the oral LD50 from 34 to 106 mg/kg bw. The brain ChE inhibition in pretreated animals was less than in control animals following an oral challenge dose. Plasma concentration following an IV dose of chlorfenvinphos was not changed by pretreatment, although liver concentration was decreased. Plasma and liver chlorfenvinphos concentrations were decreased by pretreatment following an oral challenge dose. Brain chlorfenvinphos levels were proportional to plasma levels, regardless of administration route (Ikeda et al, 1992)

Acute Toxicity

There was an obvious species difference in the oral toxicity of chlorfenvinphos with the rat being the most sensitive. The decrease in species sensitivity was: rat (oral LD50 ranging from 9.7-39 mg/kg bw), mouse (117 mg/kg bw), guinea pig (125 - 500 mg/kg bw), rabbit (300 mg/kg bw), dog (>5000 mg/kg bw). Reported signs included: salivation, lacrimation, diarrhoea, tremors and convulsions. These are considered to be typical signs of organophosphorous poisoning (ie responses to increased cholinergic activity). There was no sex-related difference in sensitivity. The sensitivity of the rat to chlorfenvinphos in the various formulations was similar. Compared with the toxicity of the active ingredient, chlorfenvinphos formulations were equivalent or in some cases slightly more toxic. Chlorfenvinphos was highly toxic in the rat following dermal (LD50 30 mg/kg bw) or inhalational (133 mg/m^3) exposure. Chlorfenvinphos technical was not irritating to the eye, although a formulation left in contact with the rabbit eye over an extended period caused severe irritation. Chlorfenvinphos was a weak skin sensitiser.

Atropine sulphate alone, or in combination with oximes was a useful antidote for acute chlorfenvinphos poisoning.

Short-Term Repeat-Dose Studies

Chlorfenvinphos was administered to mice in the diet for 2 weeks at doses of 0, 1, 10, 100, 1000 or 3000 ppm. There were no deaths or treatment-related clinical signs. Body weight was reduced in high-dose animals, along with a slight decrease in food consumption. Plasma ChE was inhibited at >25% from 10 ppm, erythrocyte ChE was inhibited at >25% from 100 ppm and brain ChE was inhibited from 1000 ppm, all in a dose-related manner. There were no abnormalities on histopathological examination. No effects were seen at 1 ppm, equal to 0.2 mg/kg bw/day (Tennekes, 1989).

Chlorfenvinphos was fed in the diet to NMRI mice for 4 weeks at doses of 0, 1, 10, 100 or 1000 ppm. There were no treatment related clinical signs observed, and ophthalmoscopic examination was normal. Plasma ChE was inhibited from 10 ppm while erythrocyte ChE was inhibited from 100 ppm. Brain ChE was inhibited in females by 52% at 1000 ppm and by 24% - 29% at all doses. Hence, effects were seen at all dose levels, with brain ChE inhibition in females at 1 ppm (0.21 mg/kg bw/day) and above (Tennekes et al, 1991).

Chlorfenvinphos was fed in the diet to Wistar rats at 0, 0.3, 1, 3 or 30 ppm for 4 weeks. There were no mortalities or abnormal clinical signs during this time, and no changes in body weight or food

consumption related to compound composition. Plasma, erythrocyte and brain ChE were inhibited at the highest dose, with the inhibition in females greater than that seen in males. No inhibition was seen at lower doses. Aliesterase activity in plasma and liver was inhibited from 3 ppm, with inhibitions of 36% in plasma and 30% in liver. Animals maintained on the control diet for 4 weeks after dosing showed no inhibition of ChE or aliesterase. No effects were seen at 1 ppm, equivalent to 0.05 mg/kg bw/day (Pickering, 1978).

Wistar rats were maintained on optimal (26% protein) or low (4.5%) protein diets containing 0, 5, 100 or 1000 ppm chlorfenvinphos for 11 or 31 days. Unspecified signs of toxicity were seen in rats at 1000 ppm in the first 5 - 8 days of the trial. Food consumption was decreased to day 5 at 100 and 1000 ppm. Body weight in rats on 1000 ppm decreased, with the decrease more marked in animals on 26% protein than on 4.5% protein. In the recovery period, the body weight began to return to normal, however the recovery was relatively slow. Gross pathological examination showed congestion of the heart, spleen and kidney at 100 and 1000 ppm. There was no determination of ChE activity in this trial. No effects were seen at 5 ppm in the diet, equivalent to 0.25 mg/kg bw/day (Puzynska, 1984a).

Chlorfenvinphos was fed to Wistar rats on an optimal (26% protein) or low (4.5%) protein diet at doses of 0, 5, 100 or 1000 ppm for 11 or 31 days. Plasma ChE activity was inhibited 60% - 80% at the 2 highest doses. Inhibition of plasma ChE was also seen in males on the optimal diet at 5 ppm in the diet, with an inhibition of 28% in comparison to controls. Brain ChE activity was significantly inhibited at 100 ppm, but no inhibition was seen at 5 ppm. After a recovery phase, males on the low protein and females on the optimal diet showed plasma ChE inhibition, with other rats being normal. Inhibition of plasma ChE was seen at 5 ppm and above in this study (0.25 mg/kg bw/day) (Puzynska, 1984b).

Dogs were maintained on chlorfenvinphos at 0, 3, 100 or 3000 ppm in the diet for 4 weeks. There were no deaths or treatment-related clinical signs. Bodyweights were not affected, and no changes were noted on ophthalmoscopic examination. Plasma ChE activity was inhibited from 100 ppm, erythrocyte ChE activity from 3000 ppm, while brain ChE activity was not affected by treatment. There was a slight increase in absolute and relative liver weights in high-dose dogs. No effects were seen at 3 ppm, equal to 0.12 mg/kg bw/day based on plasma ChE inhibition (Allen et al, 1992).

Dogs were fed chlorfenvinphos at a range of doses over an 8-week feeding period, with each dose used for a maximum of 2 weeks. Plasma ChE inhibition was seen from a dose of 16 ppm, while erythrocyte ChE inhibition was only seen from 1000 ppm. There was no brain ChE inhibition at any dose (Allen et al, 1992).

Chlorfenvinphos was applied dermally to the shorn dorso-lumbar region of male “P” strain guinea-pigs at 1, 10 or 100 mg/kg bw/day for 14 days. It was estimated (from a graph) that there was inhibition of plasma ChE activity of more than 20% at all doses from approximately day 4. The maximum inhibition was approximately 90% in the high dose group on day 15. In the lower two doses, there was recovery to less than 20% inhibition by the final week of the trial. The experiment was then repeated using doses of 0.01, 0.1 or 1 mg/kg bw/day. The lower two doses produced plasma ChE inhibition of 15%, which was not considered significant. Based on the inhibition of plasma ChE seen at 1 mg/kg bw/day, the NOEL for this dermal study was 0.1 mg/kg bw/day (Brown, 1965).

Chlorfenvinphos (50% seed dressing formulation) was applied to the clipped dorso-lumbar region of "P" strain guinea-pigs and New Zealand White rabbits, with application made 5 days/week for 23 applications. Guinea-pigs received 0.5 g of formulation while rabbits were treated with 1.0 g formulation. Skin reaction was assessed daily. Guinea-pigs showed very mild skin irritation in the second week of the trial, and rabbits did not show any irritation. The formulation was determined to be non-irritating to rabbit and guinea-pig skin (Stevenson, 1974a).

Chlorfenvinphos (granular formulation) was applied to clipped dorso-lumbar region of "P" strain guinea-pigs and New Zealand White rabbits, with application made 5 days/week for 23 applications. Guinea-pigs received 0.5 g of formulation while rabbits were treated with 1.0 g formulation. The material was moistened prior to application. Skin reactions were assessed after 1, 2, 3 and 9 weeks, and there was no irritation seen at any time. The formulation was determined to be non-irritating to non-occluded rabbit and guinea-pig skin (Stevenson, 1974b)

Beagle dogs were fitted with two collars for a 14-day period. Dogs wore two control collars, a control collar and a collar containing 15% chlorfenvinphos or two collars containing 15% chlorfenvinphos. The collars were stapled together to ensure simultaneous contact with the fur for both collars. Plasma ChE activity was determined prestudy, on 4 occasions during the study and up to 17 days after exposure. There were no abnormal clinical signs or signs of dermal irritation associated with the collar use. Plasma ChE activity was significantly inhibited during and following the use of either one or two collars, with inhibition of more than 30% present 17 days after the removal of the collar (Zeman & Johnston, 1980).

Chlorfenvinphos was administered by SC injection to female Wistar rats at doses of 0 or 1.85 mg/kg bw/day for 28 days. Body weight in treated females decreased in comparison to controls by day 20, with a 17% decrease seen in the latter part of the trial. ChE activity in the liver was decreased relative to control, with the microsomal fraction most affected (Gajewski, 1980).

Chlorfenvinphos was administered by IP injection to male Wistar rats at 0, 1 or 3 mg/kg bw/day. Body weight was not decreased over the trial. In rats assessed for activity using a rotating wheel, there was a decrease in activity in high dose rats on days 1 to 5; no other changes were seen. Following a challenge dose of 3 mg/kg bw given 7 days after the last day of dosing there was a decrease in activity in low-dose and control rats, but no observed change in the high-dose rats. Plasma and erythrocyte ChE was inhibited by the lowest dose, with inhibition of 45 - 65% in plasma and 45% in erythrocyte ChE activity. Plasma ChE activity in this group returned to normal over the recovery period, while erythrocyte ChE remained significantly inhibited in all treated rats. Inhibition following the challenge dose was marginally higher in high dose rats than in low dose. Brain ChE activity was also significantly inhibited throughout the treatment period, with 30% inhibition in the low dose group and 80% inhibition in the high dose group (Luczak, Gralewicz & Gorny, 1992).

Subchronic Toxicity

Chlorfenvinphos was fed in the diet to Wistar rats at doses of 1, 3, 10, 30, 100 or 1000 ppm for 12 weeks, after which 5 rats/sex/group were maintained on control diet for 5 weeks. No individual animal data or detailed summary data were supplied. No effects on mortality or food consumption were reported. Growth was depressed in both sexes at 1000 ppm. Plasma and erythrocyte ChE activities were significantly depressed at 30, 100 and 1000 ppm, although the degree of depression was not reported. Sporadic decreases were also seen at 10 ppm. In females, absolute spleen and kidney weights at 30 ppm were decreased; this resolved in animals maintained on control diet for 4

weeks. Based on the sporadic inhibition of plasma and erythrocyte ChE activities at 10 ppm, the NOEL was 3 ppm in the diet, equivalent to 0.15 mg/kg bw/day (Ambrose et al, 1970).

Wistar rats were fed chlorfenvinphos in the diet at 0, 3, 10, 30, 100 or 1000 ppm for 12 weeks, with 5 rats/sex/group maintained on the control diet for 4 weeks after the feeding period. Muscle fasciculations, tremors and bloody discharge from the nose and eyes were seen in high-dose rats. Body weight was decreased in animals on 1000 ppm, with decreases of 17% - 20% in females and 12% - 22% in males. Body weight was also decreased 10% - 12% in males on 100 ppm. Plasma ChE activity was inhibited 47% - 62% in females and 10% - 45% in males on 10 ppm. Erythrocyte ChE activity was inhibited at 30 ppm (25% - 35%) in both sexes, and in females on 10 ppm. Histopathological examination was limited, however one instance of testicular atrophy in a high-dose male was seen. The NOEL was 3 ppm, equivalent to 0.15 mg/kg bw/day (Anon, 1963b).

Chlorfenvinphos was fed to Wistar rats at 0, 1 or 3 ppm in the diet for 12 weeks. No individual animal results were reported for this study, however it was stated there were no treatment related effects on mortality, body weight or food consumption. No abnormal clinical signs were reported. Plasma ChE activity was decreased at 3 ppm throughout the study, with a mean inhibition in females of 22% and in males or 24%. No inhibition of plasma ChE activity was seen at 1 ppm, or of erythrocyte ChE activity at either dose. There were no abnormal findings on histopathological examination. Based on plasma ChE inhibition, the NOEL was 1 ppm, equivalent to 0.05 mg/kg bw/day (Anon, 1963c)

Chlorfenvinphos was fed in the diet at 1, 10, 100 or 1000 ppm to mongrel dogs for 12 weeks, using 2/sex/group. One dog/sex/group was euthanised after 12 weeks, with the other maintained on control diet for 8 weeks. No individual animal results or detailed summaries were reported. There were no reported effects on mortality, food consumption or weight gain. Plasma ChE activity was statistically significantly inhibited at all dose levels, while erythrocyte ChE was intermittently depressed. There were no abnormal histopathological findings. Given the plasma ChE inhibition at all doses, no NOEL could be established for this study; the LOEL was 1 ppm, equivalent to 0.025 mg/kg bw/day, however given the lack of data replied the study was not considered suitable for regulatory purposes (Ambrose et al, 1970).

Chlorfenvinphos was administered in the diet to Beagle dogs at levels of 0, 0.5, 1 or 3 ppm for 16 weeks. No abnormal clinical signs were reported. Bodyweight appeared to be normal, although was not reported in detail. Haematological examination was normal, and there were no abnormal histopathological findings. No inhibition of plasma, erythrocyte or brain ChE activity was seen. The NOEL for the study was 3 ppm in the diet, equivalent to 0.075 mg/kg bw/day based on the absence of any abnormal signs (Walker, 1965).

Chronic Toxicity

Chlorfenvinphos was fed in the diet to NMRI mice at 0, 1, 25 or 625 ppm for 90 weeks (females) or 104 weeks (males). There were no treatment-related clinical signs, and no effect on bodyweight. Food consumption was decreased in all treated females from weeks 8 to 52. Plasma ChE was inhibited from 25 ppm, while erythrocyte and brain ChE were inhibited at 625 ppm. There were a number of histopathological changes in the adrenal cortex of high-dose males, including focal hypertrophy and nodular hyperplasia. There was no increase in neoplastic lesions related to chlorfenvinphos treatment. Based on the plasma ChE inhibition seen at 25 ppm, the NOEL was 1 ppm (equivalent to 00.15 mg/kg bw/day) (Schmid et al, 1993).

Chlorfenvinphos was administered in the diet to Wistar rats at 0, 10, 30, 100 or 300 ppm for 2 years. Bodyweight in females at 100 and 300 ppm was decreased 12% - 19% from week 26. No bodyweight decreases were seen in males throughout the study. Plasma ChE activity was significantly decreased in both males and females from 10 ppm, while erythrocyte ChE activity was decreased from 30 ppm in males, with sporadic inhibition at 10 ppm. In females, erythrocyte ChE activity was significantly inhibited from week 26 to week 78 at 10 ppm. There were no treatment-related findings on histopathological examination. Based on the inhibition of plasma ChE activity in both sexes at 10 ppm, and the inhibition of erythrocyte ChE activity in females at 10 ppm, no NOEL could be established. The LOEL was 10 ppm (equivalent to 0.5 mg/kg bw/day) (Larson & Ambrose, 1965, Ambrose et al, 1970).

Chlorfenvinphos was administered in the diet to Wistar rats at 0, 0.3, 1, 3 or 30 ppm for 2 years. There were no treatment-related clinical signs observed, and no increase in mortality related to treatment. Body weight and food consumption were not affected by treatment throughout the study. There were no significant effects on haematology or clinical chemistry parameters. Plasma ChE activity was significantly inhibited in females from 3 ppm, and in males from 10 ppm. Erythrocyte ChE activity was inhibited in both sexes at 30 ppm, as was brain ChE activity. There were no abnormal findings related to treatment on gross postmortem examination, and no treatment related findings on histopathological examination. Histological examination of the sciatic nerve did not reveal any evidence of an acceleration of fibre degeneration, or an increase in the severity of degeneration related to chlorfenvinphos treatment. Based on the plasma ChE inhibition seen in females at 3 ppm, the NOEL was 1 ppm in the diet (equivalent to 0.05 mg/kg bw/day) (Pickering et al, 1980).

Chlorfenvinphos was fed in the diet to Beagle dogs at doses of 0, 30, 200 or 1000 ppm for 2 years. There were no treatment related effects on mortality, with all treated animals surviving until the end of the trial. Plasma ChE activity was significantly inhibited at all doses during the first 39 weeks; after this there was inhibition of plasma ChE activity at the top two doses. Erythrocyte ChE was significantly inhibited at the highest dose throughout the study. There were no abnormal gross or histopathological findings seen. Given the plasma ChE inhibition at the lowest dose, no NOEL could be established. The LOEL was 30 ppm in the diet, equivalent to 0.75 mg/kg bw/day (Larson & Ambrose, 1965 and Ambrose et al, 1970).

Reproductive Toxicity

Chlorfenvinphos was fed in the diet to Wistar rats at doses of 0, 30, 100 or 300 ppm for 3 generations with 2 litters bred per generation. Body weights of treated females were reported as being lower than controls; at 300 ppm the decrease was 11% in the F0 generation and 19% in the F1 generation. Plasma and erythrocyte ChE activities were reduced in rats at 30 and 100 ppm, however the reduction was not quantified. The fertility index (rats pregnant/rats mated) in the second generation was decreased at 100 and 300 ppm, and in the third generation (F2/F3) at 30 and 100 ppm, while there were no offspring in the 300 ppm group. As an additional test, F2b animals of both sexes on 30 ppm were cross-mated with control animals of both sexes. The fertility indices from these mating were reduced, with female control animals having a fertility index of 42% and treated females having a fertility index of 25%. The ratio of pups surviving to 5 days to pups born alive was decreased in F1a animals at 300 ppm, and in F1b animals at 100 and 300 ppm. In the F2 animals at 300 ppm, no pups survived to 5 days. There were no gross abnormalities noted in weanlings in any generation. Surviving F2b females examined histopathologically showed normal follicle formulation, corpora lutea and ova formation. In males, testes showed normal development; therefore there was

no explanation for the decreased fertility seen in animals in the test or cross-mated animals. No NOEL could be established, given the decreased fertility index and plasma and erythrocyte ChE inhibition at the lowest dose tested. The LOEL was 30 ppm, equivalent to 1.5 mg/kg bw/d (Ambrose et al, 1970).

Chlorfenvinphos was fed in the diet to Long-Evans rats at doses of 0, 1, 5 or 15 ppm for 3 generations. There were no changes in appearance or behaviour in treated groups in comparison to controls. ChE activity was not determined in this study. There was no effect seen on the litter sizes or mean survival of litters from the rats which became pregnant. The fertility index was slightly reduced with chlorfenvinphos treatment at the 2 highest doses with the mean fertility index being 98%, 98%, 93% and 92%. This was not considered biologically significant. The mean survival of pups was not affected by treatment, nor was the mean weight of weanlings. There were no gross or histopathological abnormalities detected on examination of the adults or of weanling rats. Based on the absence of effects on reproduction, the NOEL for this study can be set at 15 ppm in the diet, equivalent to 0.75 mg/kg bw/day (Eisenlord et al, 1967).

In a preliminary study, chlorfenvinphos was fed in the diet to Wistar rats at 0, 5, 25 and 125 ppm for one generation. No test article-related clinical signs were noted in any group and there were no deaths. Chlorfenvinphos treatment did not statistically significantly affect any mating parameters (mating index, time to conception, conception rate, or gestation indices), although the conception index (pregnancies/matings) was decreased by 10% in all treated groups. This was not considered significant as there were only 10 animals/group. The implantation numbers in all treated groups in comparison to controls were decreased when the non-pregnant animals were considered as having no implantations. There was no effect on post-implantation loss; the birth indices were similar for all groups. A slightly reduced mean pup number was recorded for the mid-dose group due to a low number of pups for one female; other groups were unaffected. There was significant plasma ChE inhibition in females from 25 ppm, while no inhibition of plasma ChE was seen in males at any dose. Erythrocyte ChE was inhibited in males from 25 ppm, and in females at all doses ($p<0.01$). Brain ChE activity was inhibited in males from 125 ppm and in females from 5 ppm. Based on the inhibition of erythrocyte ChE activity, and the statistically significant inhibition of brain ChE in females at 5 ppm, no NOEL was established in this study. The LOEL based on ChE inhibition was 5 ppm (0.4 mg/kg/day). For pups the NOEL was 25 ppm (approximately 2.5 mg/kg bw/day), based on reduced bodyweight gain during lactation only (ChE was not measured for F1 pups) (Dotti et al, 1993a)

In a 2-generation reproduction study, chlorfenvinphos was fed to Wistar rats at 0, 1, 10 or 100 ppm in the diet for 2 generations. Body weight gain was decreased in high-dose males during premating and in high-dose females during gestation. In the second generation, there was an increase in the mean pre-coital interval in the high dose group. The fertility index was slightly decreased at the high dose, while the birth index (pups born/implantations) was significantly decreased in both the mid- and high-dose in the first generation. The weaning index (pups alive on day 21/pups alive on day 4) was significantly lower in the mid- and high-dose groups in the first generation, and in the high-dose group in the second generation. There was statistically significant inhibition of plasma ChE in both males and females on 10 ppm. Erythrocyte ChE was inhibited at 100 ppm, while brain ChE was statistically significantly inhibited in females on 10 ppm. The NOEL for parental toxicity was 1 ppm (0.05 mg/kg bw/day), based on reduced food consumption and reduced plasma and brain ChE activity at 10 ppm. The NOEL for foetal toxicity and toxic effects on pups was 1 ppm (0.05 mg/kg bw/day), based on increased post-implantation losses at 10 ppm and 100 ppm and reduced

bodyweight gain and increased postnatal losses during lactation at 100 ppm (ChE was not measured for F1 or F2 pups) (Dotti et al, 1993b).

Developmental Toxicity

Chlorfenvinphos was administered to Charles River rats at doses of 0, 0.3, 1 or 3 mg/kg bw/day from days 4 - 15 of gestation. There were no abnormal clinical signs seen, and no consistent reduction in food consumption or body weight was observed. Pregnancy rate was comparable between groups, and there were no treatment-related effects on pre- and post-implantation loss, litter size or fetal weight. There was a non-significant dose-related decrease in the percentage of male foetuses (51.8%, 51%, 50.2% and 46.9%). There was no evidence of any significant effect on embryonic or foetal development, and there were no increases in malformations or visceral or skeletal variants. The NOEL for foetal and maternotoxicity was 3 mg/kg bw/day (Mayfield & John, 1986).

Chlorfenvinphos was administered to pregnant Dutch banded rabbits orally at doses of 0, 25, 50 or 100 mg/kg bw/day from days 6 - 18 of gestation. One rabbit in the 50 mg/kg bw/day group showed salivation and tremors shortly after dosing; no other animals showed clinical signs throughout the trial. A number of rabbits in the control and mid-dose groups aborted foetuses, however on post mortem examination these dams showed signs of enteric disease, thought to be the cause of the abortions. There was significant plasma and erythrocyte ChE inhibition in all dose groups, with the inhibition at 25 mg/kg bw being 37% in plasma and 55% in erythrocytes. There was a non-significant decrease in the number of live foetuses in the high dose group, and the percentage of male foetuses was also slightly decreased with increasing doses of chlorfenvinphos. The mean length of foetuses was increased in the high dose group (8.3, 8.1, 8.2, 8.6). There was an increase in the incidence of hydrocephalus with treatment (0%, 3.33%, 2.08%, 5.56%). This incidence is markedly above the historical control value for this laboratory (0.3%). Given the inhibition of plasma and erythrocyte ChE, and the increased incidence of hydrocephalus at the lowest dose, no NOEL can be set for this study. The LOEL was determined to be 25 mg/kg bw/day (Dix et al, 1979).

Chlorfenvinphos was administered to pregnant rats, rabbits and hamsters either as a single oral dose on day 8, 9 or 10 or gestation, or as 3 doses between days 6 and 12 of gestation. Some effects on survival and foetal weight and length were seen, however the study was not suitable for regulatory purposes, due to the lack of detailed reporting (Dzierzawski & Minta, 1979)

Genotoxicity

Chlorfenvinphos did not produce any genetic changes in a number of studies using *Escherichia coli*, *Salmonella typhimurium* or *Saccharomyces cerevisiae*, either with or without metabolic activation. No chromosomal damage was produced in a study using bone marrow cells of Chinese hamsters, at doses of 0, 25 or 50 mg/kg bw, and there was no effect in a dominant lethal assay in male mice treated with 0, 10, 20 or 40 mg/kg bw. Based on these findings chlorfenvinphos was not considered to be genotoxic.

Special Studies

Chlorfenvinphos was administered to White Leghorn hens at doses of 0, 100, 150, 200 or 300 mg/kg bw/day, while additional hens received 100 or 200 mg/kg bw/day with 1 mg/kg bw atropine for 10 days, followed by a 20-day observation period. After injection, hens showed

salivation, retching and an inability to stand which were not prevented by atropine treatment. Deaths occurred at all doses, with no high-dose animals surviving the 10-day treatment period. Survivors did not show neurotoxic signs. Histopathological examination showed no sign of demyelination or other neural toxicity (Ambrose et al, 1970)

Female hens were given a single oral dose of chlorfenvinphos by gelatin capsule at 40 mg/kg bw. Tri-ortho-cresyl phosphate was used as a positive control; negative controls received empty capsules. Birds were euthanised 24 and 48 h after dosing, with 10/group maintained for 21 days observation. There were no signs of delayed ataxia in any chlorfenvinphos treated birds; 6 birds in the positive control group developed ataxia. Brain and spinal cord ChE activity was decreased following chlorfenvinphos treatment, while no effect was seen on NTE. Positive controls showed decreased NTE levels. There was no evidence of delayed neurotoxicity on histological examination of chlorfenvinphos-treated birds (Redgrave & Cameron, 1996).

Chlorfenvinphos, fenchlorphos and a mixture of both was administered orally to mature hens on two occasions 21 days apart. Atropine and P-2-S were administered by IM injection as required to counteract the acute poisoning effects. Hens were observed for 21 days following each treatment, and were euthanised 21 days after the second dose. There were no gait abnormalities observed. Histopathology examination was not reported (Wallwork & Malone, 1974b).

Chlorfenvinphos at unspecified doses was administered to White Leghorn chickens premedicated with atropine sulphate. No paralysis was observed (Gaines, 1969).

Male Wistar rats were given chlorfenvinphos orally, and the erythrocyte and brain ChE activity was determined. Erythrocyte ChE was inhibited 80% 3 h after a dose of 4 mg/kg bw. Brain ChE was maximally inhibited 38% 3 h after a dose of 2 mg/kg bw, while a 4 mg/kg bw dose produced 82% inhibition at this time, and 33% inhibition after 72 h. Rats were also prepared for EEG and EMG examination. Recordings were made for 8 h/day, and the sleep patterns classified into 4 stages. A dose of 1 mg/kg bw chlorfenvinphos did not affect the sleep patterns, however there was a dose-dependent increase in wakefulness and decrease in slow-wave and parasleep at dose of 2 mg/kg bw and higher. Sleep patterns returned to normal on day 2, and there was a rebound increase in parasleep on days 3 and 4. Atropine treatment 2 h after exposure resulted in an earlier return to normal sleep patterns. The mechanism of these effects was not characterised (Osumi et al, 1975).

Chlorfenvinphos administered to anaesthetised rats at unspecified doses produced a rise in blood pressure and respiratory failure, antagonised by toxogonin or large doses of atropine. 2-PAM did not antagonise these effects. Neuromuscular transmission in rat gastrocnemius muscle was increased by chlorfenvinphos. The contractile response of guinea-pig ileum to acetylcholine or electrical stimulation were increased by low doses of chlorfenvinphos. This article was only submitted in abstract (Kaga et al, 1973).

Chlorfenvinphos was given either IV or PO to rats to compare the effects of direct ChE inhibitors (chlorfenvinphos and dichlorvos) and indirect ChE inhibitors (diazinon and fenthion). Rats given lethal IV doses of the indirect ChE inhibitors showed tonic convulsions and opisthotonos with only slight ChE inhibition. There were also differences in the cardiorespiratory and EEG responses between diazinon and chlorfenvinphos-treated rats. The lethality of the direct ChE inhibitors was considered to be related to ChE inhibition, while that of the indirect inhibitors may be independent of ChE inhibition (Takahashi et al, 1991).

Chlorfenvinphos was fed in the diet at 0 or 150 ppm to Sprague Dawley rats for 12 months. Muscle action-potential amplitude was measured in treated and control animals prior to treatment and at 3-monthly intervals. Blood and plasma ChE activity was determined at 3 and 6 months. Treated rats had decreased body weights, and ChE activity of less than 50% of control animals. On EMG examination, there were late action-potentials, including prolonged negative potentials and repetitive activity, with these effects increasing over the dosing period. Repetitive activity was decreased in chlorfenvinphos treated animals following a double stimulus (Maxwell & Le Quesne, 1982).

The effect of simultaneous exposure of rats to vibration, noise and chlorfenvinphos on the synthesis and content of ChE in brain neurons was investigated. Vibration and noise decreased acetylcholine levels to less than 50% of untreated controls, while the rate of synthesis increased to 120% of controls. Noise alone had no effect. Chlorfenvinphos alone increased acetylcholine levels to 180% of controls and the decreased rate of synthesis to 50% of controls. When chlorfenvinphos was administered in conjunction with either noise or noise and vibration, the levels of acetylcholine and rate of synthesis were similar to those in untreated controls (Gorny & Miszcak, 1981).

Chlorfenvinphos was administered PO to male Wistar rats at 6.2 mg/kg bw, and the rats killed within 24 h of dosing. Corticosterone and aldosterone levels were determined, as were blood and brain ChE levels Both corticosterone and aldosterone levels were increase at 1 h after treatment, with aldosterone levels returning to normal over 24 h and corticosterone levels returning to normal after 6 h. Brain ChE activity was comparable to controls after 1 h, but was decreased to 10% of controls at 2 h; levels had returned to 50% of controls at 24 h. Blood ChE was 50% of control values after 1 h, and remained at this level for 24 h (Osicka-Koprowsk et al, 1984).

The acute oral and dermal LD50 of chlorfenvinphos was determined to be 14.2 and 33.6 mg/kg bw respectively in female Wistar rats. Rats were then treated with 3/4 of this dose, and killed at intervals to determine blood, liver and brain ChE activity. Following oral dosing, inhibition of ChE activity was seen in the hypothalamus, cerebral cortex, erythrocytes, liver and muscle within 15 min, while there was inhibition in the pontomedullary area after 30 min. At 72 h, there was still inhibition in the brain, while levels in the erythrocytes, liver and muscle had returned to normal. Following dermal treatment there was marked inhibition of all tested tissues within 15 min, with inhibition persisting until 72 h except in erythrocytes (Kisielinski et al, 1980).

Chlorfenvinphos was administered to male Wistar rats by IP injection, and ChE activity in plasma, erythrocytes and brain were determined. Behavioural studies, including an open-field trial and a maze trial were done. Plasma ChE was inhibited for 3 h after a dose of 1 mg/kg bw, and for 48 h after a 3 mg/kg bw injection. Erythrocyte ChE remained inhibited for 48 h after 3 mg/kg bw. Brain ChE activity was inhibited for 24 h by the low dose, and for 14 days by a dose of 3 mg/kg bw. Clinical signs were seen in high dose rats, including slowness of movement. Locomotor and exploratory behaviour were decreased in the open-field test in high-dose rats for the first 5 days after treatment, while there was a decrease in choices made in the maze for the first 3 days following dosing at 3 mg/kg bw. Additionally, responses to the introduction of a novel object to the open field was reduced in chlorfenvinphos-treated animals. While most of these changes may have been related to the acute physiological effects of chlorfenvinphos, the decrease of response to a novel object was not, and may have been related to a change in the brain function in these rats (Socko, Gralewicz & Gorny, 1989).

Chlorfenvinphos was administered to male Wistar rats by IP injection at 1 or 3 mg/kg bw. Rats were tested on reactivity to a hot-plate before and after electric shock treatment on day 18 and 19

following treatment, when it was assumed ChE activities would be near-normal. Abnormal clinical signs were seen in the high-dose rats, with motor deficits persisting for 3 days after treatment. On the first exposure to the hot plate there was no observed difference between treated and control rats. Following shock treatment, the latency period from being put on the hot-plate to foot-licking was increased by 50% in the high-dose group in comparison to controls. On the second day of treatment, the latency period prior to foot-licking was longer both before and after the shock treatment in both treated and control rats. The results suggested an increase in stress-induced analgesia, with a slower dissipation of stress effects in treated rats (Gralewicz & Socko, 1990).

Male rats were given chlorfenvinphos by IP injection for 2 weeks, and killed at intervals after treatment. Plasma and erythrocyte ChE activity was determined, as was ChE activity in a number of brain areas. Hippocampal and neocortical EEG recordings were made, initially to determine baseline levels and then in association with a sporadic stimulus on days 39 - 41 after treatment. In the middle session, the stimulus was associated with an electric shock. Plasma ChE activity was inhibited at 3 h after treatment, and had recovered by 4 days. Erythrocyte ChE activity was inhibited for 14 days, while brain ChE was inhibited for 35 days by a dose of 1 mg/kg bw. The EEG recordings indicated that high-dose rats were less aroused generally, but had more response to a threatening stimulus than other rats (Gralewicz et al, 1991).

The effect of chlorfenvinphos of neocortical seizure activity was investigated in rats. There were no effects on the duration of spontaneous epileptic bursts, or the hippocampal theta rhythm. The effect of cardiazol was slightly decreased when it closely followed treatment with chlorfenvinpos. It was suggested that chlorfenvinphos had an antagonistic action on cholinergic postsynaptic receptors (Gralewicz, Tomas & Socko, 1989).

The effects of chlorfenvinphos administration on brain noradrenaline levels in relation to ChE inhibition were investigated in Wistar rats. The changes in noradrenaline levels were found to be separate to the cholinergic action of pesticides. The correlation was with the structure and toxicodynamics of the pesticide tested, rather than with the degree of ChE inhibition (Brzezinski & Wysocka-Paruszerska, 1980).

Rats and rabbits were treated with IP injections of either chlorfenvinphos or physostigmine, and the effects on hippocampal and cortical EEG and flash evoked potentials in the occipital cortex were investigated. There was a prolonged decrease in cortical or hippocampal activity in rabbits on 50 mg/kg bw, while changes in rats were not as notable. There were changes in the flash evoked potentials in both rats and rabbits in the short term after treatment, however these effects only persisted for a short period of time (Tomas & Gralewicz, 1992).

Physiological changes in rats, rabbits and cats following chlorfenvinphos treatment were investigated. The pesticide produced a rise in blood pressure in rats, with respiratory failure and cardiac collapse within 10 - 15 min. Symptoms were abolished by atropine sulphate treatment. An adrenergic blocking agent reversed the effect on blood pressure, as did sectioning the spinal cord at C1. Rabbits also showed an increase in blood pressure and heart rate, as well as dyspnoea. Rabbits also showed congestion of the lungs with blood loss from the nose and mouth. A cat showed a decrease in blood pressure 30 min after treatment with chlorfenvinphos, with typical signs of OP poisoning and a decrease in respiratory rate. Atropine sulphate was effective in reversing these effects (Chambers & Reiff, 1965).

Chlorfenvinphos was administered to cats, and the effectiveness of glutathione, atropine sulphate and 2-PAM as antidotes were tested. The right eye of each cat was stimulated with light flashes, ERG and EEG readings recorded and the blood pressure monitored. The a-wave of the ERG was increased by low doses of chlorfenvinphos, while the b-wave was increased by high doses. The N-wave in the optic tract was decreased, as were the N and P waves in the lateral geniculate nucleus. N and P waves in the visual cortex were not affected. Erythrocyte ChE activity was decreased, while there were only marginal decreases in ChE activity of the retina, lateral geniculate nucleus and visual cortex. All antidotes tested were individually effective at reversing the ERG and EEG effects (Takeda, Tsukahara & Takaori, 1976).

In vivo pharmacological studies indicated that chlorfenvinphos did not interact with central muscarinic receptors in rabbits, either as an agonist or an antagonist (Gralewicz, Tomas & Socko, 1995).

Rabbits were treated twice with chlorfenvinphos by IP injection at a variety of doses over an interval of 80 to 90 days. Plasma and erythrocyte ChE activity, body temperature and EEG readings were monitored following each injection. Plasma ChE inhibition was seen from 22 mg/kg bw, persisting for at least 96 h at all doses. Erythrocyte ChE activity was also seen at all doses. No behavioural changes were seen at the lowest dose, with abnormal clinical signs seen from 33 mg/kg bw. Clinical signs had returned to normal by 24 h after injection. Body temperature decreased after the first injection, however this effect was not noted after the second injection. There was an increase in the immobility-related rhythmic slow activity in the hippocampus which was less pronounced after the second treatment (Gralewicz et al, 1988).

Chlorfenvinphos was administered to rabbits at 14 mg/kg bw for 2 weeks, and ChE activity and electrophysiological activity were determined. Plasma ChE activity was inhibited, with levels recovering by 2 days after the last treatment. Erythrocyte ChE activity inhibition did not return to normal until 3 weeks after the end of treatment. Brain ChE activity was still inhibited after erythrocyte ChE activity had normalised. There were no changes in passive hippocampal activity between treated and control animals. An acoustic stimulation produced a response of greater magnitude in treated rabbits than in controls, with the effect more marked when the stimulus was associated with a foot-shock. These results suggested that functional brain changes outlasted ChE depression (Gralewicz et al, 1990).

Human Studies

Male volunteers were given a daily oral dose of 3 mg chlorfenvinphos for 53 days. Urine samples were analysed on days 10, 24, 38 and 52 of exposure, and it was determined that the average excretion of desethyl chlorfenvinphos was approximately 4.7% of the administered dose. There were no reports of any abnormal clinical signs following this dose, which was estimated to be approximately 0.04 mg/kg bw. No monitoring of ChE levels was done (Hunter et al, 1972).

Chlorfenvinphos was administered to male volunteers in 2 trials, one involving oral exposure, the other dermal. In the first trial, the volunteer ingested 1 mg/kg bw chlorfenvinphos in a gelatin capsule. Blood samples were taken 1.5, 3, 6, 24, 48 and 120 h and 9, 19, 26 and 54 days after exposure. Plasma and erythrocyte ChE activity, and blood chlorfenvinphos levels were determined. An EEG was done prior to dosing and 4 h after dosing. There were no abnormal clinical signs following dosing, and the EEG pattern did not change. Plasma ChE was inhibited more than 50% for 19 days after dosing, while erythrocyte ChE was inhibited more than 40% for 6 h after dosing.

Chlorfenvinphos was detectable in the blood for 6 h after dosing. In the dermal study, a 24% emulsifiable concentrate formulation of chlorfenvinphos was applied to the forearm of the volunteer at 5 mg/kg bw, and covered with an occlusive dressing. The dressing was removed after 6 h, and the skin washed. It was estimated that of the 380 mg applied, no more that 120 mg was absorbed. Blood samples were collected 3, 6, 24 and 96 h and 8 and 18 days after dosing. There were no abnormal clinical signs observed. Plasma ChE was inhibited by 45% at 96 h, and was normal at 18 days. Erythrocyte ChE was not inhibited by this dose. Effects were seen at 1 mg/kg bw for an oral dose, and 5 mg/kg bw for a dermal dose, with a possible maximal absorption of 31%, based on the amount of chlorfenvinphos recovered from the applied dressing (Brown, 1966).

Chlorfenvinphos was formulated as an 80% emulsifiable concentrate, a 24% EC and a 25% wettable powder, and applied to the forearm of 9 healthy male volunteers at either 5 or 10 mg/kg bw and covered with an occlusive bandage for approximately 4 h. The absorbed dose varied between volunteers for each formulation, as did the extent of plasma ChE inhibition. There was no significant erythrocyte ChE inhibition seen, and no cardiovascular or EEG changes were noted. Where the applied chemical remained liquid, an irritant dermatitis was noted. For the 80% EC formulation (equivalent to 4 mg active/kg bw of active) no significant plasma ChE inhibition was seen at a dose of 5 mg/kg bw. Other formulations and/or doses resulted in inhibition of plasma ChE of up to 76% compared with baseline levels (Hunter, 1969).

Chlorfenvinphos in a 0.05% solution was tested for dermal toxicity using 2 healthy male volunteers. A pre-test blood sample was taken, then each volunteer immersed his hands, wrists and forearms in the solution. The volunteers immersed their hands every 2 - 5 min over a 7 h 30 min period, with a break in the middle of the day, and were permitted to wash their hands 4 h after the end of exposure. Blood samples were taken in the middle and at the end of exposure, and the following morning and afternoon. Plasma and erythrocyte ChE activity was determined. In both subjects, plasma ChE was 50% inhibited by the end of exposure, but had returned to normal by the next afternoon. No erythrocyte ChE inhibition or abnormal clinical signs were seen in either volunteer (Stevenson & Pickering, 1965).

Chlorfenvinphos was applied to paddy rice under normal conditions, using 3 groups of volunteers. One group applied a dilute liquid formulation (0.06% of a 24% EC formulation); the second group applied a 10% chlorfenvinphos granular formulation, while the 3rd group were controls. Workers did not wear any protective clothing or shoes. Blood samples were taken prior to the trial, then every morning of the trial and for 2 days at the end of the trial. Following the analysis of the pretrial samples, it was found no intergroup comparison could be made. Plasma ChE activity was inhibited in both groups applying chlorfenvinphos at the end of the first days work (81% inhibition in the first group, and 51% in the second group). The group applying the liquid formulation continued to show inhibition until day 3, while the group applying the granular formulation did not show any inhibition after the first day. Erythrocyte ChE was not significantly inhibited at any time (Blok, Mann & Robinson, 1977).

An epidemiological study of workers exposed to chlorfenvinphos was performed, comparing workers exposed for more than 5 years with those exposed for 2 years, and workers never exposed. Analysis of ChE activity, haematological parameters and EMG voltages was done. There were no significant differences in ChE activities between groups. It was determined that workers exposed for more than 5 years had lower EMG voltages in comparison to those exposed for 2 years or controls. This effect was reversible, with either improvements in industrial hygiene, or removing

the worker from the workplace effective at increasing the values to normal. A lowering of EMG voltage was also seen in workers assessed over 8 years in another study (Ottevanger, 1976).

Pesticide factory workers were examined using EMG. Workers exposed to organophosphorus compounds (including chlorfenvinphos) showed a low voltage EMG following supra-maximal ulnar nerve stimulation. The effect was reversible, with results returning to normal after a 3-week break from exposure. Workers with a low voltage EMG also had low conduction velocities in both fast and slow motor nerve fibres. Exposed workers had approximately a 10% decrease in conduction velocities in comparison to non-exposed workers (Roberts, 1976).

Pet groomers applying organophosphate flea products with no protective clothing and without regard to label directions reported signs consistent with organophosphate poisoning (Anon, 1988).

A poisoning case in a young female child presented with upbeat nystagmus in addition to the normally observed signs of organophosphorus toxicity. Treatment with atropine and pralidoxime chloride was effective, and the child was discharged 4 days after admission (Jay et al, 1982)

DISCUSSION

Metabolism and Toxicokinetics

Chlorfenvinphos was relatively rapidly excreted in the urine following oral administration in all species, with 86 - 89% of the administered dose excreted over 4 days in rats and dogs, and 94% of the administered dose excreted in a human volunteer over 26 h. Chlorfenvinphos was hydrolysed by plasma in both rats and dogs, with the metabolism rate much higher in dogs. Liver metabolism rates showed species variation with relative reaction rats of: rats, 1; mice, 8; rabbit, 24; and dog, 88. Tissue residues were low in sheep three days after dermal application of chlorfenvinphos.

Acute Toxicity

The acute toxicity of chlorfenvinphos shows a wide variation between species, with oral LD50s from 9.7 mg/kg bw in the rat to >5000 mg/kg bw in the dog. The clinical signs were typical of anti-ChE pesticides, and were similar in all species although occurring at differing dose levels. The dermal toxicity of chlorfenvinphos was also extreme, with an LD50 of about 30 mg/kg bw in rats. The dermal LD50 in the rabbit was around 400 mg/kg bw suggesting a similar range of species sensitivity as was seen in the oral studies. Chlorfenvinphos formulations showed similar oral toxicity; the dermal toxicity was varied by the formulation type, with granules or wettable powder having a lower toxicity than emulsifiable concentrates. The inhalation toxicity of chlorfenvinphos was also extreme, being 133 mg/m^3. The differences in species sensitivity may be related to differences in metabolism

Cholinesterase Inhibition

As ChE inhibition is a primary target for chlorfenvinphos, a summary of the NOEL findings for ChE inhibition in a range of repeat-dose studies is shown in the Table below. NOELs are presented for plasma, erythrocyte and brain ChE activity.

Table 1: Summary of doses (mg/kg bw/day) at which no inhibition of ChE activity following chlorfenvinphos administration was seen

Species	Duration	Plasma ChE	Erythrocyte ChE	Brain ChE
Mice	2 weeks	0.2	2	20
Mice	4 weeks	0.2	1.9	<0.2
Mice	2 years	0.15	3.7	3.7
Guinea pig	14 days dermal	0.1		
Rat	2 weeks IP	<1	<1	<1
Rat	4 weeks	0.15	0.15	0.15
Rat	4 weeks	<0.25		0.25
Rat	12 weeks	0.15	0.15	
Rat	12 weeks	0.15	0.5	
Rat	12 weeks	0.05	0.15	
Rat	1-generation	0.4	<0.4	<0.4
Rat	2-generations	0.05	0.5	0.05
Rat	2 years	0.05	0.15	0.15
Dog	4 weeks	0.12	3.9	105
Dog	12 weeks	<0.025		
Dog	16 weeks	0.075	0.075	0.075
Dog	2 years	<0.75	5	
Human	Single oral	<1	<1	
Human	Single dermal	<1.6	1.6	
Human	Single dermal	1.81	1.81	

There was no clear difference in binding affinity with plasma (a pseudo- or butyryl-ChE), erythrocyte or brain ChE (acetyl- or true ChE). There was considerable variation between studies, with brain ChE on occasions the most sensitive (as in the 4-week mouse oral study, or the least sensitive (16-week dog study). Other studies showed no difference in sensitivity between the different ChE activities.

Neurotoxicity and behavioural studies

There was no evidence for delayed neurotoxicity in hens following a single oral dose or a 10-day administration. No longer-term dosing studies were supplied. A 2-year rat study investigated neurotoxic effect in a limited light-microscopy examination of sciatic nerves. There was no evidence of a treatment related effect; it should be noted that the examinations were of a limited nature, although performed on a large number of animals.

Evidence for neurotoxic effects were seen in a range of special studies. Workers in contact with chlorfenvinphos were tested for alterations of EMG using nerves in the forearm. It was found that

there was depression of conduction velocity and EMG voltage in these subjects, even though their plasma ChE was within normal population limits. The voltages and conduction velocities normalised after the workers were removed from the work environment for a few weeks. There was no evidence of 'intermediate syndrome' associated with the limited reports of chlorfenvinphos poisoning received. The regulatory significance and validation of such studies is unclear.

There were alterations observed in rat behavioural studies following chlorfenvinphos administration. Rats tested on a hot-plate 18 or 19 days after a single oral dose of chlorfenvinphos showed altered reactions to pain and stress in comparison to controls. Rats treated with chlorfenvinphos also showed an altered response to the introduction of a new object in an open field trial. The hippocampal EEG of rats exposed to an acoustic stimulus was altered in treated rats, particularly after the stimulus was associated with an electric shock. This effect was seen at times where brain ChE activity had returned to normal levels.

Therefore there was evidence for altered neurological functioning associated with exposure to chlorfenvinphos. At this stage there is not clear evidence for any neurotoxic effect from chronic feeding studies. The submission of a detailed study, including a functional observation battery and detailed examination of neural tissue would be useful in assisting to resolve the issue.

Genotoxicity

There was no evidence that chlorfenvinphos had any genotoxic potential.

Reproduction and Development

There was evidence of decreased fertility, evidenced by a decreased pregnancy rate, at high doses, particularly in later generations of a multi-generation study. Decreased fertility was also seen when treated animals (both males and females) were mated with untreated controls in one study. There were also effects seen on pup survival after birth. Effects were only seen at doses producing ChE inhibition. The decreased fertility following treatment with chlorfenvinphos was seen without any histopathological evidence of abnormality in the reproductive organs of either sex and the possible mechanistic basis of these findings is unknown.

In another study using the same strain of rats, with a slightly less pure chlorfenvinphos formulation, a slight increase in the pre-coital interval in high-dose animals in the second generation was noted. This was not associated with a decrease in fertility. The relationship of this finding, if any, with changes in other reproductive parameters is unknown.

In a rabbit teratology study, there was evidence of an increased incidence of hydrocephalus at all doses tested. The lowest dose tested was 25 mg/kg bw/day, a relatively high dose for rabbits. There was no dose-response relationship, and so the toxicological relevance of this finding is unclear. In a rat teratology study, there was no evidence of malformations. An additional rabbit study conducted at lower doses would be useful to resolve this issue.

Carcinogenicity

There were no carcinogenic effects seen following treatment with chlorfenvinphos over 2 years in mice, rats or dogs.

Human Studies

Chlorfenvinphos produced signs of poisoning consistent with cholinergic effects. Epidemiological studies provided evidence for reversible changes in nerve conduction velocities. There were no reports of Intermediate Syndrome associated with poisoning with chlorfenvinphos.

NOEL considerations

Table 2: Summary of the NOELs determined for chlorfenvinphos

Study	NOEL (mg/kg bw/day)	LOEL and Toxic Effects
NMRI mice 2-week dietary	0.2	Plasma ChE inhibition at 1.9 mg/kg bw/day
NMRI mice 4-week dietary	<0.2	Brain ChE inhibition at 0.2 mg/kg bw/day, NOEL for plasma ChE inhibition - 0.2 mg/kg bw/day, for erythrocyte ChE inhibition - 1.9 mg/kg bw/day
NMRI mice 90/104-week dietary	0.15	Plasma ChE inhibition at 3.9 mg/kg bw/day, brain and erythrocyte ChE inhibition at 3.7 mg/kg bw/day
Wistar rat 31-day dietary	0.25	Decreased food consumption, congestion of heart, spleen and kidney at 5 mg/kg bw/day - ChE activity not determined
Wistar rats 31-day dietary	<0.25	Plasma ChE inhibition at 0.25 mg/kg bw/day (lowest dose tested) Brain ChE inhibition at 5 mg/kg bw/day
Wistar rat 4-week dietary	0.05	Aliesterase inhibition in plasma and liver at 0.15 mg/kg bw/day
Wistar rat 12-week dietary	0.15	Plasma and erythrocyte ChE inhibition at 0.5 mg/kg bw/day
Wistar rat 12-week dietary	0.15	Plasma ChE inhibition at 0.5 mg/kg bw/day
Wistar rat 12-week dietary	0.05	Plasma ChE inhibition at 0.15 mg/kg bw/day
Wistar rat 2-year dietary	<0.5	Plasma ChE in both sexes and erythrocyte ChE inhibition in females at 0.5 mg/kg bw/day
Wistar rat 2-year dietary	0.05	Plasma ChE inhibition in females at 0.15 mg/kg bw/day
Wistar rat one-generation dietary	<0.4	Erythrocyte and brain ChE inhibition at 0.4 mg/kg bw/day
Wistar rat 2-generation dietary	0.05	Plasma and brain ChE inhibition, post-implantation and post natal losses at 0.5 mg/kg bw/day
Wistar rat 3-generation dietary	<0.15	decreased fertility index and plasma and erythrocyte ChE inhibition at the lowest dose tested
Long-Evans rats 3-generation dietary	0.75	No significant effects seen

Dutch banded rabbit teratology gavage study	<25 mg/kg bw/day	Plasma and erythrocyte ChE inhibition, increase in incidence of hydrocephalus
Beagle dog 16-week dietary	0.075	No notable plasma or erythrocyte ChE inhibition seen at highest dose
Beagle dog 2-year dietary	<0.75	Plasma ChE inhibition at lowest dose
Human 53-day oral study	approximately 0.04	No abnormal clinical signs, but ChE levels not determined. Dose only stated as 3 mg/day, weights of volunteers not supplied.
Human single oral dose	<1	Plasma and erythrocyte ChE inhibition
Human single dermal dose	<5	Plasma ChE inhibition

Determination of Public Health Standards

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.002 mg/kg bw/day, based on a NOEL of 0.15 mg/kg bw/day established for plasma ChE inhibition in a 2-year rat dietary study. In the current review, a number of lower NOELs have been identified, including 0.075 mg/kg bw/day in a 16-week dog dietary study (highest dose tested), 0.05 mg/kg bw/day in a 4-week (plasma ChE inhibition), 2-year dietary study (plasma ChE inhibition) and a 2-generation reproduction study (plasma and brain ChE inhibition) in the rat, and approximately 0.04 mg/kg bw/day in a 53-day oral dosing in humans.

The human study was not adequate to establish an ADI, as there was no monitoring of plasma or erythrocyte ChE, and it was not possible to accurately determine the dose delivered in mg/kg bw/day, as all volunteers received 3 mg of chlorfenvinphos daily with no reporting of the weight of each volunteer.

Based on the NOEL of 0.05 mg/kg bw/day obtained in a number of rat studies it is recommended that the ADI be amended to 0.0005 mg/kg bw/day. This is consistent with the current JMPR recommended ADI.

Public exposure

In Australia, there are currently no registered domestic uses for chlorfenvinphos, and the greatest potential for exposure is via ingestion of chlorfenvinphos residues in food. Current registrations include uses for control of external parasites in large animals, as well as uses in potatoes and for fly control around farm buildings. Chlorfenvinphos has MRLs established in a wide range of food, including fruit, vegetables, cereal grains and meat products; the current Australian MRL list is outlined below.

Table 3: Australian Maximum Residue Limits for Chlorfenvinphos

Commodity	**MRL (mg/kg)**	**Commodity**	**MRL (mg/kg)**
Broccoli	0.05	Maize	0.05

Brussels sprouts	0.05	Milks [in the fat]	0.2
Cabbages, Head	0.05	Mushrooms	0.05
Carrot	0.4	Onion, Bulb	0.05
Cattle, Edible offal of	0.2	Peanut	0.05
Cattle meat [in the fat]	0.2	Potato	0.05
Cauliflower	0.1	Radish	0.1
Celery	0.4	Rice	0.05
Cotton seed	0.05	Sheep, Edible offal of	0.2
Egg plant (aubergine)	0.05	Sheep meat [in the fat]	0.2
Goat, edible offal of	0.2	Swede	0.05
Goat meat [in the fat]	0.2	Tomato	0.1
Horseradish	0.1	Turnip, Garden	0.05
Leek	0.05	Wheat	0.05

Dietary Exposure Considerations

In estimating dietary exposures, The "Guidelines for Predicting Dietary Intake of Pesticide Residues(Revised)" circulated by the Codex Alimentarius Commission in November 1996 recommend the use of National Theoretical Maximum Daily Intakes (NTMDI) as an initial estimate, while admitting that these can produce a gross overestimate of the exposure for a number of reasons. The calculation involves the use of the MRL as an estimate of the amount of pesticide in the food, and national estimates of consumption for the quantity of food consumed.

When this procedure is followed for chlorfenvinphos, using the 1983 survey of average food consumption in Australia, it was calculated that a 75 kg adult male (weight as used by the Australian Market Basket Survey) could possible consume 0.0016 mg/kg bw/day, as would a 60 kg adult female. This is less than the current ADI, but is approximately 3 times the proposed new ADI of 0.0005 mg/kg bw/day.

A more reliable estimate of chlorfenvinphos intake may be derived from the Australian Market Basket Survey, a procedure which uses the measure of chlorfenvinphos residues found in various foodstuffs, rather than assuming that the pesticide is present at the MRL. In 1994, no residues of chlorfenvinphos was found in any produce. In 1992, the estimated intake in the group with the highest consumption of chlorfenvinphos residues (toddlers aged two), based on the average energy intake was 0.0000058 mg/kg bw/day. This makes up approximately 1% of the ADI.

Market Basket Survey

The 1994 Market Basket Survey did not find detectable levels of chlorfenvinphos in any foods tested. In 1992, chlorfenvinphos was found in celery, at a maximum level of 0.08 mg/kg, and in watermelon at a maximum level of 0.03 mg/kg. The residues in celery were lower than the

established MRL of 0.4 mg/kg; no MRL has been established for watermelon, and this level was therefore a violation of the Australian Food Standard Code.

Acute Reference Dose

To reflect safe/acceptable exposure from a single or short exposure to chlorfenvinphos, an acute reference dose (acute RfD) may be derived using appropriate data. The most suitable study submitted was a 2-week mouse dietary study, with a NOEL of 0.2 mg/kg bw/day, based on plasma ChE inhibition occurring at 1.9 mg/kg bw/day. Applying a safety factor of 100, using a factor 10 for individual variation, and a factor of 10 for interspecies variation, the acute RfD was 0.002 mg/kg bw/day.

Safety Directions

Table 4: Current chlorfenvinphos safety directions:

AL 3 g/L or less, with hydrocarbons 755 g/L or less	
Product is very dangerous, particularly the concentrate	120, 100, 101
Poisonous if absorbed by skin contact inhales or swallowed.	130, 131, 132, 133
Will irritate the eyes and skin.	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect.	190
Obtain an emergency supply of atropine tablets 0.6 mg.	373
Avoid contact with eyes and skin.	210, 211
Do not inhale vapour.	220, 222
When opening the container and pouring large quantities, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat, PVC or rubber apron, elbow-length PVC gloves, goggles and water resistant footwear.	279, 280, 286, 290, 292a, 293, 294, 297, 298b.
When using the product wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and elbow length butyl rubber gloves (if excessive splashing or contamination is likely when using the prepared dip wear protective waterproof clothing, elbow length butyl rubber gloves and water resistant footwear).	279, 283, 290, 292a, 294a (if excessive splashing or contamination is likely 279, 282, 290, 291, 294a, 298b)
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water.	340, 342
If product in eyes, wash it out immediately with water.	340, 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves, goggles and contaminated clothing.	360, 361, 363, 366

EC all strengths except as separately specified	
Very dangerous, particularly the concentrate	100, 101

Product is poisonous if absorbed by skin contact inhales or swallowed	120, 130, 131, 132, 133
Will irritate the eyes, nose throat and skin.	161, 162, 163, 164
Avoid contact with eyes and skin.	210, 211
Do not inhale vapour or spray mist.	220, 222, 223
Repeated minor exposure may have a cumulative poisoning effect.	190
Obtain an emergency supply of atropine tablets 0.6 mg.	373
When preparing spray and pouring large quantities wear cotton overalls buttoned to the neck and wrist and a washable hat, PVC or rubber apron, elbow-length PVC gloves, goggles, impervious footwear and a half facepiece respirator.	279, 281, 286, 290, 292, 293, 294, 297, 298, 300
When using the prepared spray wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves.	279, 282, 290, 292, 294
If clothing becomes contaminated with product or wet with spray remove clothing immediately	330, 331, 332
If product on skin, immediately wash area with soap and water.	340, 342
If product in eyes, wash it out immediately with water.	340, 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves, goggles, respirator and if rubber wash with detergent and warm water and contaminated clothing.	360, 361, 363, 364, 366

EC 215 g/L or less with liquid hydrocarbons 650 g/L or less, with surfactants	
Product is very dangerous, particularly the concentrate	120, 100, 101
Poisonous if absorbed by skin contact inhales or swallowed.	130, 131, 132, 133
May irritate the eyes and skin.	160, 162, 164
Repeated minor exposure may have a cumulative poisoning effect.	190
Obtain an emergency supply of atropine tablets 0.6 mg.	373
Avoid contact with eyes and skin.	210, 211
Do not inhale vapour or spray mist.	220, 222, 223
When opening the container, preparing the spray and pouring large quantities, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat, PVC or rubber apron, elbow-length PVC gloves, goggles and water resistant footwear.	279, 280, 281, 286, 290, 292a, 293, 294, 297, 298b.
When using the prepared spray wear protective waterproof clothing (or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron) (if excessive splashing or contamination is likely when using the prepared dip wear protective waterproof clothing, (or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron) elbow length butyl rubber gloves and water resistant footwear).	279, 282, 290, 291 [or 292a, 293] (if excessive splashing or contamination is likely 279, 282, 290, 291, [or 292a, 293] 294a, 298b)
If clothing becomes contaminated with product or spray remove clothing immediately	330, 341, 332

If product or spray on skin, immediately wash area with soap and water.	340, 341, 342
If product or spray in eyes, wash it out immediately with water.	340, 341, 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves, goggles and contaminated clothing.	360, 361, 363, 366

EC 1000 g/L, or less, with liquid hydrocarbons 20 g/L or less	
Product is very dangerous, particularly the concentrate	120, 100, 101
Poisonous if absorbed by skin contact inhales or swallowed.	130, 131, 132, 133
May irritate the eyes and skin.	160, 162, 164
Repeated minor exposure may have a cumulative poisoning effect.	190
Obtain an emergency supply of atropine tablets 0.6 mg.	373
Avoid contact with eyes and skin.	210, 211
Do not inhale vapour or spray mist.	220, 222, 223
When opening the container, preparing the spray and pouring large quantities, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat, PVC or rubber apron, elbow-length PVC gloves, goggles and water resistant footwear.	279, 280, 281, 286, 290, 292a, 293, 294, 297, 298b.
When using the prepared spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and elbow-length PVC gloves (if excessive splashing or contamination is likely when using the prepared dip wear protective waterproof clothing, (or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron) elbow length PVC gloves and water resistant footwear).	279, 282, 290, 292a, 294 (if excessive splashing or contamination is likely 279, 282, 290, 291, [or 292a, 293] 294, 298b)
If clothing becomes contaminated with product or spray remove clothing immediately	330, 341, 332
If product or spray on skin, immediately wash area with soap and water.	340, 341, 342
If product or spray in eyes, wash it out immediately with water.	340, 341, 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves, goggles and contaminated clothing.	360, 361, 363, 366

First Aid Instructions

The current first aid instructions are a, h. No changes to the first aid directions are recommended. The T-value is currently 1. No change is recommended.

Committee Considerations

The draft review of chlorfenvinphos has been considered by ACPH with the following advice.

The Committee:

- CONSIDERED the public health assessment for chlorfenvinphos, an organophosphate insecticide;

- NOTED that cholinesterase (ChE) inhibition appears to be the most sensitive toxicological endpoint for chlorfenvinphos with both plasma and brain ChE having a similar level of sensitivity in the rat;

- WAS ADVISED that reinterpretation of the pivotal 2-year Wistar rat study had led to the no-observable-effect-level (NOEL) being reduced from 0.15 to 0.05 mg/kg bw/day for plasma ChE inhibition;

- A NOEL of 0.05 mg/kg bw/day was also seen for both plasma and brain ChE inhibition in a 2-generation rat reproductive toxicity study;

- SUPPORTED a reduction in the existing acceptable daily intake (ADI) from 0.002 to 0.0005 mg/kg bw/day based upon the NOEL for ChE inhibition observed in these rat studies; and

- APPRECIATED that this ADI was the same value as the one established by the1994 Joint FAO/WHO Meeting on Pesticide Residues on the basis of reduced brain ChE activity observed in the 2-generation rat reproductive toxicity study;

- AGREED that an acute reference dose (acute RfD) for acute dietary exposure assessments be set at 0.002 mg/kg bw/day on the basis on plasma ChE inhibition observed in a 2-week mouse dietary study; and

- HIGHLIGHTED that the acute RfD may not be suitable for occupational health and safety risk assessment

RECOMMENDATIONS FOR PUBLIC HEALTH STANDARDS

1. Acceptable Daily Intake

The current acceptable daily intake (ADI) for chlorfenvinphos is 0.002 mg/kg bw/day. This ADI was derived from a NOEL of 0.15 mg/kg bw/day, based on plasma ChE inhibition seen in a 2-year rat study. In the current review, a number of lower NOELs have been identified.

Based on the NOEL of 0.05 mg/kg bw/day for plasma ChE inhibition obtained in two rat studies (2-year and a 12-week dietary studies), and for plasma and brain ChE inhibition in a 2-generation dietary study, it is recommended that the ADI be lowered to 0.0005 mg/kg bw/day.

2. Acute Reference Dose

An acute reference dose for chlorfenvinphos may be set at 0.002 mg/kg bw/day, based on plasma ChE inhibition seen in a 2-week mouse dietary study at 0.2 mg/kg bw/day, using a 100-fold safety factor.

3. Poisons Scheduling

No change to the current Schedule 7 of the SUSDP is proposed for chlorfenvinphos.

4. First Aid and Safety Directions

No change to the current safety directions are recommended.

Note: Safety Directions recommendations relating to the use of personal protective equipment are to be provided by the National Occupational Health and Safety Commission.

No change to the current first aid directions (chlorfenvinphos: a, h) and T-value (currently 1) are recommended.

5. Clearance Status

No change is recommended to the clearance status of chlorfenvinphos.

6. Additional Data

If additional long- and/or short-term studies addressing the potential neurotoxicity and/or the reproductive toxicity potential of chlorfenvinphos become available, that they should be submitted to Australian authorities.

SUMMARY OF ACUTE TOXICOLOGY HAZARD

Date of Preparation:	April 1998
Chemical name:	Chlorfenvinphos
Worst oral LD50 in rats:	9.7 mg/kg bw
Worst oral LD 50 in other species:	117 mg/kg bw, in mice
Worst dermal LD50, rat:	30 mg/kg bw
Worst inhalation LC50, rat:	133 mg/m^3
Skin irritation:	Slight
Eye irritation:	Slight
Skin sensitisation:	Negative
T-value:	1
NOEL:	0.05 mg/kg bw/day (2-year rat)

1. MAIN TOXICOLOGY REPORT - Introduction

1.1 Regulatory History of Health Considerations in Australia

Chlorfenvinphos is a contact insecticide with anti-cholinesterase activity. It is currently registered in a range of products for treating sheep and cattle, including uses against buffalo fly and flystrike.

A history of the consideration of chlorfenvinphos by regulatory committees in Australia is detailed below:

Table 5: Chlorfenvinphos regulatory history

Date	Regulatory Activity
21/07/69	MRL and WHP recommended for meat, milk and milk products and potatoes
7/07/71	Amend MRL for fat of meat of cattle and sheep, milk and milk products
7/7/71	New entry Section 3 – pastures
20/2/74	Delete Section 3. New entry: Appendix 2 part 2 - insecticides on pastures
8/5/74	Amend MRL and WHP for fat of cattle and sheep, milk and milk products
9/2/77	Add MRLs for carrots, celery, cauliflower, radish, tomato, horseradish, brussel sprouts, cabbage, broccoli, swede turnips, turnips, sweet potato, onion, leeks, eggplant, mushroom, peanuts, maize, wheat, cottonseed, rice
18/5/77	Amend MRL and WHP for potatoes
9/5/79	MRL for water recommended
6/11/80	MRL for goat meat recommended
4/2/82	Delete 3 day WHP for meat of sheep cattle and goats
17/2/83	Amend WHP for potatoes to 1 day
11/9/86	Delete: Appendix 2, part 1 - insecticide on pastures
11/9/86	Amend MRL for water

Health Standards

NOEL/ADI

The current acceptable daily intake (ADI) is 0.002 mg/kg bw/day, based on a no observable effect level (NOEL) of 0.15 mg/kg bw/day for plasma ChE inhibition in a 2-year rat study. This ADI was established in October 1985**.**

Poisons Schedule

Chlorfenvinphos is currently listed in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

MRLs

In Australia, there are currently no registered domestic uses for chlorfenvinphos, and the greatest potential for exposure is via ingestion of chlorfenvinphos residues in food. Chlorfenvinphos has MRLs established in a wide range of foods, including fruit, vegetables, cereal grains and meat products.

1.2 International Toxicology Assessments

Chlorfenvinphos has been evaluated by the Joint FAO/WHO Expert Committee on Pesticide Residues (JMPR) in 1971 and 1994. An ADI of 0.002 mg/kg bw/day was established in 1971, and was amended to 0.0005 mg/kg bw/day in 1994.

This ADI was based on the following levels causing no toxicological effects.

Mouse: 25 ppm, equal to 3.7 mg/kg bw/day (2-year carcinogenicity study)
Rats: 3 ppm, equivalent to 0.15 mg/kg bw/day (2-year toxicity/carcinogenicity study) 1 ppm, equivalent to 0.05 mg/kg bw/day (2-generation study of reproductive toxicity).
Rabbit: <25 mg/kg bw/day (teratogenicity study)
Dog: 100 ppm, equal to 2.8 mg/kg bw/day (1-year toxicity study)

2. KINETICS AND METABOLISM

2.1 Toxicokinetics

Hutson DH & Hathway DE (1967) Toxic effects of chlorfenvinphos in dogs and rats. Biochem. Pharmacol. 16: 949 - 962

The metabolism of chlorfenvinphos (source: Shell Development Co. NY; purity and batch no. not specified) and ^{14}C-labeled chlorfenvinphos (source: Shell Development Co, Modesto, CA; purity and radiochemical purity, batch no. not specified) was investigated in Porton rats (Tunstall Laboratory, Sittingbourne, Kent) and in Beagle dogs (source not specified) in a number of trials.

The uptake of unlabeled chlorfenvinphos in the blood and brain of rats was investigated. Rats were given 25 - 30 mg/kg bw chlorfenvinphos in olive oil by stomach tube. Blood samples were taken 25, 50 and 70 min after dosing. Defecation, urination, salivation, lacrimation, fasciculation, muscle weakness and prostration were observed within 25 min of dosing. Death occurred 70 - 85 min after dosing. Blood concentrations of chlorfenvinphos at 25, 50 and 70 min after treatment differed between males and females. The concentrations are detailed in the table below.

Table 6: Individual animal chlorfenvinphos concentration in peripheral blood

Time after dosing (min)	Concentration of Chlorfenvinphos (µm)	
	Males	**Females**
25	0.14, 0.19, 0.06 (mean 0.13)	0.17, 0.44, 0.08 (mean 0.23)

50	0.61, 0.14, 0.06, 0.22, 0.14, 0.03 (mean 0.20)	0.56, 0.33, 0.61 (mean 0.50)
70	0.14, 0.06, 0.14, 0.01 (mean 0.09)	0.78, 1.00, 1.17 (mean 0.98)

It was noted that the two male animals with blood concentrations of 0.01 or 0.03 µM showed only mild clinical signs. The blood concentration of chlorfenvinphos in males was decreasing 70 min after dosing, while that of females was still increasing.

^{14}C-Labeled chlorfenvinphos was administered to female rats at 25 - 30 mg/kg bw (total dose of 6.05 µCi) by stomach tube. Rats were killed 25, 50 and 70 - 85 min after dosing. Brains were excised, weighed and homogenised with water. Part of the homogenate was assayed for ChE activity, while the rest was analysed for radioactivity. At 25 min after dosing, the concentration of ^{14}C in the brain, expressed as chlorfenvinphos equivalents, ranged between 0.05 and 1.52 µM (mean 0.43 µM), and ChE inhibition (in comparison to laboratory control) was 2% - 63% (mean 25%). At 50 min, chlorfenvinphos concentrations ranged between 0.26 and 0.73 µM (mean 0.50µM), and the ChE inhibition ranged from 31% to 90% (mean 67%). At 70 - 85 min, the concentration ranged from 0.29 to 1.20 µM (mean 0.54µM), and the ChE inhibition was between 42% and 92% (mean 70%). These results suggested that the brain levels of chlorfenvinphos increased relatively rapidly, while ChE inhibition developed more slowly.

A female Beagle (11.5 kg) with an indwelling hepatic portal cannula was given a single oral dose by gelatin capsule of 1 g unlabeled chlorfenvinphos (88 mg/kg bw). Blood samples were taken from a peripheral vein and from the portal cannula for 6 h after dosing and chlorfenvinphos concentration determined. No abnormal clinical signs were observed over this period. Results are detailed below.

Table 7

Time after Dosing (h)	**Concentration of Chlorfenvinphos (µm)**	
	Portal Circulation	**Peripheral Circulation**
0.25	0.15	0.02
0.5	0.33	0.04
1	1.00	0.26
2	0.87	0.14
4	1.09	0.30
6	0.68	0.16

The chlorfenvinphos concentration in the portal blood was much greater than that in the peripheral blood. Levels in the peripheral blood of the dog in this experiment were comparable to those seen in rats in the earlier dosing study, although the dose administered to the dog was 3 times that given to the rat.

^{14}C-Labeled chlorfenvinphos was administered by stomach tube to a male Beagle dog at a dose of 3180 mg/kg bw, and peripheral blood samples were taken at intervals for 24 h. Over this time there

were no abnormal clinical signs, although the levels of chlorfenvinphos in the circulation were very high (detailed below)

Table 8

Time after Dosing (h)	Chlorfenvinphos Concentration (µm)
2	8.5
4	3.7
6	2.4
24	0.32

^{14}C-Labeled chlorfenvinphos was administered by stomach tube to a male Beagle dog at 80 mg/kg bw, and blood samples were taken over the following hour. Samples were analysed for chlorfenvinphos by thin layer chromatography and for total radioactivity. At the end of this period, the dog was euthanised, the brain removed and a slice homogenised for determination of ChE activity by the pH method. Over the observation period, chlorfenvinphos levels were 0.55 µM at 20 min, 0.70 µM at 40 min and 0.83 µM at 1 h. At 1 h, the radioactivity level was 30 µM. The radioactivity level in the brain at this time was 2.4 µM, however the chlorfenvinphos component of this was not able to be determined. ChE activity in the brain was not reported. Comparing the levels of radioactivity and chlorfenvinphos, the compound was readily metabolised over a relatively short time period (1 h duration), with metabolites present in peripheral circulation.

Fresh rat and dog brains were obtained from untreated animals, homogenised with water and added to solutions containing variable concentrations of chlorfenvinphos. The homogenates were incubated for 30 min prior to acetylcholine being added. The rate of hydrolysis was determined, and the concentration of chlorfenvinphos required to decrease the reaction rate by 50% was assessed. The results indicated that rat brain ChE was 10 times more sensitive to the presence of chlorfenvinphos than dog brain ChE.

^{14}C-Labeled chlorfenvinphos was incubated with dog or rat plasma from untreated animals. Samples were analysed with thin layer chromatography for the presence of chlorfenvinphos and 2 hydrolysis products, 2,4-dichlorophenacyl chloride and 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate. In this trial, dog plasma hydrolysed 5% of the insecticide in 6 h, while rat plasma hydrolysed 4% in 16 h. In both cases, the main hydrolysis product was the vinyl hydrogen phosphate metabolite. This indicated that dog plasma was more efficient at metabolising chlorfenvinphos than rat plasma.

^{14}C-Labeled chlorfenvinphos was mixed with either rat or dog heparinised plasma containing 2.8% glucose, or in an artificial serum containing 2.8% glucose, 0.4% sodium chloride and 3% bovine serum albumin. Rat and dog erythrocytes were well washed in salt solution, and added to the plasma and chlorfenvinphos solutions. The radioactivity in plasma and haemolysed erythrocytes was analysed. The ratio of radioactivity in erythrocytes to plasma was similar in both rat and dog erythrocytes when they were incubated with artificial serum. Dog erythrocytes incubated in dog plasma had a ratio of radioactivity (erythrocytes:plasma) of 9:91. Rat erythrocytes incubated in rat plasma had a ratio of radioactivity (erythrocytes:plasma) of 23:77. Therefore it appears that dog plasma binds chlorfenvinphos to a greater extent than rat plasma, resulting in less of

the insecticide penetrating erythrocytes. There was no difference in the permeability of the erythrocyte membrane between dogs and rats, as indicated by their similar uptake when incubated with artificial serum (containing bovine serum albumin).

^{14}C-Labeled chlorfenvinphos was mixed with dog or rat serum and dialysed against stirred horse serum for 24 h. Over this time 15% of the chlorfenvinphos in the dog serum, and 25% of the chlorfenvinphos in the rat serum, diffused into the horse serum. This suggested that chlorfenvinphos was more strongly bound in dog serum than in rat serum.

Overall, this set of experiments indicated that dogs could tolerate a higher level of chlorfenvinphos in plasma than rats, and developed less severe clinical signs at these doses. It appears that the metabolism of chlorfenvinphos following oral dosing in dogs was more efficient than in the rat, given the difference in concentration in peripheral and portal circulation, and also that the availability of the compound in dogs was less, as a significantly higher dose was required to produce similar plasma levels in dogs. *In vitro* studies indicated that chlorfenvinphos in dog plasma was less available than in rat plasma, which may have contributed to the lower brain levels seen in dogs. The greater tolerance of the dog to chlorfenvinphos appeared to be a combination of increased metabolism in the liver, an increased binding by plasma protein and a decreased sensitivity of the brain to chlorfenvinphos.

Hutson DH, Akintonwa AA & Hathway DE (1967) The metabolism of 2-chloro-1-(2',4'-dichlorophenyl)vinyl diethyl phosphate (chlorfenvinphos) in the dog and rat. Biochem J 102: 133-142

Chlorfenvinphos (purity 90%, source: Shell Development Company, NY), purified by column chromatography to 99% purity, and ^{14}C-labeled chlorfenvinphos (purity 95%, radiochemical purity 2.8 µCi/mg, source: Shell Development Company, Modesto) were used to investigate the metabolism of chlorfenvinphos in the rat and dog. Other metabolites of chlorfenvinphos were synthesised as required.

Radiolabeled chlorfenvinphos in olive oil was administered by gavage to fasted Porton rats (Tunstall Breeding Unit) at 2 mg/kg bw, using 6 rats/sex. After dosing, food and water were available *ad libitum*. Rats were maintained in metabolism cages; urine, faeces and expired air were collected for 4 days following treatment. At this time, rats were killed, and the GI tract, skin, hair, carcass and other organs examined for radioactivity. There was basically complete excretion of the ingested radioactivity over the 4-day period, mainly in the urine. In the first day, 67.5% of the administered dose was excreted in the urine, with 1.4% excreted in the faeces. On day 2, 14.4% was excreted in the urine, with 6.2% excreted in faeces. On day 3, 3.6% was excreted in urine and 2.3% in faeces, while on the final day 0.87% was excreted in urine and 1.25% excreted in faeces. The total measured recovery was >98.5%, with 86.4% in the urine, 11.1% in the faeces and 1% as expired gases.

Beagle dogs (2/sex) were fasted for 24 h, then dosed orally with 0.3 mg/kg bw radiolabeled chlorfenvinphos by gelatin capsule, and were maintained in metabolism cages for the following 96 h, with collection of urine and faeces. Over the 4-day observation period, 94% of administered radioactivity was excreted, mainly in the urine. Urinary excretion on each of the four days was 86%, 2.3%, 0.63% and 0.47% respectively. In the faeces, the daily excretion was 4.1%, 0.43%, <0.1% and <0.1% respectively. Therefore total excretion of chlorfenvinphos in dogs was more rapid than in rats, and faecal excretion was higher in rats than in male dogs. When a female dog was given a dose

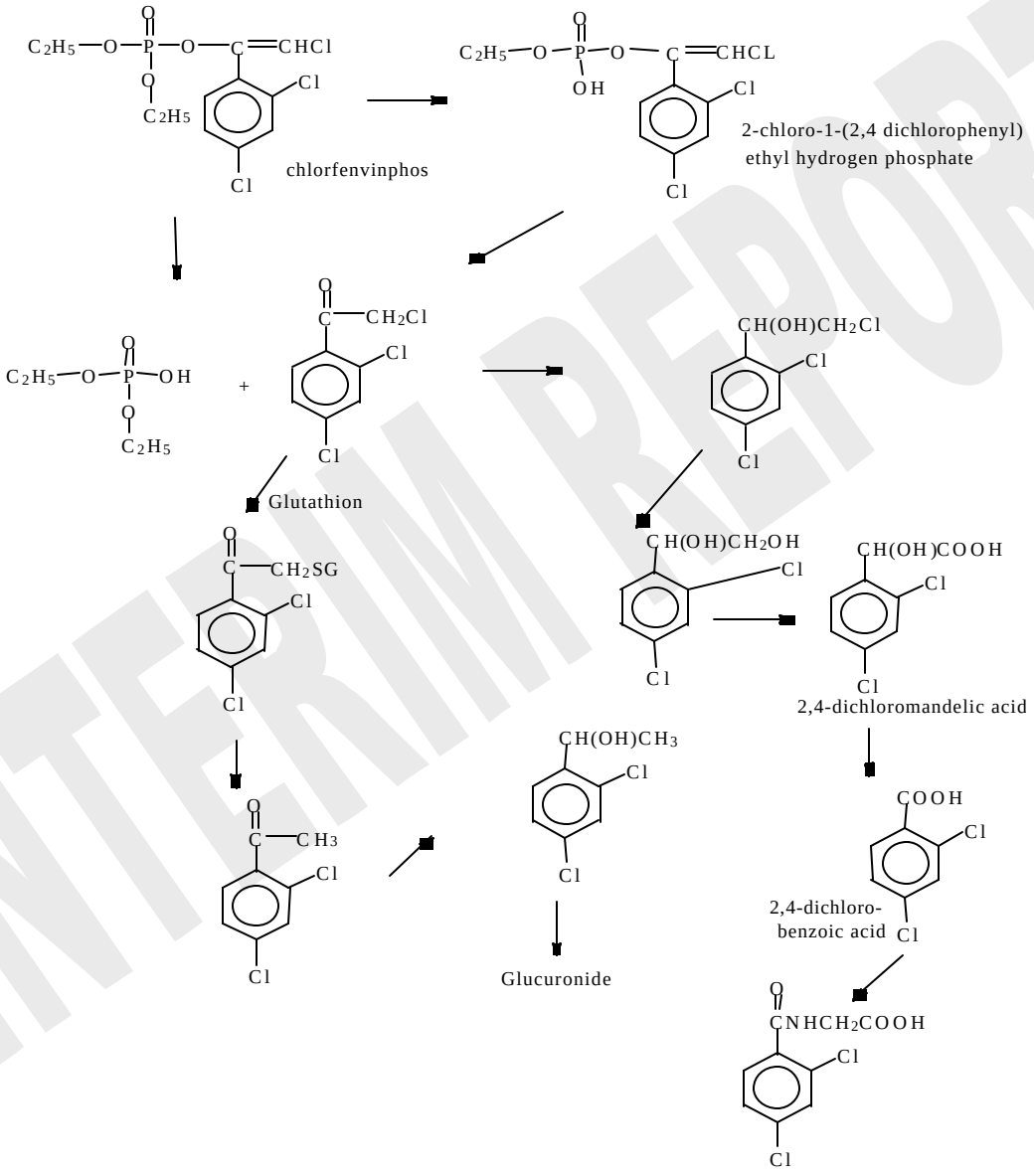
of 2.6 mg radiolabeled chlorfenvinphos, followed by 5.5 g of unlabeled chlorfenvinphos, the excretion pattern was approximately the same as with the radiolabeled dose alone, with 50% of the administered radioactive material excreted in the urine in the first 24 h. A greater proportion of the radiolabel was excreted in the faeces, with 35% excreted over 48 h, mainly as unmodified chlorfenvinphos.

Pooled urine from a number of orally dosed dogs and rats was analysed for a range of metabolites. The metabolite forming the greatest proportion in dogs was O-desethyl chlorfenvinphos, accounting for 78% of excreted metabolites. This metabolite formed 37% of excreted metabolites in rats. In rats, the greatest proportion (47%) was made up of [1-(2,4', dichlorophenyl) ethyl -D-glucopyranosid]uronic acid. Lesser metabolites in dogs and rats were 2,4 dichloromendelic acid (15% and 8% respectively), 2, 4-dichloro-hippuric acid (0 and 5% respectively) and 2,4-dichlorophenylethanediol glucuronide (3% in both species). When dogs and rats were dosed with O-desethyl chlorfenvinphos, 2,4-dichlorophenyl acyl chloride and 2-chloro-(2',4'-dichlorophenyl) ethanol, the urinary metabolites were similar to those seen following dosing with chlorfenvinphos, indicating that these chemicals may be major early metabolites of chlorfenvinphos.

These results indicated that the excretion pattern differed between rats and dogs, with a more rapid urinary excretion seen in dogs.

Hutson D & Wright AS (1980) The effect of hepatic microsomal mono-oxygenase induction on the metabolism and toxicity of the organophosphorus insecticide chlorfenvinphos. Chem Biol Interactions 31:93-101

The metabolism of chlorfenvinphos (Shell Development Company, Modesto) was investigated in Carworth Farm E rats (source not specified) following induction of hepatic microsomal monooxygenases by a 12-day treatment with dieldrin (dose not specified). An initial determination of the LD50 in pretreated and untreated rats was made. Control rats (5/group) were dosed with chlorfenvinphos (purity 90%) in olive oil by gavage at doses from 5 - 40 mg/kg bw. Rats pretreated with dieldrin were dosed with chlorfenvinphos in olive oil by gavage at doses from 40 - 240 mg/kg bw, using 5/group. Animals were observed for 72 h after dosing; all deaths observed were recorded within the first 12 h after dosing. The LD50 for rats not receiving dieldrin was 15.4 mg/kg bw, while the LD50 for rats receiving dieldrin was 157 mg/kg bw.



Metabolism of chlorfenvinphos in the rat

The metabolism of chlorfenvinphos, and the effects of pretreatment with dieldrin were investigated by dosing animals (2/group; both treated with dieldrin and untreated) with 2.5 or 13.2 mg/kg bw radiolabeled chlorfenvinphos (not otherwise specified) by gavage. Animals were housed in metabolism cages with food and water available *ad libitum*. The urine was collected for 72 h, and

analysed for radioactivity and for the presence of a number of metabolites. The percentage of administered radioactivity excreted in the urine is detailed below.

Table 9: Percentage of administered radioactivity excreted in the urine over 72 h.

Dose	0 - 12 h	12 - 24 h	24 - 30 h	30 - 72h	Total
2.5 mg/kg bw No pretreatment	49.6	11	4.7	4.6	69.8
2.5 mg/kg bw plus Dieldrin	62.4	8.1	1.4	2.0	73.8
13.2 mg/kg bw No pretreatment	16.1	5.4	6.4	1.1	28.8
13.2 mg/kg bw plus Dieldrin	66.3	32.5	1.1	3.6	74.2

At the lower dose, there was little difference in the radioactivity excreted in urine over the 72-h observation period with or without dieldrin pre-treatment, although there was a slight increase in urinary excretion over the first 12 h in the pre-treated rats. At the higher dose, the excretion both during the first 24 h and in total was much greater in the animals pretreated with dieldrin, where activation of the hepatic microsomal enzymes would be expected. This indicates that at the lower dose, there may have been sufficient enzyme present in the liver to metabolise the chlorfenvinphos, while at the higher doses in normal rats the detoxification systems are overloaded. Abnormal clinical signs were observed in rats which had not received dieldrin.

The metabolites found in the urine included 2,4-dichlorophenyl ethandiol, 1-(2,4-dichlorophenyl) ethanol, 2,4-dichloromandelic acid, 2,4-dichlorobenzoylglycine, de-ethylchlorfenvinphos, and an unidentified non-polar metabolite. Following dieldrin pretreatment, there was little change to the metabolites present at the lower dose of chlorfenvinphos. After the higher dose, the relative quantity of de-ethylchlorfenvinphos was twice that seen in the non-treated animals.

In this study, the results suggested that activation of the hepatic microsomal enzymes produced a protective effect against high doses of chlorfenvinphos by increasing the rate and extent of the metabolism, while not significantly changing the metabolism and excretion at lower doses.

Brown VKH, Moffett JA, Rees RO & Richardson A (1964) Preliminary studies on the toxicology of the chlorinated-aryl-vinyl phosphate insecticide, SD 7859 (GC 4072). Shell Research Limited, Sittingbourne TOX 4/64

Chlorfenvinphos (purity 92%, source: Woodstock Agricultural Research Centre) in dimethyl sulphoxide was administered by gavage to 12 male NZW rabbits (Tunstall Laboratories) at 12 mg/kg bw/d for 5 days. Two rabbits died on the fifth day due to injury from dosing. Four rabbits were euthanised on day 6, 3 on day 7 and 3 on day 8. The residues of chlorfenvinphos and trichloro-acetophenone (a metabolite) in the body fat were analysed by gas-liquid chromatography.

The concentrations of chlorfenvinphos in body fat were 1.17 - 2.51 ppm in the animals dying on the last treatment day. By day 1 after cessation of treatment, levels were 0.07 - 1.02 ppm, and decreased further to be between 0.07 ppm and 0.28 ppm on the 3rd day after cessation of treatment. The levels of the metabolite analysed were lower, being 0.13 - 0.18 ppm on the last day

of treatment and 0.03 to 0.15 ppm on the 3rd day after the cessation of treatment. The estimated biological half-life of chlorfenvinphos in the body fat of rabbits was one day.

Hutson D (1969) The metabolism of [^{14}C]chlorfenvinphos in man. TLGR.0006.69 , Shell Research Institute, Sittingbourne
and
Donninger C, Hutson DH & Pickering BA (1972) The oxidative dealkylation of Insecticidal Phosphoric Acid Triesters by mammalian liver-enzymes. Biochem J 126:701-707

Radiolabeled chlorfenvinphos (source, batch, purity not specified) was administered as a single oral dose to a male human volunteer, as 12.5 mg chlorfenvinphos (35.1 µCi) in 0.75 mL olive oil. The radioactivity excreted in the urine over the next 26.5 h was measured. In the first 2 h after dosing, 35.4% of the administered dose was excreted, with an additional 36.7% excreted in the next 2 h 30 min. Over the 26 - 27 h observation period, 94.2% of the administered dose was excreted in the urine.

The first 3 urine samples obtained were pooled and analysed using paper chromatography to identify the metabolites present. In the samples, 23.8% was 2-chloro-1-(2,4-dichlorophenyl) vinyl ethyl hydrogen phosphate (compared with 32.3% in the rat and 69.6% in the dog seen in earlier dosing studies (Hutson, Akinotonwa & Hathway, 1967)). 2,4-Dichloromandelic acid made up 23.9% of the metabolites in the urine of man, in comparison to 7% in the rat and 13.4% in the dog (Hutson, Akinotonwa & Hathway, 1967). Chlorfenvinphos appeared to be rapidly metabolised in the single subject in this study, with excretion rates higher than those seen in the rat or dog. There were relatively minor differences in metabolites produced. The study also indicated that these metabolites could be analysed as a measure of chlorfenvinphos exposure.

Hutson DH, Hoadley EG & Donninger C (1969) Excretion of metabolites of chlorfenvinphos in the milk of a cow treated with the insecticide. TLTR.0009.69 Shell Research Institute, Sittingbourne

^{14}C-labeled chlorfenvinphos (2.8 µCi/mg, source: Shell Development Company, Modesto), was purified to 99% purity by Tunstall Laboratories, Sittingbourne UK. The purified mixture was administered by a single IM injection to a 400 kg Friesian cow at 233 mg radiolabeled chlorfenvinphos (approximately 0.6 mg/kg bw). The cow was tethered in a metabolism cage, with water and hay available *ad libitum*, and food concentrate fed twice daily. The cow was milked twice daily at 10 am and 4 pm. Excretion of radioactive material in the milk was measured for 8 milkings after dosing. In the first milking 0.13% of the administered dose was excreted in the milk. This had decreased to 0.04% of the administered dose by the second milking, and remained at levels <0.01% of administered dose over the observation period. In the first milking, 75% of the radioactive material was unchanged chlorfenvinphos, with the rest consisting mainly of 2,4 dichloroacetophenone and 1-(2,4-dichlorophenyl) ethanol. A small amount of 2,4-dichloromandelic acid was also present. Levels of radioactivity expressed as chlorfenvinphos equivalents did not exceed 0.076 ppm throughout the observation period.

Koeman JH, Debets FMH & Strik JJTWA (1980) The porphyrogenic potential of pesticides with special emphasis on organophosphorous compounds. In: Tordoir W.F & van Heemstra EAH (ed); Field worker exposure during pesticide application, 157-162. Amsterdam, Elsevier Scientific Publishing Company

A number of pesticides were examined for their possible porphyrinogenic properties by means of an assay procedure based on the use of primary liver cell cultures of chicken embryos. Three out of twelve compounds tested appeared to be able to induce porphyria in chicken embryo liver cell culture without previous treatment of the cells with a drug enzyme inducing compound (viz. HCB, -HCH and chlorfenvinphos). Eight out of the twelve compounds responded in the induced system (viz. HCB, -HCH, chlorfenvinphos, temephos, azinphos-methyl, benzoyl prop-ethyl, pentachlorophenol and photomirex). No effect was found with 2,4,5-T, carbaryl, captan and trichlorfon. Enzyme induction markedly enhanced the porphyriniogenic potential of HCB, -HCH and chlorfenvinphos. It was suggested that the measurement of the urinary porphyrin pattern may be a valuable parameter for the purpose of human biological monitoring in relation to occupational exposure to pesticides, especially when there is some likelihood that the workers are also affected by the drug enzyme inducing properties of pesticides and/or other chemicals.

Brown VKH (1964) The in vitro effects of some halophenyl vinyl phosphates on blood acetylcholinesterases from different mammalian species. R(T)-1-64. Shell Research Institute, Sittingbourne

The *in vitro* sensitivity of cholinesterases from different species to chlorfenvinphos was examined. Chlorfenvinphos (source: Shell Development Company, Modesto CA, batch no, purity not specified) was incubated with plasma and washed erythrocytes from 'P' strain guinea pigs, NZW rabbits, Beagle dogs, Hooded Lister Rats, CF1 mice, and a human volunteer. The ChE activity of the samples was determined by measuring the change in pH after the addition of acetylcholine chloride. Initial dilutions of 10 mM, 100 µ M and 1 µM chlorfenvinphos were used: other dilutions were used as appropriate. It was calculated that the molar concentration required to produce a 50% inhibition of ChE activity in blood was: 1.6 µM in mice, 14 µM in rats, 3 000 µM in guinea-pigs, 3.9 µM in rabbits and 400 µM in humans. Hence mice were the most sensitive to the anticholinesterase effects of chlorfenvinphos, while guinea-pigs were the least sensitive.

Additionally, chlorfenvinphos was incubated with liver slices from mice, rats and dogs. The inhibition of ChE activity was measured over 30 mins. The concentration of chlorfenvinphos remaining in the incubation mixture was determined by chromatography. Inhibition of ChE activity in rat liver decreased over the period of metabolism, from an inhibition of 62.1% to a final inhibition 45 min later of 30.6%. In mouse liver, the inhibition increased from an initial level of 54% to a peak of 72% at 30 min, dropping to 63% at 45 min. In dog liver there was an initial drop in inhibition from 40% to 18% at 10 min. This effect was also seen in a later experiment with another dog which had been killed without the use of barbiturates. In this experiment, the inhibition increased from 20 min to 44% at 45 min. Over the 30-min incubation period, the concentration of chlorfenvinphos in the rat decreased by 38%. In the mouse it decreased by 30%, while in the dog it decreased by 11%. There was no attempt to measure metabolites produced in this experiment. The rat liver was efficient at metabolising chlorfenvinphos; given the decrease in inhibition, it seems that the metabolites were less active than the parent compound. The situation in mice and dogs was less clear, given that inhibition increased while the concentration of chlorfenvinphos decreased.

Hutson DH (1970) The oxidative dealkylation of insecticidal phosphoric acid triesters by mammalian liver enzymes. TLGR.0094.70. Shell Research Institute, Sittingbourne

Chlorfenvinphos, radiolabeled at either the vinyl carbon or ethyl carbon atoms, was obtained from the Shell Development Company, Modesto CA. Male CFE rats, male CFI mice, male NZW

rabbits and a beagle dog (sources not specified) were used as sources for liver enzymes. The rats, mice and rabbits were killed by a blow; the dog was exsanguinated under general anaesthetic. Livers were removed, washed in a buffer solution and sliced. Slices of 1g wet weight were incubated in a buffer solution with ^{14}C chlorfenvinphos. Following incubation, the material was homogenised and the metabolites present were evaluated. In each of the species, only one major metabolite was identified, which was 2-chloro-1-(2,4 dichlorophenyl) vinyl ethyl hydrogen phosphate. This was produced by a de-ethylation reaction. The relative reaction rates were: rats 1, mice 8, rabbit 24 and dog 88. The reaction rate may be compared with the LD50 of the compound in these species (rat 10 mg/kg bw, mice 100 mg/kg bw, rabbit 500 mg/kg bw and dog >12,000 mg/kg bw).

Following this trial, the rabbit was selected as a suitable species for further investigation. A male rabbit was given 3.9 mg ^{14}C chlorfenvinphos/kg bw by gavage and maintained in a metabolism cage. The urine was collected over a 4-day period, assayed for radioactivity, and the metabolites identified using paper chromatography. The rabbit excreted 65% of the administered radioactive dose in the first 48 h after dosing. The metabolites present were mainly the de-ethylation product indicated above, with only a small amount of 2,4-dichloromandelic acid produced. Therefore the metabolic rate in rabbits was mid-way between that of the dog and the rat.

Rabbit liver slices were homogenised in potassium phosphate with nicotinamide. There was a high activity of chlorfenvinphos de-ethylase which was associated with the microsomal fraction of the liver, but required co-factors present in the supernatant for activity, particularly NADPH and oxygen. The rate of the enzyme activity was investigated over a range of pH and temperatures. It was found that the pH requirements were fairly specific, centring around the physiological pH. The optimum temperature was normal body temperature, however a wider range of values was tolerated.

The major reaction in detoxifying chlorfenvinphos was a de-ethylation reaction. The rate of this reaction in different species roughly correlated with the LD50, however there may be other factors affecting the LD50, as the correlation was not exact. Detoxification occurred in the microsomal fraction of the liver, requiring co-factors present in the supernatant to maintain normal reaction rates.

Robinson J, Malone JC & Bush B (1966) Residues of SUPONA in sheep. J Sci Food Agric 17: 309 - 312

Chlorfenvinphos (source: Shell Research Ltd; batch no, purity not specified) was applied to sheep either at 0.05% w/v or 0.1% w/v chlorfenvinphos in a dip bath, or at 0.2% w/v chlorfenvinphos in a spray race. Tissue samples were analysed for the presence of chlorfenvinphos and trichloroacetophenone (a lipophilic metabolite). Samples of omental fat were taken by laparotomy prior to treatment. Sheep were then killed 3, 7, 14 or 21 days after treatment. The pericardial, perirenal and omental fats were collected and analysed from all sheep. In sheep treated at 0.1% chlorfenvinphos, the liver, spleen, adrenals, kidneys, heart, lungs, uterus, ovaries, brain, subcutaneous tissue and muscle were collected 7 days after treatment, and analysed for chlorfenvinphos and trichloroacetophenone.

At 3 days after treatment, the highest levels of chlorfenvinphos detected were in omental fat following a 0.1% dip treatment, with mean levels in omental fat of 0.043 ppm. At 7 days after treatment, levels in omental fat of one sheep were 0.093 ppm, which was much higher than samples from other areas of the body. Levels decreased over the 21-day observation period, with the mean concentration of chlorfenvinphos in the fat after 21 days being 0.005 ppm. In the major organs

examined 7 days after treatment, the levels of chlorfenvinphos and trichloroacetophenone were below the limit of detection (0.003 ppm and 0.001 ppm respectively).

Sitkiewicz D, Skonieczna M, Orlowska E & Bicz W (1978) The effect of organophosphorus insecticides on the oxidative processes in rat brain mitochondria. Comparative studies of chlorfenvinphos and its chemical analogs. Neuropat Pol 16: 487-495

Male albino rats (source, strain not specified) were used in tests of chlorfenvinphos, IPO-62 and IPO-63 (not otherwise specified). Mitochondria were prepared from rat cerebral hemispheres. Succinate dehydrogenase activity, protein and oxygen consumption were all measured. Chlorfenvinphos (purity 96%, source Shell Chemicals) was used, at concentrations of 50 µM, 75 µM and 100 µM. State-4 respiration of mitochondria was almost unchanged by chlorfenvinphos, while state-3 respiration was decreased by almost a third ($p<0.05$) by doses of 50 µM, and to 20% by 100 µM. The ADP/O ratio was decreased by the lowest dose of chlorfenvinphos and could not be determined at higher doses. Cytochrome oxidase activity was not consistently altered by increasing concentrations of chlorfenvinphos, while succinate dehydrogenase activity was slightly decreased by increasing concentrations of chlorfenvinphos (up to 100 µM, changes not significant). It is possible that chlorfenvinphos was acting by inhibition of coupled oxidative phosphorylation, however the mode of action is not clear. No individual animal results were presented in this paper.

Hutson DH & Logan CJ (1986) Detoxification of the organophosphorus insecticide chlorfenvinphos by rat, rabbit and human liver enzymes. Xenobiotica 16 (1): 87 - 93

Chlorfenvinphos has been found to differ markedly in toxicity between species, with dogs much less sensitive than rats. The major detoxification reaction of chlorfenvinphos is the conversion to de-ethyl chlorfenvinphos, which has a much lower toxicity. The rate of de-ethylation in the rat, rabbit and human liver was investigated using ^{14}C-labelled chlorfenvinphos (batch no 1264C, radiochemical purity 99.5%, source: Shell Development Co, Modesto CA).

The livers of Wistar rats and New Zealand white rabbits (source and sex not specified) were removed as quickly as possible after the animals were killed. The tissue was homogenised and centrifuged and the microsomal fraction isolated. Human liver samples were obtained from road accident victims and stored frozen until required. After thawing overnight, the microsomal fractions were prepared from the tissues.

The labelled chlorfenvinphos was incubated with the microsomal proteins, and the 2-chloro-1-(2,4-dichlorophenyl) vinyl ethyl hydrogen phosphate was separated into the aqueous phase, with unchanged chlorfenvinphos remaining in the organic phase. The phases were separated and the amount of chemical quantified by liquid scintillation counting. In rats, the rate of de-ethylation was 0.095 nmoles/min/mg protein, while in rats with liver enzymes induced by pre-treatment with Aroclor (dose, regime not specified) the rate was 4.00 nmoles/min/mg protein. In rabbits, the rate of de-ethylation was 0.62 nmoles/min/mg protein, while in humans the rate was 0.36 nmoles/min/mg protein. Therefore in this study the detoxification reactions in humans were approximately half the rate of that in rabbits, and approximately 4 times that seen in rats. This suggested that the LD50 in humans would be likely to be in the order of 50 to 150 mg/kg bw, based on the comparative metabolic rates.

Ikeda T, Kojima T, Yoshida M, Takahashi H, Tsuda S & Shirasu Y (1990) Pretreatment of Rats with an Organophosphorus Insecticide, Chlorfenvinphos, Protects against Subsequent Challenges with the Same Compound. Fund & Appl Toxicol 14: 560 - 567

Male Fischer 344 rats (Charles River Japan, Kanagawa) were used to investigate the interactions between chlorfenvinphos (purity 92.7%, source: Shell International Chemical Co. London; batch no was not specified), diazinon (purity 98.7%, source: Nippon Chemical Industrial Co. Tokyo; batch no not specified) oxytremorine and carbachol. The acute toxicity was investigated by an LD50 determination using 4 - 5 doses and 5 - 10 animals/dose. Clinical signs were observed. In further investigations, blood was collected prior to euthanasia and the brain removed and weighed immediately following euthanasia. The plasma, erythrocyte and brain ChE activities were determined, as was the concentration of chlorfenvinphos in the plasma.

When rats were given chlorfenvinphos in olive oil by gavage, they presented with salivation, muscle fasciculation, lacrimation, tremors, irregular respiration and prostration. These signs were seen from 1 h after administration and had virtually disappeared in survivors by 24 h after administration. The oral LD50 was 34.6 mg/kg bw.

Rats were pretreated with chlorfenvinphos at 15 mg/kg bw, and the oral LD50 determined 4, 8, 24 and 48 h later. The pretreatment dose did not produce any toxic signs. The LD50 was slightly increased 8 h after the initial dose ($p<0.05$), and maximally increased to around 100 mg/kg bw at 24 h after pretreatment. By 48 h, the LD50 was returning to normal levels (around 35 mg/kg bw), although it was still slightly increased.

Brain ChE activity was maximally inhibited 4 h after oral administration, with approximately 75% inhibition. This recovered over the next 48 h to approximately 35% inhibition. Pretreatment with chlorfenvinphos resulted in an observable inhibition of plasma, erythrocyte and brain ChE at the time of the challenge dose. A challenge dose only minimally inhibited brain and erythrocyte ChE activity in pre-treated animals, resulting in the ChE activity in the brain and erythrocytes being greater in animals pretreated with chlorfenvinphos than in those not treated. Plasma ChE activity was inhibited by the pretreatment dose; this pretreatment did not decrease the inhibition seen following the challenge dose, and the plasma ChE inhibition was thus greater in animals pretreated with chlorfenvinphos. In another experiment, animals were pretreated with a dose of 15 mg/kg bw prior to administration of doses of 7.5, 15, 30 or 60 mg/kg bw. Pretreatment increased brain ChE activity after a challenge dose of 15 and 30 mg/kg bw. Animals which had not been given pretreatment were not given a dose of 60 mg/kg bw, as it was presumed that many would die. The brain ChE activity in animals pretreated with chlorfenvinphos was higher at 60 mg/kg bw that the ChE activity in non-pretreated animals dosed with 30 mg/kg bw. The concentration of chlorfenvinphos in plasma was decreased by pretreatment with chlorfenvinphos at 15 mg/kg bw when a challenge dose was given 24 h later. This decrease was only significant at 1 h after dosing.

Pretreatment with chlorfenvinphos did not significantly change the IV LD50 of carbachol, but significantly ($p<0.05$) increased the IV LD50 of oxotremorine. The oral LD50 of dichlorvos was significantly ($p<0.05$) decreased from 97.5 mg/kg bw to 38.1 mg/kg bw.

Ikeda T, Tsuda S & Shirasu Y (1991) Metabolic Induction of the Hepatic Cytochrome P450 System by Chlorfenvinphos in Rats. Fund & Appl Toxicol 17: 361 - 367

Male Fischer 344 rats (Charles River Japan, Kanagawa) were given either 15 mg/kg bw chlorfenvinphos (purity 92.7%; source: Shell International Chemical Co, London; batch no not specified) in olive oil or 50 mg/kg bw phenobarbital in water by gavage 24 h prior to euthanasia. Immediately after euthanasia (by decapitation), the liver and kidneys were removed, weighed and homogenised in phosphate buffer. The homogenates were centrifuged and the supernatant fractions and microsomal pellets were saved. Aliquots of these fractions were added to a phosphate buffer solution either with or without an NADPH-generating system and EDTA. The material was pre-incubated for 2 min, then 5 µL chlorfenvinphos was added. A sample was removed immediately to determine the chlorfenvinphos concentration, then the material was incubated for 30 min (liver fraction and serum) or 40 min (kidney homogenate). The cytochrome P450 content in the microsomal fraction was determined.

The metabolism of chlorfenvinphos in both liver fractions was negligible without the addition of the NADPH-generating system, regardless of any pretreatment. The addition of NADPH markedly increased metabolism. In this system, rats pre-treated with chlorfenvinphos metabolised at approximately twice the rate of controls, and phenobarbital-induced rats metabolised at 5 times the rate of controls. There was very little metabolism of chlorfenvinphos in the kidneys. Chlorfenvinphos pre-treatment increased the cytochrome P450 content by approximately 30%, while phenobarbital pre-treatment increased it by 80% in comparison to untreated controls. Increases of 20 - 40% were also seen in the cytochrome b5 and cytochrome P450 reductase activity. The protein content in the microsomal fraction was increased by both pre-treatments, and aminopyrine N-demethylation and aniline 4-hydroxylation were increased by 40% and 27% respectively by chlorfenvinphos pre-treatment. Pre-treatment with chlorfenvinphos did not alter the hexobarbital sleeping times of rats.

Ikeda T, Tsuda S & Shirasu Y (1992) Pharmacokinetic Analysis of Protection by an Organophosphorus Insecticide, Chlorfenvinphos, against the Toxicity of its Succeeding Dosage in Rats. Fund & Appl Toxicol 18: 299 - 306

Male Fischer 344 rats (Charles River Japan Inc, Kanagawa) were treated with chlorfenvinphos (purity 92.7%, Shell International Chemical Co Ltd, London; batch no not specified) in olive oil. A 15 mg/kg bw dose was administered by gavage 24 h before either a second dose of chlorfenvinphos or tissue sampling to determine the unbound chlorfenvinphos fraction. The challenge dose of chlorfenvinphos was either 5 mg/kg bw IV or 30 mg/kg bw PO.

At an unspecified time after the challenge dose, rats were lightly anaesthetised and blood samples taken. The rats were then killed and the brain and liver removed, weighed and stored on ice prior to homogenisation. The brain ChE activity was determined. The concentration of chlorfenvinphos in the brain, liver and plasma was determined using high pressure liquid chromatography, and the unbound fraction of chlorfenvinphos in plasma and liver was also determined.

Pretreatment with chlorfenvinphos by the oral route increased the LD50 following IV dosing from 6.51 to 9.16 mg/kg bw, and the oral LD50 from 34.3 to 106 mg/kg bw. Clinical signs included salivation, fasciculation, lacrimation, tremors, irregular respiration and prostration, however the doses required to produce these signs were not indicated. Deaths were seen between 30 min and 2 h after IV dosing, and 2 h to 1 day after PO dosing.
Oral pretreatment with chlorfenvinphos markedly decreased the inhibition of brain ChE seen after an oral challenge dose, with pretreated animals showing 50 - 70% inhibition, while control animals (ie not pre-treated before dosing) had approximately 90% inhibition. There was no notable change in

the inhibition in brain ChE after IV dosing between control animals and those pretreated with chlorfenvinphos. Furthermore, the plasma concentration following IV dosing with chlorfenvinphos was not altered by pretreatment, although the liver concentration was decreased.

Following the oral challenge dose, both plasma and liver chlorfenvinphos concentrations were lower than those seen in animals not pretreated with chlorfenvinphos. Brain concentration of chlorfenvinphos was proportional to the plasma concentration, regardless of the method of administration. These results suggested that brain levels rapidly equilibrated to the plasma level, and the change in inhibition of brain ChE resulting from pretreatment may have been directly related to the decrease in plasma concentration. No explanation for the decrease in plasma concentration of chlorfenvinphos-pretreated animals was given, but it may have been a result of enzyme induction.

3. ACUTE STUDIES

3.1 Technical Grade Active Constituent

3.1.1 Median Lethal Dose Studies

Table 10: Mean lethal dose studies

Species	Sex	Route	Vehicle	LD50 or LC50*	Reference
mouse	?	PO	peanut oil	155	Newell, 1961
mouse	?	PO	peanut oil	145	Shellenberger & Newell, 1963
mouse	M	PO	peanut oil	133	Witherup & Schlecht, 1963
mouse	M	PO	PEG	117	Pickering, 1965
rat	M	PO	peanut oil	9.7	Ambrose *et al*, 1970
rat	F	PO	PEG	23.8	Pickering, 1965
rat	F	PO	peanut oil	12.8	Witherup & Schlecht, 1963
rat	?	PO	peanut oil	25	Shellenberger & Newell, 1963
rat	?	PO	peanut oil	33	Shellenberger & Newell, 1962
rat	M F	PO	peanut oil	15 13	Gaines, 1969
rat	?	PO	peanut oil	39	Newell, 1961
rat	M, F	PO	corn oil	31	Gardner, 1993
rabbit	M, F	PO	undiluted	500 - 1000	Brown, 1965
rabbit	?	PO	peanut oil	1250 - 2500	Newell, 1961
rabbit	M	PO	peanut oil	324	Witherup & Schlecht, 1963
rabbit	M	PO	peanut oil	300	Ambrose *et al*, 1970
guinea pig	M,F	PO	CMC**	125 - 500	Brown, 1965
fowl	M,F	PO	undiluted	44 - 62.5	Brown, 1965
dog (mongrel)	M,F	PO	corn oil	>12000	Ambrose *et al*, 1970
rat	M	IV	Lipomul	6.6	Ambrose *et al*, 1970
dog	?	IV	Lipomul	50.5	Ambrose *et al*, 1970
rat	M F	Dermal	xylene	31 30	Gaines, 1969
rat	M, F	Dermal	xylene	91.8	Brown *et al*, 1964
rat	F	Dermal	xylene	108	Pickering, 1965

rat	M F	Dermal	corn oil	358 450	Gardner, 1993
rabbit	M	Dermal	?	>200	Witherup & Schlecht, 1963
rabbit	M	Dermal		400	Ambrose *et al*, 1970
guinea pig	M,F	SC	Undiluted	500	Brown, 1965

* LD50's expressed as mg/kg bw, LC50's as mg/m^3

** carboxymethyl cellulose

? = not stated

3.1.1.1 Oral

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1-(2,4-dichlorophenyl)-2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (96% purity, source not specified) as a 1% solution in peanut oil was administered by gavage to fasted male Wistar rats (Albino Farms, Red Bank NJ) at doses of 5 - 20 mg/kg bw using 10/group. Signs included salivation, lacrimation, muscle fasciculation and diarrhoea (doses and severity not specified). The LD50 was 10 mg/kg bw.

Chlorfenvinphos (as above) as a 10% solution in peanut oil was administered by gavage to fasted male albino rabbits (source not specified) at doses of 160 - 400 mg/kg bw (10/group). Clinical signs were similar to those observed in rats, and the LD50 was 300 mg/kg bw.

Additionally, chlorfenvinphos was administered to mongrel dogs at doses of 1, 3, 6 or 12 g/kg bw by gavage, followed by 10 mL corn oil. Clinical signs were as described for the rat. Dogs also vomited. The LD50 was 12 g/kg bw (12 000 mg/kg bw). This higher LD50 was likely to have been influenced by emesis.

Newell GW (1961) Letter Report No. 73. Project PB 1008. Sponsor: Shell Development Company

The acute oral toxicity of chlorfenvinphos (code: 7859 1-3-0-0; source, purity not specified) in peanut oil was determined in mice (10/group), rats (10/group) and rabbits (1/group). Sources, sexes and strain of animals were not specified. Chlorfenvinphos was administered to mice at doses of 119 to 236 mg/kg bw; to rats at doses of 30 to 60 mg/kg bw; rabbits received 1250 or 2500 mg/kg bw. In rabbits, this was considered preliminary data, with more work to be done. Clinical signs observed in rats and mice included salivation, lacrimation, diarrhoea, tremors and convulsions; the doses at which clinical signs appeared or deaths occurred were not specified. The LD50 in mice was 155 mg/kg bw, in rats 39 mg/kg bw and in rabbits 1250 - 2500 mg/kg bw.

Shellenberger TE & Newell GW (1962) Letter Report No. 92. Project B 1008. Stanford Research Institute, Menlo Park. Sponsor: Shell Development Company

Chlorfenvinphos (SD 7859, Code 4100, source, purity not specified) in peanut oil was administered to rats (source, sex, strain not specified) at doses of 24.8, 31.3, 39.4 or 49.6 mg/kg bw PO, with an

unknown number of rats per group. Clinical signs were not described, however the LD50 was 33 mg/kg bw.

Shellenberger TE & Newell GW (1963) Letter Report No. 95. Project B 1008. Stanford Research Institute. Sponsor: Shell Development Company

Chlorfenvinphos (SD 7859 code 5100, source, purity not specified) in peanut oil was administered to mice and rats (sources, sex, strains not specified) using 10 animals/group. Mice received doses of 100, 126, 159 or 200 mg/kg bw, and rats were given doses of 19.9, 25, 31.5 or 39.8 mg/kg bw. At all doses signs of organophosphate intoxication (salivation, diarrhoea, lacrimation, tremors and clonic convulsions) were seen. The LD50 for mice was 145 mg/kg bw, and for rats was 25 mg/kg bw.

Gaines TB (1969) Acute toxicity of pesticides. Toxicol. Appl. Pharmacol. 14:515 - 534

Chlorfenvinphos (technical grade: batch no, source, purity not given) in peanut oil was administered to Sherman rats (source not specified) at unspecified doses, with no indication of the number of rats/group. Clinical signs were not described. The LD50 was 15 mg/kg bw for males and 13 mg/kg bw for females

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos (purity 96%, source: Shell Chemical Co) in peanut oil was given by gavage to female CD rats, male C3H mice and male NZW rabbits. Mice received doses of 55 to 280 mg/kg bw (5/group). Rats received doses of 3, 5, 7, 10, 16, 24 or 36 mg/kg bw (10/group), and rabbits were given doses of 44 or 80 mg/kg bw (1/group) or 120 to 940 mg/kg bw (3/group). The LD50 was 133 mg/kg bw in mice, 12.8 mg/kg bw in rats and 324 mg/kg bw in rabbits.

Pickering WR (1965) The acute toxicity of chlorfenvinphos to sheep and cattle when applied dermally. Vet Rec 77:1140 - 1144

Chlorfenvinphos (batch no, source, purity not specified) in polyethylene glycol 200 (PEG) was administered orally to female Sprague Dawley rats, male TO mice, Thornber cockerels and Rhode Island Red hens. The source of all animals and the doses and numbers of animals per group were not indicated. The oral LD50 in mice was 117 mg/kg bw, in rats was 23.8 mg/kg bw, in chicks was 36.6 mg/kg bw and in hens was 240 mg/kg bw.

Brown VKH, Moffett JA, Rees RO & Richardson A (1964) Preliminary studies on the toxicology of the chlorinated-aryl-vinyl phosphate insecticide, SD 7859 (GC 4072). Shell Research Limited, Sittingbourne TOX 4/64

Chlorfenvinphos (purity 92%, source: Woodstock Agriculture Research Centre) was administered by gavage to fasted Carworth Farm or Hooded Lister rats (2/sex/group), or to CF1 mice (5/sex/group). Rats were dosed at 5 to 25 mg/kg bw, and mice at 50 to 200 mg/kg bw. All animals were observed for 10 days following treatment. The LD50 was not determined precisely but in rats was in the range of 10 - 15 mg/kg bw, and in mice was 150 - 200 mg/kg bw.

Brown VKH (1965) Some further data on the acute and sub-acute toxicities of the insecticide SD 7859 (GC 4072). PPR TL/1/65 Shell Research Limited, Sittingbourne, Kent

Chlorfenvinphos (batch no FC1339, purity 92%, source: Shell Development Co) was tested for acute oral toxicity in fowl, guinea-pigs and rabbits. Additionally, the effect of a single oral dose on plasma ChE in beagle dogs was investigated.

White Leghorn chickens (Appleby Farm Ltd) were fasted overnight, then dosed by gelatin capsule with 31.25 to 125 mg/kg bw using 1/sex/group. In animals which subsequently died, there was either a flaccid paralysis or opisthotonic convulsions prior to death. Animals which survived only showed 'droopiness'. The LD50 was determined to be 44 - 62.5 mg/kg bw.

"P" strain guinea-pigs (Tunstall Laboratories) were dosed with chlorfenvinphos suspended in carboxymethyl cellulose by oral gavage at 62.5, 125, 250 or 375 mg/kg bw using 1/sex/group. Clinical signs included salivation, diarrhoea and muscle fasciculations, and the LD50 was in the range of 125 to 250 mg/kg bw.

New Zealand White rabbits (Tunstall Laboratories) were dosed, either by gelatin capsule or by gavage, with 125, 250, 500 or 1000 mg chlorfenvinphos/kg bw, using 1/sex/group. At the highest dose, excessive salivation and constricted pupils were observed. In the other treatment groups diarrhoea was the only adverse clinical signs. The LD50 was in the range of 500 to 1000 mg/kg bw.

A dose of 5 g/kg bw was administered to Beagle dogs (1/sex, Tunstall Laboratories) by gavage. Blood was taken 24 h after dosing, and irregularly after this, to determine plasma ChE activity. At 24 h, the male had approximately 75% inhibition and the female >90% inhibition. Recovery was slow, with the male having >60% inhibition at 28 d after dosing (female ~25% inhibition) (all inhibitions were estimated from a graphical presentation). During this trial, there were no abnormal clinical signs observed.

Puzynska, L (1984) Relationship between dietary protein level and enzymatic changes in acute poisoning of rats with chlorfenvinphos. Die Nahrung 8:779 - 787

The acute oral LD50 of chlorfenvinphos (purity, batch no, source not specified) was determined in Wistar rats (Food and Nutrition Institute, Poland) which had been maintained for 30 or 60 days on low protein (4.5% casein) or optimal (26% casein) diets. Food and water were available *ad libitum* throughout the feeding period.

Rats were fasted overnight prior to the administration of chlorfenvinphos in lecithin by gavage using 10 rats/group in the main trial. In addition to this, a number of rats (not specified) were given 30 mg chlorfenvinphos/kg bw by gavage, and euthanised 40 min later after clinical signs were evident. An unspecified enzymatic and histological examination was done. This was repeated 14 days later on animals which survived the initial exposure.

The acute oral LD50 in animals maintained on a low protein diet for 30 days was 10.56 mg/kg bw in males and 14.69 mg/kg bw in females. This was compared to the LD50 in those maintained on an optimal diet, which was 17.74 mg/kg bw in males and 17.02 mg/kg bw in females. When rats were maintained on the diets for 60 days, the differences were more notable, with the LD50 on the low protein diet being 7.43 mg/kg bw for males and 10.22 mg/kg bw for females, while the rats on the optimal diet maintained a similar LD50 to that previously seen.

Following a dose of 30 mg/kg bw, plasma and brain ChE activities were severely inhibited in rats on both diets, with slight differences between diets (plasma ChE activity approximately 95% inhibited in all animals, brain ChE activity inhibited from 72% in females on the optimal diet to 87% in females on the low protein diet). No details of the histological examination carried out were supplied, however it was stated that there were no changes attributable to the actions of chlorfenvinphos detectable in these rats.

Gardner JR (January 1993). BIRLANE technical: Acute oral and dermal toxicity in rat, skin and eye irritancy in rabbit and skin sensitisation potential in the pig. SBTR.92.037, Sittingbourne Research Centre, Sittingbourne, Kent, UK. GLP (USEPA, UK DHSS, OECD, Japan MAFF)

Chlorfenvinphos (Birlane technical, Batch F910271, 93.1% purity) in corn oil was administered by oral gavage to groups (5/sex/group) of fasted rats (Crl:CD.BR; Charles River, UK) at doses of 23, 30 or 50 mg/kg bw. The mean LD50 was 31 mg/kg bw/day.

Deaths occurred within 2 days of dosing at the HD level and at intervals until day 4. There were deaths in all groups (1/10, 4/10 and 10/10 for the LD, MD and HD groups respectively). Clinical signs (mostly within 4 h of dosing) included tremor, fasciculations, twitching and/or thrashing, splayed gait, salivation, lachrymation, hunched posture, pallor of the eyes, diarrhoea, unkempt appearance, piloerection, tachypnoea (HD only); lethargy, hypothermia and periorbital encrustation were seen in some surviving rats at 2 days. Recovery was complete by 10 days for surviving rats.

All surviving rats gained weight at a similar rate. The principal findings at postmortem for the animals that died were lung congestion, darkening or exaggerated lobular pattern in the liver, darkening or pallor of the spleen and kidneys and gaseous/yellow liquid contents of the stomach and small intestines. For the animals killed after 14 days, postmortem findings were limited to single cases of lung congestion, hepatic pallor and gaseous contents in the caecum.

3.1.1.2 Dermal Studies

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl) -2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (86% purity, source not specified) was applied to the closely clipped, non abraded, dorso-lumbar skin of male albino rabbits at doses between 200 and 673 mg/kg bw, using 10/group. It was not stated whether the application site was covered with an occlusive dressing following application. No skin irritation was observed, and the LD50 was determined to be 400 mg/kg bw.

Gaines TB (1969) Acute toxicity of pesticides. Toxicol. Appl. Pharmacol. 14:515 - 534

Chlorfenvinphos (technical grade; batch no, source, purity not specified) in xylene was applied to the skin of Sherman rats (source not specified) at unspecified doses for an unspecified time. It was not indicated whether the skin was clipped prior to application. The treated area was left uncovered. No description of clinical signs was given. The LD50 was 31 mg/kg bw in males and 30 mg/kg bw in females.

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos (purity 96%, source: Shell Chemical Co) was applied to the clipped dorso-lumbar area of 10 male New Zealand White rabbits at 200 mg/kg bw. The area was covered with an occlusive bandage for 24 h, following which the skin was well washed with soap and water. Rabbits were maintained for a 14 day observation period. At the end of this time 1/10 rabbits had died. The dermal LD50 of chlorfenvinphos was therefore >200 mg/kg bw.

In further studies, technical chlorfenvinphos was applied in a similar fashion at doses of 940 to 4700 mg/kg bw, using 2 rabbits/group. The LD50 was 3200 - 4700 mg/kg bw.

Pickering WR (1965) The acute toxicity of chlorfenvinphos to sheep and cattle when applied dermally. Vet Rec 77:1140 - 1144

Chlorfenvinphos (batch no, purity, source not specified) in xylene was applied dermally to female Sprague Dawley rats (source not specified), at unspecified doses with an unknown number of animals per group. It was not stated whether the skin was clipped or abraded, or whether the area was covered after application. The reported LD50 was 108 mg/kg bw.

Brown VKH, Moffett JA, Rees RO & Richardson A (1964) Preliminary studies on the toxicology of the chlorinated-aryl-vinyl phosphate insecticide, SD 7859 (GC 4072). Shell Research Limited, Sittingbourne TOX 4/64

Chlorfenvinphos (purity 92% source: Woodstock Agricultural Research Centre) in xylene was applied to the clipped neck area of Hooded Lister rats (Tunstall Laboratories) at doses of 50, 75, 112.5 or 168.75 mg/kg bw, using 5 male and 15 female rats/group. Animals were confined in a holding box for 24 h to prevent licking of the area, then were observed for 10 days. The LD50 was 91.8 mg/kg bw.

Newell GW (1961) Letter Report No 75 Project PB-1008. Stanford Research Institute, Menlo Park

Chlorfenvinphos (codes 1-3-0-0 and 1-6-0-0, purity and source not specified) was applied to the skin of rabbits (source, sex, strain not specified) at doses of 1250 or 2500 mg/kg bw using 3/group. It was not stated whether the skin was clipped, or whether the treated area was covered with an occlusive bandage. This treatment produced mild diarrhoea and the LD50 was determined to be between 1250 and 2500 mg/kg bw.

Gardner JR (January 1993) BIRLANE technical: Acute oral and dermal toxicity in rat, skin and eye irritancy in rabbit and skin sensitisation potential in the pig. SBTR.92.037, Sittingbourne Research Centre, Sittingbourne, Kent, UK. GLP (USEPA, UK DHSS, OECD, Japan MAFF)

Chlorfenvinphos (Birlane technical, Batch F910271, 93.1% purity) in corn oil was applied to the clipped backs of groups (5/sex/group) of rats (Crl:CD.BR; Charles River, UK) at doses of 185, 333 or 600 mg/kg bw. The test material was held in place with a gauze dressing for 24 h before the skin was washed. The animals were then observed for 14 days.

The mean LD50 was 358 mg/kg bw for males and 450 mg/kg bw for females. Mortalities occurred among MD (1M;1F) and HD (5M; 4F) rats, mostly on day 1 after dosing (1 MD female on day 3 and 1 HD female on day 4). Clinical signs were seen between 4 h and 2 days after dosing and included muscle fasciculations, twitching and salivation among MD and HD rats and yellow staining of urogenital fur at all dose levels; recovery was complete by day 10 for survivors. The application sites were commonly discoloured (brown) and showed erythema after removal of the dressing on day 2. These effects resolved by day 3 (erythema) or day 10 (discolouration). All surviving rats gained weight during the study.

Postmortem examination of the rats that died showed lung congestion, pallor of the spleen and kidneys, pallor or exaggerated lobular pattern of the liver, abnormal contents of the gastrointestinal tract (gaseous or yellow liquid contents) and slight vascular congestion of the dermal test sites. There were no macroscopic changes in rats that survived.

3.1.1.3 Intravenous Studies

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl) -2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (96% purity, source not specified) was administered as a solution with Lipomul (not otherwise specified) by IV injection to male Wistar rats (Albino Farms, Red Bank NJ) at doses of 2.5, 3.5, 5.0, 10, 20 or 25 mg/kg bw, using 10 rats/group. Clinical signs were salivation, lacrimation, muscle fasciculation and diarrhoea, but the dose at which signs occurred was not specified. The LD50 was 6.6 mg/kg bw**.**

Chlorfenvinphos (as above) was administered to mongrel dogs (source not specified, weighing 7.5 - 18.5 kg) at doses of 25, 35, 40, 65, 75 or 100 mg/kg bw IV, using 3 dogs/group. Clinical signs were as for the rat, however dogs were also observed to vomit. The LD50 was 50.5 mg/kg bw.

3.1.1.4 Subcutaneous Studies

Brown VKH (1965) Some further data on the acute and sub-acute toxicities of the insecticide SD 7859 (GC 4072). PPR TL/1/65 Shell Research Limited, Sittingbourne, Kent

Chlorfenvinphos (batch no FC 1339, purity 92%, source: Shell Development Company) was administered by SC injection into the right flank of "P" strain guinea-pigs (Tunstall Laboratories) at doses of 250, 500 or 1000 mg/kg bw, using 2/sex at the lowest dose and 1/sex/group at the two higher doses. All treated animals showed salivation, diarrhoea and muscle fasciculation. The LD50 was determined to be approximately 500 mg/kg bw.

Stearns SM & Albert JR (1974) Determination of the acute subcutaneous toxicity and effects on blood cholinesterase of SD 7857 7-1-0-6TKH (SUPONA) in the dog. TIR 73-018-74, Shell Development Company, Houston

Chlorfenvinphos (code SD.. 7859, 7-1-0-6TKH, source, purity not specified) in corn oil was administered to Beagle dogs (Hazelton Research Animals) at doses of 0, 25, 50 or 100 mg/kg bw

SC using 2/sex/group. Blood samples were taken 24 h prior and immediately prior to dosing, and 48 h after dosing to determine plasma and erythrocyte ChE inhibition using a colorimetric method.

A number of dogs (3/4 at 100 mg/kg bw and 2/4 at 50 mg/kg bw) were observed scratching the injection site. No other adverse clinical signs were observed. Plasma ChE was inhibited 97% at all doses in comparison to controls. Erythrocyte ChE was inhibited 40% at the lowest dose tested, with inhibition of 73% at the highest dose.

3.1.1.5 Inhalation Studies

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos (purity 96%, source: Shell Chemical Co) was administered by inhalation to 10 rats and 10 mice for 65 minutes, at an average concentration of 2000 mg/m^3 using whole body exposure. Within 48 h of administration 7/10 rats had died, while no mice had died. After 14 days, 4/10 mice had died, and 8/10 rats had died. No information regarding the clinical signs observed, or the cause of death was supplied.

Takahashi H, Yoshida M, Murao N & Maita K (1994) Different inhalation lethality between micron-sized and submicron-sized aerosols of organophosphorus insecticide, chlorfenvinphos in rats. Toxicol Lett 73: 103 - 111

Male Fischer 344 rats (Charles River Japan Inc., Kanagawa) were exposed to micron (>1µm) or submicron (<1 µm) particles of chlorfenvinphos (purity, batch no, source not specified) by nose-only inhalation for 4 h. Three trials were performed. In the first, groups of rats were exposed to the compound by inhalation without other intervention. In the second, a cannula was placed in the oesophagus of rats to prevent any gastric absorption of particles which were not absorbed through the lungs, and the rats were then exposed. In the third groups, rats had indwelling electrodes placed in the frontal cortex (active electrode) and parietal cortex (reference electrode) and a catheter inserted through the femoral artery to the level of the renal artery. The EEG, ECG, arterial blood pressure, respiratory flow and tidal volume of the rats was measure for 4 h, then they were exposed to the pesticide and monitored for 4 h.

Following exposure to micron-sized particles of chlorfenvinphos, the LC50 for intact rats was 133 mg/m^3 while the LC50 for rats with an oesophageal cannulation was 489 mg/m^3. When rats were exposed to submicron-sized particles, the LC50 in intact rats was 509 mg/m^3, and in cannulated rats was 475 mg/m^3. It therefore appeared that absorption from the GI tract played a major role in the toxicity of chlorfenvinphos by inhalation exposure, with a notable reduction in toxicity with particle sizes which would be drawn deep into the lung, and also when larger particles were prevented from reaching the stomach or intestinal tract. It was not possible to observe clinical signs during exposure, given the restraint of the rats. Survivors showed salivation, urination, exophthalmus, twitches and tremors.

In rats monitored closely throughout exposure, there was an initial increase in blood pressure and bradycardia, with prolonged TP intervals detected on ECG. The EEG activity became more erratic, with a slight decrease in voltage detected. The respiratory flow became more erratic prior to apnea. These signs were similar for both particle sizes.

3.1.2 Skin irritation

Gardner JR (January 1993) BIRLANE technical: Acute oral and dermal toxicity in rat, skin and eye irritancy in rabbit and skin sensitisation potential in the pig. SBTR.92.037, Sittingbourne Research Centre, Sittingbourne, Kent, UK. GLP (USEPA, UK DHSS, OECD, Japan MAFF)

Undiluted chlorfenvinphos (BirlaneE technical, Batch F910271, 93.1% purity; 0.5 mL) was applied to the clipped backs of three NZ White rabbits (sex not stated; Froxfield Farms UK, Ltd) and covered with a lint patch for 4 h before washing. There was very slight erythema at all of the dermal test sites within 1 h of removal of the dressings. The reaction persisted for at least 48 h but was completely resolved within 7 days.

Using the EEC guidelines, which allow quantitative assessment (average number affected) at 24, 48 and 72 hours, the overall average scores were 0.9 for erythema (very slight) and 0 for oedema. Based on this, chlorfenvinphos was not a skin irritant to the rabbit.

3.1.3 Eye irritation

Gardner JR (January 1993) BIRLANE technical: Acute oral and dermal toxicity in rat, skin and eye irritancy in rabbit and skin sensitisation potential in the pig. SBTR.92.037, Sittingbourne Research Centre, Sittingbourne, Kent, UK. GLP (USEPA, UK DHSS, OECD, Japan MAFF)

Undiluted chlorfenvinphos (Birlane technical, Batch F910271, 93.1% purity; 0.1 mL) was instilled into the lower conjunctival sac one eye of each of three NZ White rabbits (sex not stated; Froxfield Farms UK, Ltd). The immediate pain response and subsequent ocular reaction were recorded for up to 7 days after treatment.

There was a slight initial pain response when the test agent was instilled into the eyes. There were also slight conjunctival irritation and chemosis, and an ocular discharge developed in all rabbits within 1 h of dosing.

There was marked constriction of the pupil (miosis) in all treated eyes 1 and 4 h after instillation of chlorfenvinphos. All iridial and conjunctival reactions had resolved on the day after treatment. The cornea was not overtly affected, and no opacities resulted from treatment.

Using the EEC guidelines, which allow quantitative assessment (average number affected) at 24, 48 and 72 h, the overall average scores were 0 for all parameters (conjunctival chemosis, conjunctival redness, corneal opacity and iridial effects). Under the conditions of this study, chlorfenvinphos was determined to be non-irritating to the rabbit eye.

3.1.4 Skin sensitisation

Rose GP (1982) Toxicology of BIRLANE : The skin sensitizing potential of BIRLANE SBGR.82.112, Shell Research Institute, Sittingbourne UK

Chlorfenvinphos (batch no 61001, source: Shell Nederland Chemie, Pernis, purity 91.3%) was investigated for skin sensitising potential. 'P' strain guinea-pigs (Tunstall Laboratories) were used, and were housed in groups of 2 or 3. Food and water were available *ad libitum*.

A range finding study was conducted using 2 male and 2 female guinea-pigs. The shoulder region of each animal was closely shorn, and 0.1 mL of several dilutions (0.05, 0.1, 0.5 or 1% in corn oil) was injected intradermally each side of the midline. The animals were examined to determine the maximum tolerated dose. A further study, using doses of 0.3 mL of 50, 75 or 100% solution applied to the shorn flank, used 2 animals/sex/group. The treated area was covered with an occlusive bandage for 24 h, after which the skin was examined for irritation using a 4 point scale.

The main test was conducted using 10/sex in the positive control and test group, with a negative control group of 5/sex. The test was divided into an induction phase and a topical challenge. During the induction phase, test animals received chlorfenvinphos in 0.1 mL of Freund's complete adjuvant (FCA), corn oil or FCA/corn oil in a 50:50 mix. Positive control animals received DNCB in the same mixture as above, while negative control animals received only solvent injections. One week after the intradermal injections, the area was clipped, and patches of filter paper were moistened with test material or positive control as appropriate, placed over the area and covered with an occlusive dressing for 48 h.

Two weeks after the topical induction described above, the challenge procedure was carried out. An area on one flank was clipped, and a patch of filter paper moistened with the appropriate chemical was placed over the area, and covered with an occlusive bandage for 24 h. After this time, the patch was removed and the area examined for a response. The skin was also examined after 24 and 48 h, with responses scored at none, slight redness (trace), pink/red area (moderate), beet red area with well defined edges (marked).

Following induction with chlorfenvinphos, there was a trace response to the challenge test immediately after removing of the dressing. All signs of reaction had disappeared by 24 h after the end of exposure. In comparison, the positive control produced moderate reactions in all animals immediately after removal of the dressing, with reactions persisting at least at the trace level until 48 h after removal of the dressing. Based on these results, chlorfenvinphos was non-sensitising in guinea-pig skin.

Gardner JR (January 1993) BIRLANE technical: Acute oral and dermal toxicity in rat, skin and eye irritancy in rabbit and skin sensitisation potential in the pig. SBTR.92.037, Sittingbourne Research Centre, Sittingbourne, Kent, UK. GLP (USEPA, UK DHSS, OECD, Japan MAFF)

Groups of guinea-pigs (10/sex for treated group; 5/sex for controls) were used for a skin sensitisation test with chlorfenvinphos with or without Freund's complete adjuvant (FCA), using standard methodology (EEC Method B6) and with undiluted chlorfenvinphos for the topical induction and challenge stages of the test.

A range-finding study was conducted using 2 male and 2 female guinea-pigs (Dunkin-Hartley strain from Harlan Porcellus). The shoulder region of each animal was closely shorn, and 0.1 mL of several dilutions of chlorfenvinphos (Birlane technical, batch F910271, 93.1% purity) (0.06, 0.2, 0.6 or 2% in corn oil) was injected intradermally on each side of the midline. The animals were examined to determine the maximum concentration that caused no more than moderate irritation (>

2%, but actual concentration used in main test was not stated). A further study, with doses of 0.3 mL of 10, 25, 60 or 100% solution applied to the shorn flank, using 2/sex/group. The area was covered with an occlusive bandage for 24 h, after which the skin was examined for irritation. The undiluted tests material proved to be non-irritant and was used for the topical induction and challenge in the main test.

The main test was conducted using 10/sex in the test group, and 5/sex in a negative control group (there was no positive control group). During the induction phase, test animals received 0.1 mL of FCA, chlorfenvinphos in corn oil or chlorfenvinphos in FCA; negative control animals received only FCA and/or corn oil. One week after the intradermal injections, the area was clipped, and patches of filter paper were moistened with 0.3 mL undiluted test material, placed over the area and covered with an occlusive dressing for 48 h.

Three weeks after the topical induction, the challenge procedure was carried out. An area on one flank was clipped, and a patch of filter paper moistened with 0.3 mL undiluted chlorfenvinphos was placed over the area, and covered with an occlusive bandage for 24 h. After this time, the patch was removed and the area examined for a response. The skin was also examined after 24 and 48 h, with responses scored at none (0), slight redness (1), pink/red area (2), beet-red area with well defined edges (3).

All the animals showed a positive response immediately following removal of the challenge patches (1–2); 10/20 of the test animals showed a positive response at 24 h (1) and 9 of the same animals showed positive response after 48 h (1). These results indicated that chlorfenvinphos was a slight skin sensitising agent in guinea-pigs.

3.2 Formulations

3.2.1 Median Lethal Dose Studies

Table 11: Median Lethal Dose Studies

Formulation	Species	Sex	Route	LD50 (mg active/kg bw)	Reference
48% in xylene	mice	Not given	PO	>36	Witherup & Schlecht, 1963
24% in xylene	mice	Not given	PO	>18	Witherup & Schlecht, 1963
48% in xylene	rat	Not given	PO	<24	Witherup & Schlecht, 1963
24% in xylene	rat	Not given	PO	>12	Witherup & Schlecht, 1963
24% in Shellsol A	rat	M,F	PO	11.5	Muir, 1970
24% wettable powder	rat	M,F	PO	5.2	Muir, 1970
5% dust	rat	M,F	PO	7.2	Muir, 1970
10% granule	rat	M, F	PO	M-30, F-17.3	Anon, 1973

48% in xylene	rabbit	Not given	PO	>24	Witherup & Schlecht, 1963
24% in xylene	rabbit	Not given	PO	>12	Witherup & Schlecht, 1963
50% seed dressing	rat	M,F	PO	5.4	Stevenson, 1974
24.5% solution	rat	M,F	PO	8.6	Reno, 1975a
0.5% dust	rat	F	PO	9.2	Wallwork & James, 1975
0.5% dust	rat	M	PO	7.1	Williams & James, 1975
1% in propylene glycol	rat	F	PO	15.5	Taylor et al, 1979b
21.2% surface spray	rat	M,F	PO	9.5	Reno, 1975b
8.84%	rat	M,F	PO	M - 18.4, F - 24	Tait, 1990a
12.77%	rat	M,F	PO	M - 16, F - 9	Collier 1982
15% collar ground in agar	rat	M,F	PO	M - 35.4, F - 22.1	Lee & Johnston, 1980
24% in Shellsol A	rat	M, F	Dermal	27	Muir, 1970
32% liquid seed dressing	rat	M,F	Dermal	32	Muir, 1970
25% wettable powder	rat	M,F	Dermal	wet - 26 dry - M>200, F - 97	Muir, 1970
5% dust	rat	M,F	Dermal	wet - 32 dry - M>800, F - 400	Muir, 1970
40% powder seed dressing	rat	M, F	Dermal	dry-M>2000, F - 1600	Muir, 1970
10% granules	rat	M,F	Dermal	dry - >800	Muir, 1970
10% granules	rat	M,F	Dermal	dry - >500	Anon, 1973
50% seed dressing	rat	M,F	Dermal	>128	Stevenson, 1974
6.5% EC	rat	M,F	Dermal	M - 85, F - 99	Tucker, 1979
49% in xylol	rabbit	M	Dermal	1087	Ambrose et al, 1970
24.5% solution	rabbit	Not given	Dermal	>49	Reno 1975a
21.2% surface spray	rabbit	Not given	Dermal	>42.4	Reno 1975b
50% seed dressing	rat	M,F	Inhalation	M - 203, F - 63.4	Moffett & Roderick, 1974
21.2% surface spray	rats	M,F	Inhalation	>1950 mg/m^3	Reno 1975b

3.2.1.1 Oral Studies

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos (source: Shell Chemical Company) in two formulations was administered to male C3H mice, female CD rats and male NZW rabbits (10 animals/group). Solutions of 48% and 24% chlorfenvinphos in xylene (XP-636 and XP-635 respectively) were administered at 75 mg/kg bw to mice, and 50 mg/kg bw to rats and to rabbits. Over the 14 day test period, 1/10 mice given XP-636 died, while none of those give XP-635 died. In rats, 8/10 died at 50 mg/kg bw XP-636 (equivalent to 24 mg active/kg bw), and 2/10 rats given XP-635 died. No rabbits died following dosing. The LD50 in mice was >75 mg formulation/kg bw (>36 mg active/kg bw), the LD50 in rats was < 50 mg/kg bw for XP-636, but > 50 mg/kg bw for XP-635 (12 - 24 mg active/kg bw), while in rabbits the LD50 was > 50 mg XP-636/kg bw (>24 mg active/kg bw).

Muir CMC (1970) The acute oral and percutaneous toxicities to rats of some currently marketed BIRLANE formulations. TLGR.0016.70 Shell Research Limited, Sittingbourne

The acute oral LD50 of a number of formulations of chlorfenvinphos was investigated using Carworth Farm E (CFE) rats (Tunstall Laboratories). The formulations tested were EF 2395 (24% chlorfenvinphos in Shellsol A), EF 2227 (24% chlorfenvinphos as a wettable powder) and EF 2353 (5% chlorfenvinphos as a wettable powder). In each trial the formulation was administered by gavage to fasted rats, using 4/sex/group. Food and water were available *ad libitum* through the 10 day observation period following dosing. The doses administered were not specified, however the LD50s were determined to be: 11.5 mg active/kg bw for EF 2395; 5.2 mg active/kg bw for EF 2227 and 7.2 mg active/kg bw for EF 2353 .

Anon. (1973) Toxicity studies on the pesticide BIRLANE. The acute oral and percutaneous toxicities of BIRLANE Granules (EF 3651) to rats. TLTR.0014.73 Shell Research Institute, Sittingbourne

A chlorfenvinphos formulation, containing 10% chlorfenvinphos in granules with 2% dichlorvos (source: Shell Research Limited) was administered by gavage to fasted CFE rats (Tunstall Laboratory) at doses of 80, 200, 320, 400, 440 or 480 mg formulation/kg bw, using 8 rats/sex/group. Animals were observed for 10 days following dosing, with food and water available *ad libitum*. Clinical signs were not reported in this study. The LD50 was 300 mg formulation/kg bw in males (equivalent to 30 mg active/kg bw) and 173 mg formulation/kg bw in females (equivalent to 17.3 mg active/kg bw).

Stevenson DE (1974) Toxicity of insecticides. Skin and eye irritancy and acute toxicity of BIRLANE 50% seed dressing. TLTR.0015.74 Shell Research Institute, Sittingbourne

Chlorfenvinphos, as a 50% seed dressing (source: Deutsche Shell Chemie GmbH) was administered to fasted CFE rats (Tunstall Laboratories) at doses of 4, 6, 8, 10, 15, 20 or 30 mg formulation/kg bw, using 4 rats/sex/group. Clinical signs were tremors, ataxia and fasciculation,

which occurred within 24 h of dosing, although it was not specified at which doses these signs occurred. The LD50 was 10.8 mg formulation/kg bw (equivalent to 5.4 mg active/kg bw).

Reno FE (1975a) Acute oral toxicity - rats, Acute dermal toxicity - rabbits, Acute inhalation toxicity - rats. Dermaton dip. Final report. Doc Code HIBG/75/0007. Hazleton Laboratories America Inc. Sponsor: Burroughs-Wellcome & Co

Dermaton dip containing 24.5% chlorfenvinphos (source, batch no not specified) was given orally to fasted Charles River Sprague-Dawley rats (10/sex/group) at doses of 21.5 to 68.1 mg formulation/kg bw. Rats were observed immediately, and at 1, 4 and 24 h after dosing, then daily for 14 days. Post-mortem examination was performed on all animals dying during the study, and all animals at scheduled sacrifice.

Abnormal clinical signs were seen at all doses, including lethargy, tremors, ataxia, salivation and laboured respiration. Post-mortem examination of animals dying during the study revealed congested livers and gastrointestinal tracts. There were no abnormalities detected at post-mortem examination of animals at terminal sacrifice. The LD50 was 35 mg formulation/kg bw (equivalent to 8.6 mg active/kg bw).

Wallwork LM & James JA (1975) 0.5% Supona Dust - Rat Oral Toxicity. Doc Code HIBG 75-13 Lab Ref No TL 38-75. Research & Development (V & A). The Wellcome Foundation Ltd

Chlorfenvinphos (0.5% dust, batch no E-103, source not specified) was given orally to fasted female Wistar rats (source not specified) at doses of 2.5 to 20 mg chlorfenvinphos/kg bw. The dust was made into a 50% aqueous suspension. Rats were observed for 14 days following treatment. Post-mortem examination was done on all animals dying during the study and on all remaining animals at terminal sacrifice. Hypersensitivity (not otherwise described) was reported at the lowest two doses, while tremors were seen at 5 mg/kg bw. At doses of 10 and 20 mg/kg bw no clinical signs were recorded, as all animals were dead at the first observation time of 4 h after dosing. On post-mortem examination, there were signs of lung congestion at the 2 highest doses, while there were no abnormal findings in the animals surviving until the end of the test at the lower doses. The acute oral LD50 was 9.2 mg active/kg bw.

Williams LM & James JA (1975) 0.5% Supona Dust - Rat Oral Toxicity. Doc Code HIBG 75-19 Lab Ref No TL 43-75. Research & Development (V & A). The Wellcome Foundation Ltd

Chlorfenvinphos (0.5% dust, batch no E-103, source not specified) was given orally to fasted male Wistar rats (source not specified) at doses of 2.5 to 20 mg chlorfenvinphos/kg bw. The dust was made into a 50% aqueous suspension. Rats were observed for 14 days following treatment. Post-mortem examination was done on all animals dying during the study and on all remaining animals at terminal sacrifice. Hypersensitivity (not otherwise specified) and muscle tremors were seen at all doses at 1 h. By 24 h, animals had either recovered or had died. On post-mortem examination, there were signs of lung congestion in animals dying during the test, while there were no abnormal findings in the animals surviving until the end of the test at the lower doses. The acute oral LD50 was 7.1 mg active/kg bw.

Kapp RW (1976a) Acute oral toxicity study in rats Cooper-Phos DF Livestock Insecticide. Final Report. Doc Cod HIBG 76-13 270-76. Hazleton Laboratories America Inc.

Chlorfenvinphos (as Cooper-Phos DF Livestock Insecticide; source, batch no, purity not specified) was administered by gavage to Sprague-Dawley caesarean-derived rats (Charles River Laboratories, Wilmington Mass.) using 5/sex/group. Male rats received between 10 and 100 mg formulation/kg bw, while female rats received between 2.5 and 15.9 mg formulation/kg bw. Rats were observed immediately after dosing, at 1 and 4 h after dosing then daily for 14 days. Post-mortem examinations were done on all animals dying during the study and all rats at terminal sacrifice.

Male rats at 10 mg formulation/kg bw showed listlessness, a hunched appearance, tremors, ataxia and chromodacryorrhea. All male rats at higher doses were found dead within 1 h of dosing. In female rats, signs were similar; additionally females showed dyspnea, excessive salivation, epistaxis and prostration. Post-mortem signs included discolouration of the kidneys and livers, and blanched stomachs and gastro-intestinal tracts. The acute oral LD50 in males was <15.9 mg formulation/kg bw, but could not be determined more accurately than this. The acute oral LD50 in females was 13.3 mg formulation/kg bw. As the percentage chlorfenvinphos in the formulation was not provided, the LD50 of the active could not be determined.

Taylor PE, Piercy DWT & James JA (1979b) Acute oral toxicity of a suspect batch of Supona. Doc Red HIBG 76/13. Wellcome Report, Berkhamsted UK

The acute oral toxicity of a suspect batch of chlorfenvinphos (believed to be off-specification) in comparison to a standard batch was determined in female Sprague-Dawley rats (source; OLAC 1976 Ltd). Chlorfenvinphos (1% w/v in propylene glycol; source: Kelvindale; batch no not specified) in both standard and suspect batches was given to fasted rats at 6.25, 12.5, 17.6 or 25 mg active/kg bw using 6 rats/group. Rats were observed for 14 days after dosing, with post-mortem examination done on all animals dying during the study and all animals at termination. Food and water were available *ad libitum* throughout the observation period after dosing.

Abnormal clinical signs were seen with the standard solution at 12.5 mg/kg bw and above, and with the suspect solution at all doses ie at 6.25 mg active/kg bw and above. They included whole body tremors, salivation, lethargy, blood stained eyes and muzzle and piloerection. Post-mortem examination of animals dying following dosing with the standard solution showed congestion of the lungs and thymus, excess fluid in the stomach and small intestine, mottling or the liver and discolouration of the spleen. Post-mortem examination of animals dying following dosing with the suspect solution showed mottling of the liver, congestion of the liver and lungs, excessive mucus and blood in the ileum and jejunum, and thymic haemorrhages. The acute oral LD50 for the standard solution was 15.5 mg active/kg bw, while the acute oral LD50 for the suspect solution was 16.6 mg active/kg bw. The toxicity of the suspect solution did not differ markedly from that of the standard solution.

Taylor PE & James JA (1984c) Acute oral toxicity of Supona/Diazinon 45/45% w/v EC (with 8.2% Lankroflex L) in the male rat. Doc Ref HIBG 84/13. Wellcome Report, Berkhamsted UK

A chlorfenvinphos/diazinon emulsifiable concentrate solution (45/45% w/v, ref no 716/71/1P, source not specified) was tested for acute oral toxicity in male Sprague-Dawley rats (source not specified) using 6/group. Fasted rats were dosed with 0.006, 0.008, 0.01 or 0.012 mL/kg bw (equivalent to 2.7, 3.6, 4.5 or 5.4 mg chlorfenvinphos/kg bw) and observed for 14 days. Rats were housed in treatment groups with free access to food and water. Post-mortem examination was conducted on all animals dying during the study and all animals at the end of the study.

At 0.008 mL/kg bw one animal showed whole body tremor at 5 h after treatment. At the high dose, two animals died, while the only other notable sign was muscle fasciculation seen in all animals at 3 h after dosing. On post-mortem examination the animals that died showed liver and lung congestion and fluid contents in the small intestine; no other abnormalities were detected on post-mortem examination. The LD50 was higher than the highest dose administered of 0.012 mL/kg bw (5.4 mg chlorfenvinphos/kg bw).

Taylor PE & James JA (1984d) Acute oral toxicity of Supona/Diazinon 45/45% w/v EC (with 8.2% w/v Lankroflex L) in the female rat. Doc Ref HIBG 84/14. Wellcome Report Berkhamsted UK

A chlorfenvinphos/diazinon emulsifiable concentrate solution(45/45% w/v, ref no 716/71/1P, source not specified) was tested for acute oral toxicity in female Sprague-Dawley rats (source not specified) using 6/group. Rats were dosed at 0.006, 0.012, 0.013, 0.015 and 0.024 mL/kg (equivalent to 2.7, 5.4, 5.9, 6.8 or 10.8 mg chlorfenvinphos/kg bw) and observed for 14 days. Rats were housed in treatment groups and food and water were available *ad libitum*. Post-mortem examination was conducted on all rats dying during the study and all rats at the end of the study.

At 0.012 mL/kg bw rats showed tremors and muscle fasciculations from 3 to 5 h after treatment. At 0.0132 mL/kg bw one animal died, while all animals showed tremors and urine staining of the perineum for 5 h after treatment, and blood staining of the eyes and muzzle for 2 days. At the 2 highest doses, all animals died in the first 2 days after treatment with convulsions, tremors, piloerection and blood staining of the eyes and muzzles. On post-mortem examination animals dying during the study showed lung and liver congestion and fluid-filled intestines. The acute oral LD50 was not precisely determined in this study, but was between 0.013 and 0.015 mL/kg bw (5.9 and 6.8 mg chlorfenvinphos/kg bw).

Reno FE (1975b) Acute oral toxicity - rats; acute dermal toxicity - rabbits, acute inhalation toxicity - rats - residual surface spray: Final report. Doc Code HIQG 75/0001

Chlorfenvinphos (as Residual Surface Spray containing 21.2% chlorfenvinphos; source Cooper USA Inc. batch no. not given) was administered orally to fasted Sprague-Dawley rats (source not specified) at doses of 10, 17.8, 31.6, 56.2, 100 or 1000 mg formulation/kg bw, using 5 rats/sex/group. Observations were made immediately after dosing and at 1, 4 and 24 h after treatment, then once daily for 14 days. Post-mortem examination was performed on all animals dying during the study and on all animals at terminal sacrifice.

In males at 17.8 mg formulation/kg bw there was laboured respiration and depression on day 2; no animals died at this dose. At 31.6 mg formulation/kg bw, 2/5 died within 4 h after dosing while survivors showed depression, tremors and laboured respiration through day 5. At 56.2 mg

formulation/kg bw, 2 animals died within 1 h of dosing. Clinical signs were similar to those seen at 31.6 mg/kg bw. At the highest two doses, all animals died within 1 to 4 h after dosing.

In females at the lowest dose 4/5 rats were slightly depressed 1 h after dosing. At the next dose, rats showed slight to severe depression, slight tremors and laboured respiration during day 1. At 31.6 mg formulation/kg bw, 2 animals died within 4 h of dosing. Survivors showed slight to severe depression, tremors, salivation and laboured respiration through day 3. At 56.2 mg formulation/kg bw, 3/5 died within 4 h after dosing. The survivors showed depression, salivation, tremors and laboured respiration through day 8. At the two highest doses all animals died within 1 h of dosing.

On post-mortem examination, there were no abnormalities noted in animals surviving until study termination. Animals dying during the study showed poor differentiation between the kidney cortex and medulla, dark red areas on the lungs and reddened pyloric areas of the stomach. The acute oral LD50 was 44.6 mg formulation/kg bw (equivalent to 9.5 mg active/kg bw). No individual animal data on clinical signs were submitted for this study.

Tait AJ (1990a) Acute toxicity and dermal irritancy of Supona Fly Dip (concentrate) Doc Ref RRS-90-22. Pitman-Moore Report, Harefield UK GLP: UK/OECD

Chlorfenvinphos (as Supona Fly Dip; 8.84% chlorfenvinphos; product code D197; batch no 2: source; Coopers, Berkhamsted) was administered by gavage to fasted CD rats (Charles River, Kent, UK) in a dose-ranging study and a main toxicity test. In the dose-ranging study using 1 rat/sex/group, the product was administered at 63, 125, 250 or 500 mg product/kg bw. Based on these results, the main study was conducted using doses of 77, 139, 250 or 450 mg product/kg bw, using 5 rats/sex/group. Rats were housed in treatment groups with food and water available *ad libitum*. Following dosing, rats were observed 3 times in the first hour, then at 3 and 5 h after dosing and twice daily for 14 days after this. Post-mortem examination of all rats dying during the study, and all rats at terminal sacrifice was done.

No deaths were seen at the lowest dose, with the only abnormal clinical signs being decreased motor activity and tremors. At 125 mg product/kg bw one rat/sex died; clinical signs observed included ataxia, salivation, tremor, bradypnoea and hyperpnoea. At 250 mg product/kg bw 3/5 male and 2/5 female rats died, with signs similar to those at 125 mg product/kg bw. At the highest rats all males and 4/5 females died. The surviving females showed ataxia and decreased motor activity. On post-mortem examination, the only abnormal clinical signs were seen at the two highest doses, and were increased fluid content in stomach and intestines. The LD50 was 208 mg product/kg bw for males and 272 mg product/kg bw for females. These doses were equivalent to 18.4 mg active/kg bw and 24 mg active/kg bw respectively.

Collier M (1982) An acute oral toxicity (LD50) study in the rat with Dermaton II (BW 0023Z61) Burroughs Wellcome Report, USA; GLP: USA

Chlorfenvinphos (Dermaton II, reformulation C, batch BW 0023Z61; 12.77% chlorfenvinphos: source; Burroughs Wellcome Co) was given by gavage to fasted Charles River CD rats (Charles River Breeding Laboratories, MA) at doses of 30, 60, 90 or 150 mg product/kg bw for males and 50, 65, 80 or 95 mg product/kg bw for females, using 10 rats/sex/group. Animals were observed on the day of dosing and for 14 days after dosing. Rats were weighed pre-dose and on days 7 and 14. All rats dying on the day of dosing were examined post-mortem to determine if mis-dosing

was the cause of death. Animals dying during the study were not examined; at the end of the study, survivors from the two highest doses were examined for macroscopic abnormalities.

On the day of dosing muscle fasciculations and tremors were seen in animals from each group. Salivation was seen in all groups of females, and in all groups of males except the lowest dose group. All groups of females also showed ataxia, while laboured breathing was also seen. During the observation period, rats had perineal staining, red material around eyes nose or mouth and rough coats. Other clinical signs included decreased activity, body tremors and laboured breathing. No post-mortem findings related to treatment were detected. The acute oral LD50 was 126 mg product/kg bw for males (equivalent to 16 mg active/kg bw) and 74 mg product/kg bw for females (equivalent to 9 mg active/kg bw).

Lee I & Johnston RE (1980) An acute oral toxicity study in the rat using ground Dermaton collars (15% Supona) Doc Ref TTEP/80/0018. Burroughs Wellcome Report, USA

Dermaton collars (15% chlorfenvinphos; batch no VW 0023Z61; Ref no FS3; source Burroughs Wellcome Co.) were ground to powder, suspended in sterile agar and administered by gavage to fasted CD rats (Charles River Breeding Laboratory, MA) at doses of 50, 250, 500 or 1000 mg product/kg bw, using 10 rats/sex/group. A ground collar (not containing any chlorfenvinphos) was suspended in agar and administered to 10 rats/sex at 1000 mg/kg bw as a control. Rats were observed daily for 14 days after dosing. Post-mortem examination was conducted on all rats dying during the study, and all remaining rats at the end of the study. Rats were housed in groups of five and allowed free access to food and water during the observation period.

Abnormal clinical signs were observed at doses of 250 mg product/kg bw and above, and included salivation, lacrimation, tremors, clonic and/or tonic convulsions and dyspnea. There were no abnormal clinical signs seen in the control group receiving ground collar in agar. The results of post-mortem examinations were not reported. The acute oral LD50 for males was 235 mg product/kg bw (equivalent to 35.4 mg active/kg bw) and for females was 214 mg product/kg bw (32.1 mg active/kg bw).

3.2.1.2 Dermal Studies

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl) -2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (49% formulation in xylol) was applied to the shorn dorso-lumbar skin of male albino rabbits at doses of 1.25 - 2.5 mL/kg bw (equivalent to 588 - 1177 mg active/kg bw), and covered with an occlusive bandage for 24 h. At the end of this time, the bandage was removed and the skin washed. After 3 days, the skin appeared irritated, with erythema and desquamation observed. The skin was dry and leathery. After 14 d, the skin had returned to normal. Post mortem examination of visceral and thoracic organs revealed no visible abnormalities. The LD50 was 1097 mg active/kg bw.

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos (48% or 24% in xylene, source: Shell Chemical Co) was applied to the shorn skin of 10 male NZW rabbits at 200 mg formulation/kg bw, and covered with an occlusive bandage for 24 h. The treated area was then washed with soap and water, and the animals observed for 14 d. There were no mortalities observed with the 24% formulation, and 1/10 rabbits died when the 48% solution was applied. The LD50 was >200 mg formulation/kg bw (>96 mg active/kg bw).

Muir CMC (1970) The acute oral and percutaneous toxicities to rats of some currently marketed BIRLANE formulations. TLGR.0016.70 Shell Research Limited, Sittingbourne

The acute dermal toxicity of a number of formulations of chlorfenvinphos was investigated using CFE rats (Tunstall Laboratories). The formulations tested were EF 2395 (24% in Shellsol A), EF 2502 (32% liquid seed dressing), EF 2227 (25% wettable powder), EF 2353 (5% field strength dust), EF 2424 (40% powder seed dressing) and EF 2387 (10% granules). The formulations EF 2395, EF 2502, EF 2227 and EF 2353 were applied either undiluted or in suspension, and the formulations EF 2227, EF 2353, EF 2424 and EF 2387 were applied as the dry formulation. Each formulation was applied to the shorn dorso-lumber skin of 4 rats/sex/group at unspecified doses, and covered with an occlusive dressing of aluminium foil and adhesive tape for 24 h. After this time, the bandage was removed and the skin washed with a weak detergent solution. Animals were observed for 10 days after dosing. It was recognised that there were difficulties with applying formulations in the dry state, as at high doses the formulation tends to form a layer on the skin, and the animal is not exposed to the entire dose.

The LD50 for the formulations applied as liquids were: EF 2395 - 27 mg active/kg bw; EF 2502 - 32 mg active/kg bw; EF 2227 - 26 mg active/kg bw and EF 2353 - 32 mg active/kg bw. For the formulations applied in the dry state, the LD50s were: EF 2227 - male >2000 mg active/kg bw, female 92 mg active/kg bw; EF 2343 - male >800 mg active/kg bw, female 400 mg active/kg bw; EF 2424 - male >2000 mg active/kg bw, female 1600 mg active/kg bw and for EF 2387 - >800 mg active/kg bw.

Anon. (1973a) Toxicity studies on the pesticide BIRLANE. The acute oral and percutaneous toxicities of BIRLANE Granules (EF 3651) to rats. TLTR.0014.73 Shell Research Institute, Sittingbourne

A formulation containing 10% chlorfenvinphos and 2% dichlorvos was applied as dry granules to the shorn dorso-lumbar skin of CFE rats (Tunstall Laboratories) at doses of 1000, 2500 and 5000 mg formulation/kg bw using 2 rats/sex/group. The area was covered with an occlusive dressing for 24 h, following which the bandage was removed and the skin well washed with a detergent solution. Animals were observed for 9 days following treatment. No clinical signs were reported. The LD50 was >5000 mg formulation/kg bw (>500 mg active/kg bw).

Stevenson DE (1974) Toxicity of insecticides. Skin and eye irritancy and acute toxicity of BIRLANE 50% seed dressing. TLTR.0015.74 Shell Research Institute, Sittingbourne

Chlorfenvinphos as a 50% seed dressing (source: Deutsche Shell Chemi GmbH) was applied as an aqueous solution to the shorn dorso-lumbar skin of CFE rats (Tunstall Laboratories) at doses of 4, 8, 16, 32, 67 or 128 mg formulation/kg bw, and the treated area was covered with an impermeable dressing for 24 h. Food was removed during this period, although water was

available *ad libitum*. The dressings were then removed and the skin washed with a dilute detergent solution. Rats were observed for 9 days, and no signs of toxicity were seen during this period. The LD50 was >128 mg formulation/kg bw (equivalent to > 64 mg active/kg bw).

Palmer JS (1964) Toxicologic evaluation of an insecticide, Compound 4072, in cattle. J Am Vet Med Assoc 144:268-271

Chlorfenvinphos (source: Shell Chemical Co, Modesto CA; batch no not specified) was applied to yearling cattle and young dairy calves. A number of formulations were used, including a 25% wettable powder, a 98% emulsifiable concentrate and a 24% emulsifiable concentrate. Cattle were sprayed by power spraying at 200 psi, using approximately 1 - 2 gallons (3.8 - 7.6 L) per animal.

Calves were sprayed with the formulations made up to 0.1%, 0.25%, 0.3%, 1% or 2%. At the lowest concentration, the average ChE inhibition was 31%. There were no clinical signs of poisoning at this dose. Mild clinical signs were seen at 0.25%, with ChE inhibition of 61%. Signs and ChE inhibition increased in severity with increasing doses.

Yearling cattle were treated with formulations ranging in concentration from 0.1% to 1%. Mild clinical signs were seen at 0.25%, both with the wettable powder and the emulsifiable concentrate formulation. ChE inhibition was seen from 0.15%, with inhibition between 36% and 64%. No ChE inhibition or abnormal clinical signs were seen at 0.1% in yearling cattle.

Based on these findings, the author considered that a spray concentration of 0.1% would be considered safe to apply to yearling cattle, but would not be able to be used on calves.

Palmer JS (1964) Tolerance of Brahman cattle to organic phosphorus insecticides. J Am Vet Med Assoc 144:143-145

Chlorfenvinphos (source: Shell Chemical Co, NY) as a 25% wettable powder or a 24% emulsifiable concentrate, was applied by sprayer to Brahmin-cross and mixed breed cattle of one year of age or older. Up to 7.6 L was applied to each animal, at spray concentrations of 0.15% or 0.25%. Chlorfenvinphos was also administered orally at 10 or 20 mg/kg bw. The Brahmin cattle had more ChE inhibition than the other cattle, and showed more serious clinical signs at 25 mg/kg bw PO. The Brahmin-cross animals were therefore less tolerant of chlorfenvinphos than were the other breeds.

Reno FE (1975) Acute oral toxicity - rats, Acute dermal toxicity - rabbits, Acute inhalation toxicity - rats. Dermaton dip. Final report. Doc Code HIBG/75/0007. Hazleton Laboratories America Inc. Sponsor: Burroughs-Wellcome & Co

Chlorfenvinphos (24.5% solution as Dermaton dip; source, batch no not specified) was applied to the clipped abdominal skin of New Zealand White rabbits (source and sex not specified) at 200 mg product/kg bw and covered with an occlusive bandage for 24 h. The skin sites of half of the rabbits were abraded while the skin sites of the remaining animals were left intact. After the 24 h exposure, the bandage was removed and the skin washed with tap water. Animals were observed for 14 days after treatment. Post-mortem examination was done on all animals dying during the study, and on all animals at terminal sacrifice.

No animals died during the observation period. The only abnormal clinical signs observed in animals with abraded skin were slight depression and a nasal discharge; moderate anorexia was seen in one intact animal. There was slight to moderate erythema and desquamation of the treated skin, with some atonia and blanching. On post-mortem examination there were no abnormalities seen. The acute dermal LD50 was >200 mg formulation/kg bw (equivalent to > 49 mg active/kg bw).

Kapp RW (1976c) Acute dermal toxicity study in rabbits - Cooper-Phos DF Livestock insecticide - Final Report. Doc Ref HIBG 76/14. Hazleton Laboratories, Virginia USA

Chlorfenvinphos (as Cooper-Phos DF Livestock Insecticide; purity 77.18%; source, batch no and full formulation not given) both undiluted and diluted 1:2000 in water was applied to the clipped abdominal skin of New Zealand White rabbits (Bunnyville Farms, Littlestown PA; sex not specified). The undiluted formulation was applied at 1000, 2150 and 10 000 mg formulation/kg bw (corrected for specific gravity of 0.772 g/mL)while the diluted formulation was applied at 1000 and 10 000 mg formulation/kg bw (corrected for specific gravity of 1.030 g/mL). Two rabbits/group had the application site abraded, while two had the skin left intact. The application site was covered with an occlusive bandage for 24 h. On removal of the bandage the skin was washed with water. Rabbits were observed daily for 14 days, including scoring of the dermal irritation using a company scoring system. Post-mortem examination was done on all animals dying during the study and on all animals at terminal sacrifice.

Animals treated with the low dose of the undiluted formulation had tremors on days 1 to 3, were anorexic on days 3 to 10, and were listless on days 1 to 8. In the mid-dose group, 2 animals died, while the rest showed anorexia and listlessness until day 6. Dyspnea, salivation, ataxia, prostration and tremors were seen between days 2 and 4. All animals in the high-dose group died on day 2. There were well-defined erythema in animals with abraded skin, and slight erythema on day one in animals with intact skin. This resolved to very slight erythema on days 2 to 3 and had resolved in the low-dose animals by day 4. On post-mortem examination there was congestion of the lungs and abdominal cavity in animals dying during the study, and congestion of the renal cortico-medullary junction in a few animals and the low- and mid-dose. The LD50 of the undiluted formulation was 2665 mg formulation/kg bw.

With the diluted formulation, there were no deaths, abnormal clinical signs, dermal irritation or abnormal findings on post-mortem examination.

Taylor PE & James JA (1984a) Acute dermal toxicity of Supona/Diazinon 45/45% w/v EC (with 8.2% Lankroflex L) in the male rat. Doc Ref HIBG 84/11. Wellcome Report, Berkhamsted UK

A chlorfenvinphos/diazinon mixture (45/45% w/v EC; Ref No 716/71/1P; source not specified) was applied to the shorn dorso-lumbar region of male Sprague-Dawley rats (OLAC 1976 Ltd) and covered with an occlusive bandage for 24 h. After this, the dressing was removed and the area washed with detergent and warm water. Rats were individually housed and food and water were available *ad libitum*. The material was applied at 0.707, 1 and 10 mL/kg using 6 rats/group. Rats were observed for 14 days after treatment, with all abnormal clinical signs recorded. Post-mortem examination was done on all rats dying during the study, and all surviving rats after 14 days.

In the low dose at 24 h, rats showed tremors and blood stained eyes. In the mid-dose at 24 h, rats showed blood stained eyes and muzzles, laboured breathing and muscle fasciculations and 1/6 rats died, while at the high dose, tremors and muscle fasciculations were seen from 5 h. At 24 h in the high dose, 5/6 rats were dead, while the remaining rat showed piloerection and lethargy. On post-mortem examination, high-dose rats showed congestion of the heart, lungs and liver with no other abnormalities seen. The dermal LD50 was not calculated in this study as the doses used were not suitable for this calculation.

Taylor PE & James JA (1984b) Acute dermal toxicity of Supona/Diazinon 45/45% w/v EC (with 8.2% w/v Lankroflex L) in the female rat. Doc Ref HIBG 84/12. Wellcome Report Berkhamsted UK

A chlorfenvinphos/diazinon mixture (45/45% w/v EC; Ref No 716/71/1P; source not specified) was applied to the shorn dorso-lumbar region of female Sprague-Dawley rats (OLAC 1976 Ltd) and covered with an occlusive bandage for 24 h. Rats were individually housed and food and water were available *ad libitum*. The material was applied at 0.024, 0.048 and 0.1 mL/kg, using 6 rats/group. The lower doses were achieved by diluting the formulation in water. Rats were observed for 14 days after treatment, with all abnormal clinical signs recorded. Post-mortem examination was done on all rats dying during the study, and all rats after 14 days.

At the lowest dose, rats had blood-stained muzzles at 5 h, with recovery by 24 h. There were no abnormal clinical signs seen at 0.048 mL/kg, while high-dose animals showed piloerection, whole body tremors and blood stained eyes and muzzles at 24 h. One animal died at 0.1 mL/kg bw. There were no abnormal signs noted on post-mortem examination. The dermal LD50 was greater than 0.1 mL/kg bw.

Reno FE (1975b) Acute oral toxicity - rats; acute dermal toxicity - rabbits, acute inhalation toxicity - rats - residual surface spray: Final report. Doc Code HIQG 75/0001

Residual Surface Spray containing 21.2% chlorfenvinphos (source Cooper USA Inc., batch no. not specified) was tested for dermal toxicity using 10 New Zealand White rabbits (source not specified). The test material was applied to the clipped abdominal skin at 200 mg formulation/kg bw, with 50% of animals previously having received epidermal abrasions. After application, the area was covered with an occlusive dressing for 24 h. Rabbits were restrained to prevent possible ingestion of the test material. After the exposure period, the dressings were removed and the treated skin washed with water. Rabbits were observed for mortality, toxic effects and dermal effect immediately following application and once daily for 14 days. Post-mortem examination of all animals dying during the study and all animals at the end of the observation phase was performed.

None of the treated animals with either abraded or intact skin died during the study. Diarrhoea (3/5 abraded, 1/5 intact) and anorexia (1/5 intact) were observed. Dermal effects included slight to moderate erythema, slight atonia and slight drying of the skin in both intact and abraded animals. Erythema persisted until day 6. There were no abnormal findings on post-mortem examination of treated animals. The LD50 was >200 mg formulation/kg bw (equivalent to >42.4 mg active/kg bw), and the formulation was a slight skin irritant.

Tucker WE (1979) Acute dermal toxicity (LD50) study in rats with Dermaton (6.5% Supona) Doc Ref TTEP/79/0020 Burroughs Wellcome Report, USA

Chlorfenvinphos (as Dermaton EC, 6.5% chlorfenvinphos; batch no 8E9501; source; Burroughs Wellcome Company) was administered to the clipped dorso-lumbar region of Sprague-Dawley rats (Harlan Industries Inc, Indiana) at doses of 681, 1000, 1470, 2150 and 3162 mg product/kg bw, using 5 rats/sex/group. The application site was covered with an occlusive bandage for 24 h, after which the bandages were removed and the sites wiped clean. Rats were observed for 4 h after dosing and then daily for 14 days for abnormal clinical signs and mortality. They were housed in groups of 5, with free access to food and water.

In male rats clinical signs included dyspnoea at 1000 mg product/kg bw, and tremors, ataxia, decreased activity and salivation at higher doses. In females, abnormal signs were seen at doses of 1470 mg product/kg bw and higher, and included tremors, ataxia, decreased activity, decreased muscle tone and prostration. Deaths were seen from 1000 mg product/kg bw in males and 1470 mg product/kg bw in females. On post-mortem examination, there were signs of lung congestion in animals dying during the study. There were no notable findings in animals examined following terminal sacrifice. The acute dermal LD50 in male rats was 1306 mg product/kg bw (equivalent to 85 mg active/kg bw) and in female rats was 1525 mg product/kg bw (equivalent to 99 mg active/kg bw).

3.2.2 Inhalational Route

Witherup S & Schlecht H (1963) The immediate toxicity of compound 4072 with reference to its qualifications as a class B poison. Kettering Laboratory Report, College of Medicine, University of Cincinnati, Cincinnati

Chlorfenvinphos in 2 formulations (48% and 24 % in xylene) (source: Shell Chemical Co) was administered by inhalation to 10 female CD rats and 10 male C3H mice by whole body exposure for 65 minutes. The estimated concentration was 2 000 mg/m^3 for the 48% solution and 2 400 mg/m^3 for the 24% solution. No mortalities were observed over a 14 day observation period in mice: in rats 1/10 died during the first 2 days following exposure to 48% chlorfenvinphos in xylene.

Moffett BJ & Roderick HR (1974) Toxicity studies on BIRLANE insecticide: Acute inhalation exposure of rats to BIRLANE 50% powder seed dressing. TLTR.0018.74 Sittingbourne, Shell Research Limited, Sittingbourne

Chlorfenvinphos (50% formulation as powder seed dressing, source: Deutsche Shell Chemie, Frankfurt) was administered by inhalation to CFE rats (Tunstall Laboratories) for 4 h at doses of 20.8, 62.2, 126.5 or 207 mg/m^3. Males were also treated with 258 mg/m^3 for 4 h. The median particle size of the dust was 9.0 µm, and thus much of the dust was of a particle size able to be inhaled. The rats were restrained in holders which decreased dermal contact with the dust, and also prevented grooming during exposure. At the end of the exposure period, rats were removed from the holder, washed in water and observed for 14 days. Clinical signs typical of organophosphate poisoning were reported: these were not detailed further. The LC50 was 63.4 mg/m^3 for female rats and 203 mg/m^3 for male rats.

Reno FE (1975) Acute oral toxicity - rats, Acute dermal toxicity - rabbits, Acute inhalation toxicity - rats. Dermaton dip. Final report. Doc Code HIBG/75/0007. Hazleton Laboratories America Inc. Sponsor: Burroughs-Wellcome & Co.

Rats (source, strain not specified) (5/sex) were exposed to chlorfenvinphos by inhalation for 1 h in a glass inhalation chamber using whole body exposure. The nominal exposure concentration was 2500 mg/m^3. Animals were observed during the exposure period and for 14 days. After 1 min exposure hyperactivity was observed. Nasal discharge was observed after 13 min, which persisted for the exposure period, but resolved after the end of exposure. There were no abnormalities observed during the observation period, and no mortalities during the study.

Kapp RW (1976b) LC50 of Cooper-Phos of livestock insecticide in rats. final Report. Doc Ref 136-46 HIBG 76/0007. Hazleton Laboratories

Sprague-Dawley rats (source: Charles River COBS) were exposed to chlorfenvinphos (as Cooper-Phos Livestock Insecticide; source, purity, batch no not specified) by inhalation at nominal concentrations from 1000 to 6000 mg/m^3, using 5 rats/sex/group. Additionally, 5 rats/sex were exposed to diluted chlorfenvinphos at 20 mg/m^3). Rats were placed in glass inhalation chambers and exposed by whole-body exposure for 1 h. Rats were continuously observed during this period and for 1 h after exposure, and were then maintained for a 14 day observation period. At the lowest doses a slight nasal discharge was seen, while at higher doses rats showed respiratory distress with gagging, tremors and/or convulsions. All groups showed inactivity. The LC50 for a 1-h whole-body exposure was 2140 mg/m^3.

Reno FE (1975b) Acute oral toxicity - rats; acute dermal toxicity - rabbits, acute inhalation toxicity - rats - residual surface spray: Final report. Doc Code HIQG 75/0001

Residual Surface Spray containing 21.2% chlorfenvinphos (source: Cooper USA Inc., batch no. not specified) was given to rats (source, strain not specified) by inhalation (whole body exposure) at 1950 mg formulation/m^3 for 1 h using 5 rats/sex. Rats were observed for clinical signs and mortality during exposure and daily for 14 days after exposure. No abnormal clinical signs related to treatment with chlorfenvinphos were observed. There were no deaths either during exposure or for the 14 days after treatment. The LC50 was >1950 mg formulation/m^3 (>400 µg/m^3).

Zeman A (1982) An acute rat inhalation (LC50) study with Dermaton II (BW 0023Z61). Doc Ref TTEP/82/0098. Burroughs Wellcome Report, USA

Chlorfenvinphos (Dermaton II, reformulation C, batch BW 0023Z61; 12.77% chlorfenvinphos: source; Burroughs Wellcome Co) was administered to Crl: COBS (CD-SD) Sprague Dawley rats (Charles River Canada Inc.) by inhalation at 0, 1130, 1550, 1850 and 2690 mg/m^3 by whole-body exposure for 4 h using 10 rats/sex/group. Air samples were taken from the chamber each hour to determine the actual chamber concentration and particle size. Animals were closely observed during exposure, and checked at least twice daily during the 14-day observation period. At the end of the surviving period animals were killed and examined macroscopically. The liver, kidneys, gonads and any abnormalities were preserved in formalin, while the lungs were weighed prior to fixation.

Abnormal clinical signs observed included tremors, salivation, ataxia, lethargy, weakness, respiratory distress and discharge from the eyes or nose. These signs were seen in all treatment groups. On post-mortem examination, patchy reddening of the lungs in the animals which died was the only consistent treatment related finding; one treated rat had evidence of corneal damage which may have been related to treatment. The LC50 for males was 1450 mg/m^3 and for females was

1470 mg/m^3. It was not clear whether the concentrations were expressed in mg of product or as mg of chlorfenvinphos.

Johnston RE (1979) Acute inhalation toxicity study in rats with Dermaton Dip (EC 6.5% Supona) Doc Ref TTEP/79/0023 Burroughs Wellcome Report, USA

Chlorfenvinphos (as Dermaton Dip, 6.5% chlorfenvinphos in EC formulation (not otherwise specified); source; Burroughs Wellcome Company; Batch no 8E9501) was tested for inhalation toxicity using Charles River CD rats (source not specified). Rats were housed in groups in the pre-exposure period and individually throughout the post-exposure period. Rats were exposed by whole-body exposure for 4 h to concentrations of 1100, 2000, 3500, 6700 or 20700 mg/m^3 using 5 rats/sex/group. Rats were observed every 30 min during exposure and twice-daily for 14 days after exposure. Rats dying during the study were necropsied as soon as possible, while a post-mortem examination of all rats was done at the study termination.

No deaths were seen at the lowest concentration, while the only abnormal clinical signs observed were dyspnea both during and shortly after exposure. At 2.0 mg/L rats showed dyspnea, nasal discharge, tremors and decreased activity. Clinical signs persisted for up to 2 days after exposure and one male died on day 2 after exposure. At 3.5 mg/L rats showed similar signs, and 4/5 males and 3/5 females died. Signs increased in severity at 6.7 mg/L, including salivation and prostration, and all rats died within 2 days of exposure. At the highest dose, all rats died within a short period of exposure with similar clinical signs.

On post-mortem examination the most commonly observed signs were congested lungs and pinpoint haemorrhages in the stomach mucosa. The acute inhalation LC50 for male rats was 2990 mg/m^3, while the combined acute inhalation LC50 for male and female rats was 2670 mg/m^3 (the LC50 for female rats was not calculated, as the mortality data was not suitable for this calculation). It was not clear whether the concentration was expressed as mg product or mg active.

3.2.3 Skin Irritation

Stevenson DE (1974) Toxicity of insecticides. Skin and eye irritancy and acute toxicity of BIRLANE 50% seed dressing. TLTR.0015.74 Shell Research Institute, Sittingbourne

The irritancy of a dry solid chlorfenvinphos formulation, containing 50% chlorfenvinphos (source: Deutsche Schell Chemie GmbH) was assessed by applying the material to the abraded and non-abraded skin of New Zealand White rabbits (Ranch Rabbits, Sussex). The length of exposure, quantity of material applied and method of abrasion were not specified. The skin was assessed 24, 48 and 72 h and 7 days after exposure, using 4 rabbits/sex/group. One male rabbit had very mild erythema at 24 h, and 1 male and 1 female rabbit had very mild oedema at 24 h, evident in both the abraded and non-abraded patches on these rabbits. No signs or erythema or oedema were detectable at 48 h. Based on this, a 50% formulation as a seed dressing was non-irritating to rabbit skin.

Stevenson DE (1974) Toxicity of insecticides: Skin and eye irritancy of BIRLANE granules formulation EF 3651. TLTR.0014.74, Shell Research Institute, Sittingbourne

Chlorfenvinphos, as Birlane granules (source: Woodstock Laboratory, Sittingbourne, purity, batch no not specified) was applied to the skin of New Zealand White rabbits (4/sex), and covered. Three

applications were made, with 24 h between applications. No indication of the dose applied was given. It was not stated whether the skin was either shorn or abraded, and no indication was given of the time the skin was covered. Mild erythema was seen in one female and one male within 24 h of the first application. The skin had returned to normal within 30 h of the first application. No oedema was seen at any stage following application. The formulation was determined to be a non-irritant to occluded rabbit skin.

Piercy DWT & James JA (1975) Supona (23Z61) Rabbit Dermal Irritancy Test. Doc Code HIBG 75-11 Lab Ref No TL 30-75. Research & Development (V & A), The Wellcome Foundation Ltd

Chlorfenvinphos (0.5% dust formulation, batch no E-103, source not specified) was applied to the clipped abdominal skin of New Zealand White rabbits (source, sex not specified). Five rabbits had abraded skin, while 5 had intact skin. The formulation was made into a thick aqueous paste prior to application by adding sterile water to the dust formulation, and it was applied at 200 mg formulation/kg bw (1 mg active/kg bw). The application area was covered with an occlusive bandage for 24 h, after which the bandage was removed and the skin washed with water. The dermal reaction was observed at 24, 48 and 72 h and 7 and 14 days after application.

There were no abnormal clinical or behavioural signs observed during the observation period. In intact rabbits, a mild erythematous reaction was seen at 24 h; this had resolved by 48 h and no other abnormalities were observed. In rabbits with abraded skin, there was slight to well-defined erythema at 24 h, which resolved by 7 days. There was also very slight oedema in one animal on day 2. These effects were considered to be related to the abrasion technique. Based on these finding, this formulation of chlorfenvinphos was not a skin irritant to rabbits.

Mallard JR & James JA (1984a) Primary dermal irritancy of Supona/Diazinon 45/45% w/v EC in the rabbit. Doc Ref HIBG 84/2 Wellcome Report, Berkhamsted UK

A chlorfenvinphos/diazinon mixture (45/45% w/v EC: source -Development Laboratory, Berkhamsted; batch no not specified) was applied to the clipped intact back or flank skin of New Zealand White rabbits (Ranch Rabbits, Sussex). Test material (0.5 mL) was applied undiluted, and was covered with an occlusive dressing for 4 h. On removal of the dressing, the area was washed with distilled water. Rabbits were housed singly with free access to food and water, and were observed for 17 days after treatment. Examination of the treated area was done 4.5, 24, 48 and 72 h and on days 6, 7, 10, 14 and 17 after treatment. Very slight erythema and oedema was seen in 2 animals at 24 h after treatment, while very slight erythema was seen in 2 animals at 48 h after treatment. Signs of irritation had resolved by 72 h after treatment. At 6 days after treatment, there were signs of desquamation and dryness of the skin. Based on this, the formulation was a mild irritant to rabbit skin.

Johnson N & Zeman A (1982) An acute primary skin irritation study in the New Zealand white rabbit with Dermaton II (BW 0023Z61) Doc Ref TTEP/82/0021 Burroughs Wellcome Report, USA

Chlorfenvinphos (Dermaton II, reformulation C, batch BW 0023Z61; 12.77% chlorfenvinphos: source; Burroughs Wellcome Co) was applied to the abraded or non-abraded clipped dorso-lumbar skin of female New Zealand White rabbits (Dutchland Laboratory Animals, PA). A 0.5 mL aliquot of test material was applied, and covered with gauze and an occlusive dressing for 24 h. It was not

stated whether the skin was washed after the dressing was removed. Rabbits were examined for erythema and oedema at 24 and 72 h after test material application. There was no sign of irritation at either examination time, therefore the skin was not examined at any later points. Based on these results, this formulation of Dermaton II was non-irritating to rabbit skin.

Tait AJ (1990a) Acute toxicity and dermal irritancy of Supona Fly Dip (concentrate) Doc Ref RRS-90-22. Pitman-Moore Report, Harefield UK GLP: UK/OECD

Chlorfenvinphos (as Supona Fly Dip; 8.84% chlorfenvinphos; product code D197; batch no 2: source; Coopers, Berkhamsted) was applied to the shorn dorso-lumbar skin of 3 male New Zealand White rabbits (Ranch Rabbits, Sussex). Rabbits were housed individually, with free access to food and water. The test material (0.5 mL) was applied to an area on the left side of the rabbits, and covered with a gauze patch. An untreated area on the right side was also covered with a gauze patch to serve as a control. The whole area was bandaged with an elasticised bandage to ensure good contact between the test material and the skin. After 4 h, the bandages were removed and the skin washed with warm water. The sites were assessed 1, 24, 48 and 72 h and 7, 10, 13 and 16 days after treatment for signs of dermal irritation.

All rabbits showed very slight to well defined erythema until 72 h after treatment, with 2 rabbits showing erythema at day 7. All rabbits showed slight to moderate oedema until day 7, persisting until day 10 in 2 animals. Based on these findings, the formulation was a moderate skin irritant to rabbits.

Tait AJ (1990b) Acute dermal irritancy of Supona Fly Dip (Recommended In-Use Concentration. Doc Ref RRS-90-29. Pitman-Moore Report, Harefield UK GLP: UK/OECD

Chlorfenvinphos (as Supona Fly Dip; 8.84% chlorfenvinphos; product code D197; batch no 2: source; Coopers, Berkhamsted) was applied to the clipped dorso-lumbar skin of 3 male New Zealand White rabbits (Ranch Rabbits, Sussex) as the 0.5% v/v formulation, which was the concentration specified for use of the insecticide (equivalent to 0.04% chlorfenvinphos). The test material (0.5 mL) was applied to an area on the left side of the rabbits, and covered with a gauze patch. An untreated area on the right side was also covered with a gauze patch to serve as a control. The whole area was bandaged with an elasticised bandage to ensure good contact between the test material and the skin. After 4 h, the bandages were removed and the skin washed with warm water. The sites were assessed 1, 24, 48 and 72 h and 7, 10, 13 and 16 days after treatment for signs of dermal irritation. Rabbits were housed individually, with free access to food and water. There were no signs of dermal irritation (erythema or oedema) at any time during the observation period. Based on this, the 0.5% aqueous dilution of Supona Fly Dip was a non-irritant to rabbit skin.

3.2.4 Ocular Irritation

Stevenson DE (1974) Toxicity of insecticides. Skin and eye irritancy and acute toxicity of BIRLANE 50% seed dressing. TLTR.0015.74 Shell Research Institute, Sittingbourne

The ocular irritancy of a 50% formulation of chlorfenvinphos (source Deutsche Shell Chemie GmbH) was assessed using New Zealand White rabbits (Ranch Rabbits, Sussex) by applying 15 mg of undiluted seed dressing to the conjunctival sac of one eye of 4 rabbits/sex. The untreated eye of each animal served as a control. The formulation was not washed out of the eye. Irritancy was assessed immediately after application, and 1, 2, 3, and 7 days after application. On the

immediate examination, all animals showed redness of the conjunctiva with 1/8 animals having chemosis and discharge. Redness and chemosis persisted in 5/8 animals to varying degrees until 72 h. It was noted at this time that in one female rabbit there had not been sufficient lacrimation to completely remove the formulation from the eye. The remaining formulation had caused a small patch of corneal opacity, which was still present at 7 days after treatment. Based on the findings, chlorfenvinphos was determined to be mildly irritating to the rabbit eye, and it was noted that severe irritation could result if the formulation was left in contact with the eye for an extended period of time.

Stevenson DE (1974) Toxicity of insecticides: Skin and eye irritancy of BIRLANE granules formulation EF 3651. TLTR.0014.74, Shell Research Institute, Sittingbourne

Chlorfenvinphos, as Birlane granules (source: Woodstock Laboratory, Sittingbourne, purity, batch no not specified) was applied to the eye of two New Zealand White rabbits. The method used was based on the method elaborated in the US Federal Register, 1963; no additional information on the testing method was supplied. Observations were made immediately, after 24, 48 and 72 h and after 7 days. Both rabbits showed mild redness in the conjunctiva in the first 24 h after application which resolved by 48 h after the test. The formulation was non-irritating to rabbit eyes.

Anon. (1963a) Eye-irritating properties of Compound 4072 4 lb/gal., E.C. Medical College of Virginia, VA, USA

Chlorfenvinphos (Compound 4072, 480 g/L EC formulation - no other details supplied) was instilled into the right eye of 6 male albino rabbits (source, strain not specified), using 0.1 mL/rabbit. The left eye served as an untreated control. Eyes were examined at 1, 2, 3, 4, and 7 days after treatment. On the first day, 2/6 rabbits developed conjunctivitis (severity not indicated), which persisted until day 3. No miosis was observed at any time. All eyes were normal on days 4 and 7. This formulation of chlorfenvinphos was therefore a mild irritant to the rabbit eye.

Mallard JR, Woollon RM & James JA (1982) Ocular irritancy of diluted Supona/diazinon 45/45% w/v EC in the rabbit. Doc Ref HIBG 82/9 Wellcome Report, Berkhamsted UK

A chlorfenvinphos/diazinon mixture (45/45% w/v EC; source: Development Laboratory, Berkhamsted; ref. no 716/71/1F), diluted 1:1800 in water was tested for ocular irritancy using New Zealand White rabbits (Ranch Rabbits UK). Rabbits were housed individually with free access to food and water. The mixture was instilled into the right eye (0.1 mL) of 9 rabbits (sex not specified). In 3 rabbits the chemical was rinsed out after 20 to 30 seconds, while the other rabbits were left without rinsing. The left eye was maintained as an untreated control. Rabbits were observed 1 and 6 h after dosing, and on days 1, 2, 3 and 7 after dosing. The eyes were closely examined for irritation, including the use of fluorescein eye drops to detect corneal damage.

At 6 h after treatment, two animals had very slight conjunctival swelling which had resolved by 24 h after treatment. There were no other abnormalities detected. Therefore the diluted formulation was determined to be non-irritating to the rabbit eye.

Mallard JR & James JA (1984b) The assessment of the ocular irritancy of Supona/Diazinon 45/45% in the rabbit. Doc Ref HIBG 84/3 Wellcome Report Berkhamsted UK

The ocular irritation of a 45/45% EC formulation of chlorfenvinphos/diazinon (source: Development Laboratory Berkhamsted; ref. no 716/71/1P) was assessed using New Zealand White rabbits (Ranch Rabbits, Sussex). The test material (0.1 mL) was instilled into the left eye of 6 rabbits with no further treatment. The right eye was maintained as an untreated control. Rabbits were housed individually with free access to food and water, and were observed 1, 6, 24, 48 and 72 h and 6 days after application. Irritation was observed using a company scoring system.

At 1 h after treatment, 5/6 rabbits showed obvious conjunctival swelling, while all showed corneal dulling and some reddening of the conjunctiva. At 6 h signs were similar, except that the intensity of the conjunctival reddening had increased slightly. By 24 and 48 h after treatment, the conjunctival reddening was beginning to resolve, the conjunctiva was still red and there was corneal opacity on more than 50% of the corneal surface. By day 3, the eye irritation was resolving with a decrease in corneal opacity and conjunctival reddening, while on day 6 all eyes had returned to normal. The formulation therefore was a moderate irritant to rabbit eyes.

Melich D & Johnson N (1982) An acute eye irritation in the New Zealand White Rabbit with Dermaton II (BW 0023Z61). Doc Ref TTEP/82/0073. Burroughs Wellcome Report USA; GLP US

Chlorfenvinphos (Dermaton II, reformulation C, batch BW 0023Z61; 12.77% chlorfenvinphos: source; Burroughs Wellcome Co) was applied to the right eye of 6 female New Zealand White rabbits (Dutchland Laboratory Animals, PA). The eyes were examined before treatment to ensure that there were no pre-existing abnormalities. The left eye of each animal served as an untreated control; neither eye was rinsed. The eyes were examined and scored for irritation at 24, 48, 72 and 96 h and 7 and 14 days after treatment using a company scoring method. Rabbits were also examined for corneal abnormalities at 7 and 14 days after treatment by the instillation of fluorescein dye and examination under ultraviolet light.

There were no effects observed on the cornea or iris by direct examination at any time after treatment. One animal had a fluorescein stained area in the treated eye on day 7, which was possibly treatment related. This corneal damage had resolved by day 14. There was conjunctival irritation at 24 h and 48, with all rabbits showing some degree of injection of the vessels, mild swelling of the conjunctiva and 2 animals having a discharge at 24 h. Conjunctival irritation had resolved by day 7. Based on these findings, in particular the corneal irritation on day 7, Dermaton II was a moderate to severe irritant to the rabbit eye.

Lee IS & Johnston RE (1978) An acute rabbit eye irritancy test on a reformulated Dermaton Dip (12.25% Supona) without HAN. Doc Ref TTEP/78/0027. Burroughs Wellcome Report, Research Triangle Park USA

Chlorfenvinphos (as Dermaton Dip but without heavy aromatic naphthalene; 12.25% chlorfenvinphos; batch no 8B9501; source not specified) was tested for acute eye irritancy using male New Zealand White rabbits (Dutchland Laboratory Animals Inc. PA). Six rabbits were selected, and their eyes examined with fluorescein to confirm no corneal damage was present. Rabbits were housed individually, with free access to food and water. An aliquot of 0.1 mL of test material was placed into the right eye of each rabbits, while the left eye served as an untreated control; none of the eyes were washed after treatment. Rabbits were examined for eye irritation at

24, 48 and 72 h and on day 7 after treatment. Eye irritation was scored according to the Draize system.

During the observation period there were no abnormalities of the iris or cornea noted. In all rabbits, the conjunctiva of both treated and control eyes were injected more than normal; in treated eyes at 24 h in all animals there was more diffuse redness, with individual vessels not distinguishable. There was detectable swelling of the conjunctiva in all treated rabbits at 24 h after treatment, and a small amount of discharge was noted at 24 and 48 h after treatment. All eyes had returned to normal after 48 h after treatment. Based on the absence of any corneal effects, and the resolution of effects within 72 h after treatment, this formulation was a slight to rabbit eyes.

3.3 Isomers and Manufacturing Intermediates

3.3.1 Oral toxicity

Muir CMC (1970) Laboratory Report. Tunstall Laboratory, Sittingbourne, Kent

The alpha- and beta-isomer of Birlane (trade name for chlorfenvinphos) as a 2% solution in DMSO were administered orally to CFE rats. The beta-isomer was given at doses of 3.75, 7.5, 15, 22.5, 30.5, 37.5 or 45 mg/kg bw, using 5/sex/group. The alpha-isomer was administered at 25, 50, 100 or 200 mg/kg bw using 2/sex/group. No details of clinical signs observed were supplied, except for the statement that the beta-isomer produced signs typical of organophosphorus intoxication. The LD50 of the beta-isomer was 13.6 mg/kg bw, and that of the alpha-isomer was >200 mg/kg bw.

Brown VKH, Ferrigan LW & Muir CMC (1967) The acute toxicities and skin irritant properties of four intermediates used in the production of chlorfenvinphos. Technical Service Report TOX 10/67 Shell Research Institute, Sittingbourne

Four chemical intermediates used in the manufacture of chlorfenvinphos were examined from the point of view of safety in handling. These were: 2,4 dichloroacetophenone (24DA) (purity 94%, source: Luwa-Bazel, batch no not specified), , ,2,4-tetrachloroacetophenone (24TA) (source: KSLA, batch no: 67/5161, purity not specified) , m-dichlorobenzene (DB) (source: Hoechst AG, batch no 611-13.518, purity not specified) and triethyl phosphite (TEP) (source: Farbenfabriken Bayer AG, batch no: 25E 0202, purity not specified). Toxicity testing consisted of acute oral and dermal LD50's, skin sensitization, and skin and eye irritation potential tests, and were performed using CF1 mice, CFE rats, “P” strain guinea-pigs (all sourced from Tunstall Breeding Unit) and NZW rabbits (source: Matthews Ltd, Norfolk).

The acute oral LD50s were determined in fasted rats and mice, and are detailed in the table below.

Table 12: Oral LD50s of chlorfenvinphos intermediates

Species	Compound	LD50 (mg/kg bw)	Signs of Intoxication
Rat	24DA	>2,600	Salivation
	24TA	1400-2100	Depression, salivation, labored breathing
	DB	1800-2400	None

	TEP	> 1600	None
Mouse	24DA	> 2600	None
	24TA	2800	None
	DB	635-760	Trembling, convulsions
	TEP	330-670	Convulsions, limpness

The acute dermal toxicities were determined in rats, with each material applied undiluted to the shorn dorso-lumbar skin of male and female rats. The area was covered with an occlusive dressing for 24 h, after which the skin was washed with a detergent solution. The animals were observed for 9 days. The results are as detailed below.

Table 13: Dermal LD50s of chlorfenvinphos intermediates

Intermediate	**Dermal LD50 (mg/kg bw)**	**Clinical signs**
24DA	>2600	None
24TA	Male 1500 – 2900 Female 500	Tremors, excessive salivation
DB	> 2500	None
TEP	>1600	None

A test for primary skin irritation was performed in rabbits. The method used was not specified, and individual animal results were not given. The first test was a covered test, described as a Draize Test. 24DA produced a moderate to severe skin irritation after 2 or 3 exposures, which was reversible. 24TA severely affected the skin, with necrosis visible after 2 exposures. Animals died due to systemic effects. DB produced erythema with drying and wrinkling of the skin, which is consistent with an aromatic solvent, while TEP was severely irritant after 3 exposures. The effects of TEP were reversible.

In an uncovered, repeated application test using rabbits and guinea-pigs, 24DA severely irritated the skin of both species after prolonged contact. 24TA irritated the skin after only one exposure, with the irritation becoming severe on continued exposure. DB produced an erythematous condition, with cracking of the skin and bleeding. There was also some epidermal thickening between the cracks. TEP was not irritant in the uncovered test, contrasting with the severe effects seen in the covered trial.

Intradermal and topical tests were carried out in guinea-pigs using each of the compounds. The methods used were not specified, and no individual results were presented. None of the compounds produced any sensitisation reactions.

Eye irritation tests were carried out. The methods and species used were not specified. 24DA caused a transient, moderately severe conjunctivitis, which cleared within 24 h. 24TA produced a severe persistent conjunctivitis with chemosis and discharge, which persisted for 10 - 14 days. The severity of the chemosis with lid involvement produced temporary blindness. Irrigation of the eyes, even immediately after exposure did not reduce the signs. DB caused a transient conjunctivitis with

complete reversal within 24 h. TEP caused a transient but severe conjunctivitis, which cleared within 48 h.

3.4 Potentiation Studies

Witherup S & Schlecht H (1963) The immediate toxicity of various binary combinations of diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate (Compound 4072) with other organophosphorus insecticides. Kettering Laboratory, College of Medicine, University of Cincinnati Cincinnati Ohio

Chlorfenvinphos (purity 96%, batch no not specified, source: Shell Chemical Co), as well as technical grade material of a number of compounds, was tested for acute oral toxicity in female CD rats (Charles River Breeding Laboratory). The compounds, suspended in peanut oil, were administered orally by gavage, and the LD50, LD10 and LD01 of each compound determined using 10 rats/dose group for each of these trials. The acute oral LD50 of chlorfenvinphos was 12.8 mg/kg bw. The LD10 was 5.9 mg/kg bw, while the LD01 was 4.98 mg/kg bw.

Following the determination of these values, each of the compounds to be tested (a range of pesticides) were administered at the LD01 to groups of 10 rats. At this dose there were no mortalities in any of the treated groups. The compounds were then administered at the LD01 in conjunction with chlorfenvinphos at the LD01. Potentiation was observed when diazinon, malathion, parathion-methyl or Ronnel (fenchlorphos) were co-administered with chlorfenvinphos. In these combinations either 9/10 or 10/10 animals died during the observation period. A lesser degree of potentiation was observed following administration of a combination of chlorfenvinphos and Guthion (azinphos-methyl) where 6/10 animals died. No potentiation was seen with the other chemicals tested. It therefore appeared that the combination of the listed chemicals with chlorfenvinphos was more toxic than either chemical administered individually.

Harper DW, Piercy DWT & James JA (1977a) Potentiation of the acute oral toxicity of 21Z73 in female rats by Delnav, Supona, Ethion and diazinon. Doc Code HEPG 77-8 Lab Ref No Tl.29-77. Research and Development (V & A), The Wellcome Foundation Ltd

Female Sprague-Dawley rats (source: OLAC 1976 Ltd) were dosed orally with permethrin, dioxathion, chlorfenvinphos, ethion or diazinon after an overnight fast, to determine the acute oral LD50 of each compound, using 6 rats/group. Animals were housed in dosing groups with *ad libitum* food and water. The observation period was not specified. Following the determination of the oral LD50 for each compound, rats were dosed with permethrin in combination with each of the other chemicals to determine whether there was an additive effect, or a potentiation of effects. The expected LD50 for the combination was calculated to be equal to the sum of half the LD50 for each compound. The ratio of expected LD50 to observed LD50 was determined for each combination; where the ratio was greater than one, it was determined potentiation had occurred.

The acute LD50 for each compound tested individually was as follows: permethrin 2263 mg/kg bw; dioxathion 26 mg/kg bw; chlorfenvinphos 22 mg/kg bw; ethion 25 mg/kg bw; and diazinon 749 mg/kg bw.

When permethrin was administered in combination with each of the compounds in an equitoxic mixture, the ratio of expected LD50 to observed LD50 was 1.42 with dioxathion, 1.00 with

chlorfenvinphos, 0.97 with ethion and 6.33 with diazinon. Therefore there was no potentiation between permethrin and chlorfenvinphos or ethion, some potentiation with dioxathion and notable potentiation with diazinon.

Harper DW, Piercy DWT & James JA (1977b) Potentiation of the acute oral toxicity of 21Z73 in female rats by certain organophosphate compounds. Doc Code HEPG 77-26 Lab Ref No Tl.79-77. Research and Development (V & A), The Wellcome Foundation Ltd

Permethrin was tested for potentiation with coumaphos, bromophos ethyl, chlorpyrifos, dicrotophos, carbophenothion, malathion, crotoxyphos and a chlorfenvinphos/dioxathion mixture. The acute oral LD50 of each of the compounds individually in fasted female Sprague-Dawley rats (source: OLAC 1976 Ltd) were determined, using 6 rats/group. Following this, equitoxic mixtures (based on the LD50) of permethrin with each of the other chemicals were administered, and the ration of expected LD50 to observed LD50 determined. Where this ratio was greater than 1, it was determined that potentiation had occurred.

The acute oral LD50 for each of the chemicals, and the ratio of expected LD50 to observed LD50 when administered with permethrin are set out in the following table.

Table14: Acute oral LD50 and ratio of expected LD50/observed LD50

Compound	LD50 (mg/kg bw)	Ratio of exp. LD50/obs. LD50 when administered with permethrin
permethrin	2854	
coumaphos	24.3	1.7
bromophos ethyl	86.0	2.3
chlorpyrifos	185	2.5
dicrotophos	75.0	1.6
carbophenothion	36.4	1.6
malathion	1631	2.3
crotoxyphos	265	2.2
chlorfenvinphos/dioxathion	19.6	1.2

The mixing of permethrin with each of the tested compounds showed potentiation of the acute toxicity, although the chlorfenvinphos/dioxathion mixture showed the least potentiation of the tested chemicals.

Taylor PE, Woolon RM & James JA (1980) Stomoxin - acute oral potentiation with Supamix (105.5% w/v DFF) in the female rat. Doc No HEFG 81-47. Lab Ref HPAW 80-72 BAGB 82-13. Group Research and Development, The Wellcome Foundation Ltd

Permethrin was tested for potentiation with a chlorfenvinphos/dioxathion equal (by weight) mixture in female Sprague-Dawley rats. Rats (6/group) were housed in treatment groups with *ad libitum*

food and water, and were maintained for a 14-day observation period after dosing to determine the acute oral LD50 of the substances or the mixture. Animals were observed daily for abnormal clinical signs. Post mortem examination was done of all animals dying during the study, and all animals at scheduled sacrifice at the end of the observation period.

Permethrin was administered at doses from 735 to 1310 mg/kg bw. Clinical signs of toxicity were seen at all doses, with the main signs being blood staining of the eyes and muzzle, lethargy, piloerection, salivation, tremors and urine staining of the perineum. The acute oral LD50 was 854 mg/kg bw.

The chlorfenvinphos/dioxathion mixture was administered at doses from 4.9 to 9.9 mg chlorfenvinphos/kg bw, with abnormal clinical signs seen at all doses. The main signs observed were tremors, piloerection, blood-stained eyes, lethargy and urine staining of perineum. The acute oral LD50 was 5.54 mg chlorfenvinphos/kg bw.

When permethrin was administered in combination with the chlorfenvinphos/dioxathion mixture, the LD50 was 542 mg/kg bw (expressed as the content of permethrin). Clinical signs were similar to those seen with either compound in isolation, and as the ratio of expected LD50 to observed LD50 was 0.8, there was no potentiation seen.

Autopsy results were similar for each of the compounds, with signs including thymic haemorrhages, congestion of the liver, lung and kidney and excess fluid and/or blood in the gastrointestinal tract.

Wallwork LM & Malone JC (1974a) Acute toxicity and potentiation toxicity studies with Nankor and Supona in rats and mice. Doc Cod HIBG 74-1 Lab Ref No TL 1-74 Research and Development (V & A), The Wellcome Foundation Ltd

Female Wistar rats (source not specified) were treated with chlorfenvinphos, fenchlorphos or a combination of both. Animals (6/group) were fasted overnight prior to treatment, with free access to water at all times. Rats were dosed orally with chlorfenvinphos at 5% in corn oil; the LD50 was 15.2 mg/kg bw. The LD50 of fenchlorphos (30% in corn oil) was 2219 mg/kg bw. The predicted LD50 for the combination of chlorfenvinphos and fenchlorphos was 1110 mg fenchlorphos and 7.8 mg chlorfenvinphos/kg bw; the observed LD50 was 409 mg fenchlorphos and 2.9 mg chlorfenvinphos/kg bw. Potentiation was therefore seen with the combination of chlorfenvinphos and fenchlorphos.

A formulation containing 72% w/v chlorfenvinphos and 48% w/v fenchlorphos was also tested. The oral LD50 of the mixture was 12.5 mg formulation/kg bw (equivalent to 9 mg chlorfenvinphos and 6 mg fenchlorphos/kg bw). When the formulation was diluted to contain 10% chlorfenvinphos, the oral LD50 was 45 mg formulation/kg bw (equivalent to 4.5 mg chlorfenvinphos and 3 mg fenchlorphos/kg bw). The dermal toxicity of the formulation was 378 mg formulation/kg bw (equivalent to 272 mg chlorfenvinphos and 181 mg fenchlorphos/kg bw).. No details of the method used to determine the dermal toxicity were supplied, other than the exposure time which was 24 h.

Taylor PE, Piercy DWT & James JA (1979a) Decamethrin - acute oral potentiation with dioxathion, ethion or chlorfenvinphos in the female rat. Doc Ref HIBG 79/5. Wellcome Report, Berkhamsted UK

Decamethrin (2% in sesame oil) was tested for potentiation with ethion (10% in sesame oil), dioxathion (10% in sesame oil) and chlorfenvinphos (10% in sesame oil). The source and batch number of the chemicals was not specified. The acute oral LD50 of each of the chemicals in fasted female Sprague-Dawley rats (source not specified) was determined using 6 rats/group. Rats were observed for 14 days after treatment, and abnormal clinical signs recorded. Post-mortem examinations were conducted on all animals dying during the study and all animals at the end of the study.

Following the determination of each of the LD50s, female rats were dosed with an equitoxic mixture of decamethrin in combination with each of the chemicals, and the LD50 of the mixture determined. The ratio of the expected LD50 based on the LD50 of each chemical alone to the observed LD50 of the mixture was determined: if this ratio was greater than 1, it was determined the chemicals were more toxic in combination ie. that potentiation of their acute oral toxicity was occurring. Observations following dosing with the mixture were similar to those after dosing with the individual chemicals.

Decamethrin was administered at doses between 35 and 200 mg active/kg bw. At all doses signs included convulsions, blood-stained muzzles, lethargy, tremor, piloerection, ataxia, urine staining of perineum and abduction of limbs. On post-mortem examination of animals dying during the study, there was congestion of the lungs, liver, spleen and kidney, thymic haemorrhages and excess mucous and blood in the small intestine. The acute oral LD50 of decamethrin alone was 68 mg active/kg bw.

Dioxathion was given at 15.7 to 50 mg active/kg bw, and clinical signs at all doses included tremor, piloerection, blood-stained eyes and muzzle, hypersensitivity, exophthalmus, salivation and lethargy, On post-mortem examination there was congestion of the lungs, liver, spleen and kidneys and liver mottling. The acute oral LD50 of dioxathion alone was 19 mg active/kg bw.
Ethion was given at 19.8 to 50 mg active/kg bw, and the signs at all doses were similar to those seen with dioxathion. On post-mortem examination there was congestion of the lungs and liver and excess mucous and blood in the small intestine. The acute oral LD50 of ethion alone was 33 mg active/kg bw.

Chlorfenvinphos was administered at doses from 9.9 to 31.5 mg active/kg bw. Abnormal clinical signs seen were tremors, piloerection, blood staining of the eyes, hypersensitivity, exophthalmia, salivation and lethargy. On post-mortem examination there was congestion of the lungs, liver and kidney, thymic haemorrhage and mucous and blood in the small intestine. The acute oral LD50 of chlorfenvinphos alone was 20 mg active/kg bw.

When decamethrin and dioxathion were administered together, the dose at which half the animals died was 18.8 mg decamethrin/kg bw (expected 34 mg/kg bw) plus 5.3 mg dioxathion/kg bw (expected 9.5 mg/kg bw). With decamethrin and ethion, the dose at which half the animals died was 15.2 mg decamethrin/kg bw (expected 34 mg/kg bw) plus 7.4 mg ethion/kg bw (expected 16.5 mg/kg bw). With decamethrin and chlorfenvinphos, the dose at which half the animals died was 8.54 mg decamethrin/kg bw (expected 34 mg/kg bw) plus 2.73 mg chlorfenvinphos/kg bw (expected 10 mg/kg bw). Clinical signs observed included blood staining of the eyes and muzzle, tremors, piloerection, lethargy, convulsions, laboured respiration and salivation. Post-mortem examination of animals dying during the observation phase showed mottling of the liver; congestion of the liver, heart, lungs, kidney, spleen and small intestine; mucous and blood in the ileum and jejunum and thymic haemorrhages.

When each of the chemicals was administered in combination with decamethrin the observed LD50 was lower than the expected LD50 and thus potentiation was determined to have occurred.

3.5 Antidote studies

Natoff IL & Reiff B (1967) Pharmacological studies into the toxic actions of cholinesterase inhibitors: 3. The reactivation of cholinesterase after inhibition by Codrin and chlorfenvinphos in vitro. IRR TL/7/67. Shell Research Institute, Sittingbourne

The potency of a number of oximes was investigated in an *in vitro* test using rat brain homogenate as a ChE source. Chlorfenvinphos (source: Shell Development Company, Modesto, batch no, purity not specified) was incubated with 40 mg of rat brain tissue for up to 30 min. This mixture was then tested for ChE activity using the pH method immediately after incubation, and after 60 min. Following this, the effect of the addition of a range of concentrations of 2-PAM to homogenates incubated for 15 minutes with chlorfenvinphos was tested, as was the effect of monoisonitrosoacetone (MINA) and bis[4-hydroxy-iminomethyl-pyridinium-(1)-methyl]ether dichloride (Toxogonin). The percentage enzyme inhibition and percentage reversal by the oximes were calculated.

The inhibition of ChE activity of rat brain was increased following an increased incubation time, from 66% initially to 88% after 30 min incubation. ChE inhibition was reduced by increasing amounts of 2-PAM. In a solution of 10^{-7}M chlorfenvinphos inhibition was at 95% with no treatment. This inhibition was decreased to 28% when 0.0316mM of 2-PAM was added to the mixture. When 2-PAM was added to the mixture after 30 min reactivation, it was effective at reversing the established inhibition. When the 3 oximes were compared, Toxogonin was the most effective at reactivating ChE, while MINA was the weakest. Toxogonin may therefore be an effective antidote. However, as this test was conducted *in vitro* it is difficult to predict how the results may be extrapolated to *in vivo.*

Natoff IL & Reiff B (1970) Quantitative studies on the effect of antagonists on the acute toxicity of organophosphates in rats. Br J Pharmacol 40:124-134

Chlorfenvinphos (purity 92%, source: Shell International Chemical Company, batch no not specified) was administered by SC injection to female CFE rats (Tunstall Breeding Unit). A standard dosing volume of 1 mL/kg was used, while the doses used were logarithmically spaced dose increments of the LD50, using 5/group. The antidotes used were atropine methonitrate (18.02 mg/kg bw), atropine sulphate (17.4 mg/kg bw), P2S (50 mg/kg bw), and *bis*-(4-hydroxyiminomethyl pyridinium-1-methyl)ether dichloride (obidoxime) (90 mg/kg bw). The antidotes were injected SC when the first signs of intoxication became apparent. In some cases, these signs were seen 60 min after administration of chlorfenvinphos. Rats were observed for 7 days after treatment. The LD10, LD50 and LD90 values were estimated.

In this trial the LD50 of chlorfenvinphos was estimated to be 43.4 mg/kg bw. Treatment with atropine sulphate increased this to 104 mg/kg bw. A combination of atropine and P-2-S increased the LD50 to 216 mg/kg bw. Obidoxime increased the LD50 to 87 mg/kg bw, while a combination of obidoxime and atropine sulphate increased the LD50 to 800 mg/kg bw. These were the only notable effects in the trial.

Another measure of the effectiveness of antidotes was to determine the separation of the mortality curves observed with and without treatment. It was determined that an effective antidote should be able to change a dose of pesticide from very likely to produce mortality (LD90) to unlikely to produce mortality (LD10), and the most effective antidotes would be those whose LD10 value was higher than the untreated LD90 value. For chlorfenvinphos, this effect was achieved using atropine sulphate in combination with P2S, and atropine sulphate in combination with obidoxime. Atropine sulphate alone and obidoxime alone also produced results close to this effect. This study therefore indicated that for chlorfenvinphos poisoning, the most effective antidote was a combination of atropine sulphate and obidoxime, while a combination of atropine sulphate and P2S was also effective.

4. SHORT TERM REPEAT DOSE STUDIES

4.1 Oral

4.1.1 Mice

Tennekes H (1989) 14-day range-finding (feeding) study with chlorfenvinphos in the mouse. RCC project 248793. Research and Consulting Company Ltd, Itingen, Switzerland. (GLP not stated) (Study duration: 5/9/89 to 26/9/89)

Groups (5 males/group) of mice (NMRI, Hanover-derived outbred, spf; Biological Research Laboratories, Switzerland) were given technical grade chlorfenvinphos (batch not stated; purity 92.8%) at 0, 1, 10, 100, 1000 or 3000 ppm in the diet for 14 days (equal to 0, 0.2, 1.9, 20.3, 203.4 or 633.2 mg/kg/day). The mice were observed daily for clinical signs; bodyweight and food consumption was recorded weekly.

Blood samples (retro-orbital plexus) were collected after 14 days from non-fasted mice for assay of plasma and erythrocyte ChE activity. At the end of the feeding period, the mice were killed and whole brain tissue was weighed and placed on ice for ChE determination. Liver and kidney weights were also recorded; no histopathology was carried out.

The mean concentration of chlorfenvinphos in the feed was 82–120% of the nominal concentrations. Homogeneity and storage stability data were acceptable.
No deaths occurred during the study and there were no treatment-related clinical signs. Bodyweight gain was reduced in the high-dose mice during the feeding period (19%), accompanied by a slight decrease in food consumption.

At 2 weeks, there was a dose-dependent reduction in plasma ChE activity at doses of 10 ppm (25% - 93%), erythrocyte ChE activity at doses 100 ppm (25% - 79%) and brain ChE at doses 1000 ppm (28% - 40%).

Absolute organ weights were not affected by chlorfenvinphos treatment but relative brain and liver weights were significantly increased (18% and 19%, respectively) at 3000 ppm, which appeared to reflect the decreased bodyweight (19%) at this dose.

No plasma ChE inhibition was seen at 1 ppm, equal to 0.2 mg/kg bw/day.

Tennekes H, Janiak T, Stucki HP, et al (April 1991). 28-day range-finding (feeding) study with chlorfenvinphos in the mouse. RCC project 243202. Research and Consulting Company Ltd, Itingen, Switzerland. GLP (Switzerland, OECD [Guideline 403;May 1981]) (Study duration: 16/10/89 to 21/11/89)

Groups (10/sex/group) of mice (NMRI, Hanover-derived outbred, spf; Biological Research Laboratories, Switzerland) were dosed with technical grade chlorfenvinphos (batch 1331-89; purity 92.8%) at 0, 1, 10, 100 or 1000 ppm in the diet for 28 days (equal to 0, 0.18, 1.89, 18.26 or 187.89 mg/kg/day for males and 0, 0.21, 2.09, 20.84, 226.17 mg/kg/day for females). (The doses were based on a 14-day range-finding feeding study in which mice received technical grade chlorfenvinphos in the diet at doses up to 3000 ppm for 14 days.)

The mice were observed daily for clinical signs; bodyweight and food consumption was recorded weekly. Ophthalmological examination was conducted pretest and at the end of feeding. Blood samples (retroorbital plexus) were collected after 28 days from non-fasted mice for clinical biochemistry (albumin, AP, glucose, total protein, ALT, AST and plasma and erythrocyte ChE activity).

At the end of the feeding period, the mice were killed. Whole brain tissue was weighed and placed on ice for ChE determination and selected organs were weighed (adrenals, testes, kidneys, liver, spleen and heart). For control and high-dose mice, histopathological examination was carried out on the following organs and tissues: adrenals, aorta, heart, kidney, lungs, liver, lymph nodes, ovaries, pancreas, spleen, stomach, testes, thymus, thyroid (with parathyroid) uterus, small and large intestine and gross lesions. For the mice fed 1, 10 and 100 ppm chlorfenvinphos, however, only the liver, kidneys and gross findings were examined microscopically.

The mean concentration of chlorfenvinphos in the feed was 82–120% of the nominal concentrations. Homogeneity and storage stability data were acceptable. No deaths occurred during the study and there were no treatment-related clinical signs or effects on food consumption or bodyweight. Ophthalmoscopic examinations did not show any treatment-related effects.

Clinical biochemistry results showed slight increases in the albumin concentration of males fed 100 or 1000 ppm chlorfenvinphos and the total protein concentration in the high-dose males. At 4 weeks, plasma ChE activity was reduced by 18%–19%, 79%–82% and 94%–95% at 10, 100 and 1000 ppm chlorfenvinphos, respectively. Erythrocyte ChE was reduced by 30% (males only), and 65% for the 100 and 1000 ppm groups, respectively. Brain ChE was inhibited in females by approximately 52% for the 1000 ppm group, and 24–29% for all the lower doses. In males, brain ChE was inhibited by 50% at 1000 ppm; inhibition was 22 % at 10 ppm, but only 9% at 100 ppm.

Relative liver and kidney weights were significantly increased for males at 1000 ppm (9% and 12%, respectively) and absolute and relative heart weight was increased for females at 1000 ppm (15% and 10%, respectively). There were no gross or microscopic effects on any organ or tissue examined. The slight increases in liver weight, plasma albumin and total protein levels in the high-dose males indicated that the liver could become a target organ at high dose levels.

There was inhibition of brain ChE in females at the lowest dose of 1 ppm, equal to 0.2 mg/kg bw/day.

4.1.2 Rats

Pickering CE (1978) Toxicity of chlorfenvinphos: The reversibility of cholinesterase and aliesterase inhibition produced by feeding chlorfenvinphos to rats. TLGR.0169.78 , Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no 16001, purity 90.5%, source: Pernis Laboratory) was fed in the diet to Wistar rats (Tunstall Breeding Unit) at doses of 0, 0.3, 1, 3 or 30 ppm (equivalent to 0, 0.015, 0.05, 0.15 or 1.5 mg/kg bw/day) using 20 rats/sex/group. Rats were maintained on the treated diets for 4 weeks, after which 10/sex/group were euthanised. The remaining 10/sex/group were maintained on control diets for a period of 4 weeks, after which they were euthanised. During the feeding period, animals were examined daily for clinical signs and behaviour. Body weight and food consumption were determined weekly. On euthanasia, blood samples were taken and the heart and liver collected from the animals. Plasma, erythrocyte and brain ChE activities were determined. Liver and plasma aliesterase levels were also analysed.

There were no mortalities or abnormal clinical signs observed during the study. There were no changes in body weight or food consumption related to compound consumption. Plasma, erythrocyte and brain ChE activities were significantly inhibited in the high-dose group; the inhibition in plasma was >29%, in erythrocytes >44% and in brain >33%, with the activity inhibited to a greater extent in females than in males. The aliesterase activity in plasma and liver was inhibited from 3 ppm in the diet, with inhibition of 36% seen in plasma and 30% in liver. Following 4 weeks on control diet, there was no inhibition of either ChE or aliesterase seen at 3 ppm. At this time, brain ChE activity was inhibited approximately 10% in both males and females in the high-dose group, while there was no inhibition of plasma ChE.

In this study inhibition of aliesterase in plasma and liver was seen at 3 ppm in the diet, but no effects were seen at 1 ppm in the diet, equivalent to 0.05 mg/kg bw/day.

Puzynska L (1984a) Dietary protein deficiency and the influence of chlorfenvinfos on the biological parameters in rats. Die Nahrung 28: 173-183

Wistar rats (Food and Nutrition Institute) were maintained on diets of 4.5% or 26% protein and containing chlorfenvinphos (purity 91.4%, source, batch no. not specified) at 0, 5, 100 or 1000 ppm, using 25 rats/sex/group, with additional rats in the high-dose group. Food and water were available *ad libitum*. Bodyweight and food intake were recorded daily. After 10 days, 10/sex/group were killed by decapitation, and after 31 days 15/sex/group were killed. Additional high-dose rats (number not specified) were maintained on control diets for 14 days to determine recovery.

Clinical signs of poisoning (not specified) were seen in rats in the high-dose group in the first 5 to 8 days of the trial. Food consumption was decreased in rats on 100 and 1000 ppm over the first 5 days; the intake then increased until by day 10 the food consumption in treated rats was higher than that of controls. The body weight in rats at 1000 ppm decreased around 20% over the period of dosing (estimated from a graph). This decrease was more marked in rats on 26% protein in the diet than in rats on 4.5% protein. After the withdrawal of chlorfenvinphos from the diet the bodyweight began to slowly return to normal.

On gross pathological examination, congestion of the heart, spleen and kidney was seen at the two highest doses. In rats on 100 ppm and 4.5% protein in the diet, eosinophilic infiltrations around the lungs were seen. In rats on 1000 ppm and 26% protein in the diet, there were focal degenerative changes in the exocrine pancreas, and a proliferation of lymphatic nodules in the spleen. No individual animal results, or detailed summary of pathological findings were presented, so no other changes could be assessed. There was no determination of ChE activity in this trial. No effects were seen at 5 ppm in the diet, equivalent to 0.25 mg/kg bw/day.

Puzynska L (1984b) Alterations in some biochemical processes in the organism of rats being under the influence of chlorfenvinphos administered in diets with variable protein content. Die Nahrung 28(5): 473 - 482

Chlorfenvinphos (purity 91.4%, source Ciba-Geigy, batch no not specified) was fed in the diet to Wistar rats (Food and Nutrition Institute, Poland) at 0, 5, 100 or 1000 ppm (equivalent to 0, 0.25, 0.5 or 50 mg/kg bw/day) for 11 or 31 days. Rats were maintained either on an optimal diet (26% protein) or a low protein diet (4.5% protein) using 25 rats/sex/dosing group on each diet. Additional animals were maintained in the control and high dose groups. After 10 days, 10/sex/group were killed by decapitation, and after 31 days 15/sex/group were killed. High-dose rats in a recovery group were maintained on the control diet for 14 days. Blood, brain and liver were rapidly removed after euthanasia, and the activity of ChE, sorbitol dehydrogenase, glutamic dehydrogenase, glucose phosphate isomerase, AST, ALT, aromatic amino acid aminotransferases, tyrosine aminotransferase, and phenylalanine and tryptophan aminotransferases were determined. The protein levels in the brain and liver were also estimated.

Plasma ChE activity was inhibited 60% in males and 80% in females at both 100 and 1000 ppm at both time periods. In rats on 5 ppm, plasma ChE inhibition was only seen in rats on the optimal protein diet, where there was 28% inhibition in males and 18% inhibition in females. Brain ChE activity was not significantly inhibited at 5 ppm, but was inhibited significantly (approximately 60%) in both diets at 100 ppm. Chlorfenvinphos also affected brain carbohydrate metabolism, with glucose phosphate isomerase activity decreased. There were no changes to the other enzymes assayed in serum, liver or brain. After the recovery period, high-dose males on the 4.5% protein and females on the 26% protein diet showed significant plasma ChE inhibition, while other rats did not show any inhibition. Inhibition of plasma ChE was seen at 5 ppm and above, equivalent to 0.25 mg/kg bw/day.

4.1.3 Dog

Allen TR, Corney SJ, Frei TH, Luetkemeier H, Biedermann K and Springall CJ (July 1992) 4-week oral range-finding toxicity (feeding) study with chlorfenvinphos in the dog. RCC project 271934. Research and Consulting Company Ltd, Itingen, Switzerland. GLP (Switzerland) (Study duration: 10/9/90–7/11/90)

Groups (2/sex/dose) of beagle dogs (BRL Biological Research Laboratories Ltd, Switzerland) were given technical grade chlorfenvinphos (batch F 890355; purity 92.8%) mixed in the diet at 0, 3, 100 or 3000 ppm for four weeks (equal to 0, 0.12, 3.9 and 105 mg/kg bw/day, respectively). Clinical signs were observed twice daily, food consumption was recorded daily, bodyweight was recorded weekly (including first and last day of treatment) and ophthalmoscopic examinations were performed at pretest and at the end of the feeding period.

Blood (jugular vein) and urine samples were obtained from all dogs after an overnight fast at pretest, and at 4 weeks for clinical laboratory investigations (see table below). Additional blood samples were also collected at pretest, and weeks 1, 2 and 3 for analysis of plasma and erythrocyte ChE activities.

At the end of the feeding period the dogs were killed and selected organs were weighed (adrenals, brain, testes with epididymis, kidneys, liver, spleen, thyroid with parathyroid, heart, pituitary gland, prostate). Histopathological examination was carried out on the following organs and tissues: adrenals, aorta, bone, bone marrow, brain, epididymis, eyes with optic nerve, gall bladder, tongue, heart, kidney, lungs, liver, lymph nodes, mammary gland, skeletal muscle, sciatic nerve, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus, vagina, small and large intestine and gross lesions). A piece of brain tissue from the cerebellum sagittal region was recovered for assay of brain ChE activity.

The clinical chemistry parameters examined were: albumin (protein electrophoresis), AP, bilirubin (total), calcium, chloride, cholesterol (total), ChE (plasma and erythrocyte), creatinine (blood), creatine kinase (CK), gamma-glutamyl transpeptidase, globulin (protein electrophoresis), glucose (blood), glutamate dehydrogenase, iron, serum lactate dehydrogenase (LDH), lipids (total), magnesium, ornithine decarboxylase, phospholipids, phosphorus, potassium, protein (total), ALT, AST, sodium, triglycerides and BUN.

The haematology parameters examined were APTT, PT, erythrocyte count, Hct, Hb,
leucocyte differential count, leucocyte total count, platelet count, reticulocyte count, MCH, MCHC, MCV and a blood smear. Urinalysis was performed for appearance/colour, specific gravity, osmolality, glucose, ketones, sediment, occult blood, pH, protein, bilirubin and urobilinitrogen.

The mean concentration of chlorfenvinphos in the feed was 86% - 99% of nominal concentrations. Homogeneity and storage stability data were acceptable.

No deaths occurred during the study and there were no treatment-related clinical signs. One high-dose dog of each sex had reduced food intake during the study, particularly during the first week. Bodyweights were unaffected by chlorfenvinphos intake and ophthalmoscopic examinations did not reveal any treatment-related effects.

Haematology and urinalysis parameters were unaffected by treatment. Clinical biochemistry results showed a dose-dependent reduction in plasma ChE activity at the mid- and high-doses (>60% and >80%) for both sexes. Erythrocyte ChE was also reduced at 3000 ppm (49% and 39% in week 1; 63% and 77% in week 4, for males and females, respectively). Brain ChE was unaffected by treatment.

The only organ weight, gross or microscopic pathological finding that could be related to chlorfenvinphos treatment was a slight increase in absolute and relative liver weight in HD dogs. The slightly increased liver weight in the high-dose dogs at postmortem may indicate some involvement of the liver as a target organ at higher doses.

No effects were seen at 3 ppm (equal to 0.12 mg/kg bw/day).

Allen TR, Corney, SJ Luetkemeier H and Biedermann K (June 1992) Preliminary dose-range finding (feeding) study with chlorfenvinphos in the dog. RCC project 271844. Research and Consulting Company Ltd, Itingen, Switzerland. GLP (Switzerland) (Study duration: 2/8/90–7/10/90)

Three groups (2/sex/group) of beagle dogs (BRL Biological Research Laboratories Ltd, Switzerland) were given technical grade chlorfenvinphos (batch F 890355; purity 92.8%) mixed in the diet as follows:

Table 15

Group	Dose	Days administered	Average intake (mg/kg bw/day)
1	0	1 - 60	0
2	1	1 - 12	0.037
	2	13 - 26	0.072
	4	27 - 35	0.143
	8	36 - 41	0.281
	16	42 - 50	0.545
	32	51 - 60	1.13
3	1000	48 - 54	31
	2000	55 - 60	63

Dogs in group 2 were given progressively increasing doses as indicated in the table, with the same two dogs used in this group throughout the study. The group 3 dogs had their dose doubled after 54 days.

Clinical signs were observed twice daily, food consumption was recorded daily and bodyweight was recorded on the first and last day of treatment. Blood (jugular vein) samples were obtained from all dogs after an overnight fast at pretest, every 2 to 8 days thereafter, and at each dose change for analysis of plasma and erythrocyte ChE activities. No other clinical biochemistry, urinalysis or haematology examinations were performed.

At the end of the feeding period the dogs were killed and selected organs were weighed (adrenals, brain, testes with epididymis, kidneys, liver, spleen, thyroid with parathyroid, heart, pituitary gland, prostate). Histopathological examination was carried out on the following organs and tissues: adrenals, aorta, bone [femur and sternum], bone marrow, brain, epididymis, eyes with optic nerve, gall bladder, tongue, heart, kidney, lungs, liver, lymph nodes, mammary gland, skeletal muscle, sciatic nerve, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, skin, spinal cord, spleen, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus, vagina, small and large intestines and gross lesions). A piece of brain tissue from the cerebellum sagittal region was used for assay of brain ChE activity.

The mean concentration of chlorfenvinphos in the feed ranged from 82% to 100% nominal concentrations. Homogeneity and storage stability data were acceptable.

No deaths occurred during the study; there were no treatment-related clinical signs. Food intake was markedly reduced for Group 3 females after dosing started, particularly during the first three days. This was accompanied by a slight weight loss over these days. Bodyweight gain was unaffected in Group 2 dogs and males of Group 3.

A progressive and dose-dependent reduction in plasma ChE activity (13% - 50%) was seen for all dogs of Group 2 from day 47 onwards (16 ppm). A severe reduction in plasma ChE activity (68% - 87%) also occurred for all Group 3 dogs from the start of treatment (day 48). Erythrocyte ChE activity was unaffected for Group 2 dogs but in Group 3 there was a moderate reduction (45% - 55%) on day 57. Brain ChE activity was unaffected by treatment for both groups.

Organ weights were not affected by chlorfenvinphos treatment. The only pathological finding was reddish discolouration of the mucosa of the duodenal or ileum of one male and one female of Group 2.

The only significant toxic effect of chlorfenvinphos on beagle dogs at doses up to 3000 ppm in the diet (equal to 63 mg/kg/day for males and females) was ChE inhibition. No effect on plasma ChE was seen until 47 days when the dose reached 16 ppm (0.545 mg/kg bw/day).

4.2 Dermal

Brown VKH (1965) Some further data on the acute and sub-acute toxicities of the insecticide SD 7859 (GC 4072). PPR TL/1/65 Shell Research Limited, Sittingbourne, Kent

Chlorfenvinphos (batch no FC 1339, purity 92%, source: Shell Development Company) was applied dermally to the shorn dorso-lumbar region of male "P" strain guinea-pigs (Tunstall Laboratories) at 1, 10 or 100 mg/kg bw/day for 14 days using 10/group. Blood samples were taken to determine plasma ChE activity 24 h after the first dose, then weekly for 5 weeks. Results were only presented in a graph form, but it was estimated that there was inhibition >20% at all doses from approximately day 4, with maximum inhibition of approximately 90% seen in the high-dose group on approximately day 15. Plasma ChE remained inhibited at the highest dose until the end of the study, when inhibition was approximately 37%. Recovery to less than 20% inhibition in the two lower doses had occurred by the final week of the study.
The experiment was repeated using doses of 0.01, 0.1 or 1 mg/kg bw/day, with chlorfenvinphos suspended in olive oil to allow application of these smaller doses. Doses of 0.01 or 0.1 mg/kg bw/day produced approximately 15% inhibition of plasma ChE. It was noted that no abnormal clinical signs were observed in either trial, despite the severe plasma ChE inhibition observed in the first trial. The inhibition of plasma ChE activity seen at 0.1 mg/kg bw/day was not considered to be toxicologically significant.

Stevenson DE (1974a) Toxicity of insecticides. Skin and eye irritancy and acute toxicity of BIRLANE 50% seed dressing. TLTR.0015.74 Shell Research Institute, Sittingbourne

Chlorfenvinphos (as a 50% seed dressing formulation) was applied to the clipped dorso-lumbar region of 'P' strain guinea-pigs (Tunstall Laboratory) (5/sex) and New Zealand White rabbits (Ranch Rabbits, Sussex) (1/sex). Applications were made 5 days/week for 23 applications, with 0.5 g of formulation for guinea-pigs and 1.0 g formulation for rabbits, moistened with water,

applied to the test area daily. It was not stated whether the area was occluded. Assessments of skin reactions were made daily throughout the study. In the second week of the trial, 4/8 guinea-pigs showed very mild skin irritation, which was not found in subsequent observations. Rabbit skin was not irritated at any time during the trial. Based on this, the seed dressing formulation of chlorfenvinphos was determined to be non-irritating to rabbit and guinea-pig skin.

Stevenson DE (1974b) Toxicity of insecticides: Skin and eye irritancy of BIRLANE granules formulation EF 3651. TLTR.0014.74, Shell Research Institute, Sittingbourne

Chlorfenvinphos, as Birlane granules (source: Woodstock Laboratory, Sittingbourne, purity, batch no not specified) was applied to the shorn dorso-lumbar skin of New Zealand White rabbits (Ranch Rabbits, Sussex, 1/sex), and 'P' strain guinea-pigs (Tunstall Laboratories, 5/sex) once daily (0.5 g/day for guinea-pigs, 1.0 g/day for rabbits), 5 days/week for 23 applications. The test material was moistened with water prior to application, and the area was not covered following application. Assessments were made after 1, 2, 3, and 9 weeks, and no irritation was seen in any animal at any time. Therefore this formulation was determined to be non-irritating to non-occluded rabbit and guinea-pig skin.

Zeman A & Johnston RE (1980) Plasma cholinesterase levels and dermal irritation with Supona Dog Collars in Dogs. Doc Ref TTEP/80/0035. Burroughs Wellcome Report, USA

Beagle dogs (Marshall Research Animals Inc, NY) were tested for plasma ChE activity and divided into 3 groups, ensuring mean plasma ChE levels were comparable between each group. Three groups of 4 dogs/sex were exposed to 2 control collars, 1 control and 1 chlorfenvinphos-containing collar or 2 chlorfenvinphos-containing collars for 14 days. The treated collars contained 15% chlorfenvinphos (not otherwise specified) and were obtained from Burroughs Wellcome Co. The collars were attached around the neck loosely (but securely enough to prevent removal), and were stapled together to ensure simultaneous contact with the fur for both collars.

All animals were observed daily for changes in activity, behaviour and skin condition at the application site. Body weights were determined twice before the study and on days 7 and 14 of the study. Plasma ChE activity was determined 4 times pre-study, on exposure days 1, 3, 7 and 14 and on days 3, 7, 10, 14 and 17 after exposure. Blood samples were taken in the morning on each testing day.

No abnormal clinical signs or signs of dermal irritation were detected at any time during the study. There were no dose-related changes in body weight or food consumption during the study. Inhibition of plasma ChE occurred with either 1 or 2 chlorfenvinphos collars; results are presented in the table below.

Table16: Mean plasma ChE inhibition (expressed as a % of concurrent controls)

Study day	1 collar		2 collars	
	male	**female**	**male**	**female**
1	8	8	21	18
3	28	32	48*	40*

7	55*	54*	65*	60*
14	58*	60*	68*	60*
3+	54*	52*	62*	56*
7+	47*	41*	50*	47*
10+	44*	38*	46*	47*
14+	38*	35*	38*	39*
17+	36	34	36	41*

+ - days post treatment, * - statistically significantly inhibited ($p<0.05$)

Plasma ChE activity was inhibited in both sexes with either one or two collars from study day 3, and remained inhibited at >20% for 17 days after the removal of the collar. Therefore even a single collar containing 15% chlorfenvinphos produced significant plasma ChE inhibition under normal conditions of use.

4.3 Subcutaneous

Gajewski D (1980) Acetylcholinesterase activity in several rat liver cell fractions after repeated poisoning with some organophosphates. Acta Physiol Pol 31: 576-580

Chlorfenvinphos (purity, batch no, source not specified) was administered to female Wistar rats (source not specified) at doses of 0 or 1.85 mg/kg bw/day by SC injection, using 8 rats per group for 28 days. The dose was selected following a determination of the LD50 using 6rats/group (doses not specified). Bodyweights were determined on day 10, 20 and 28 of the experiment. Rats were killed by decapitation 1 h after the last dose was administered. The liver was immediately removed, weighed and homogenised in a solution of saccharose in phosphate buffer. The soluble, microsomal and mitochondrial fractions were obtained following centrifugation. The nitrogen levels in the tissues was determined, as was the ChE activity.

A decrease in ChE activity in the liver relative to controls was noted, with the activity in the liver homogenate 69.9% of controls; in the soluble fraction 75.1% of controls; in the microsomal fraction 52% of controls and in the mitochondrial fraction 61% of controls. Body weight in treated females decreased in comparison to controls by day 20, with a 17% decrease by day 28.

4.4 Intraperitoneal

Luczak C, Gralewicz S & Gorny R (1992) Tolerance to chlorphenvinphos in rats assessed on the basis of changes in locomotor behaviour in rotating wheels. Pol J Occ Med Env Health 5(1) 43 - 54

Chlorfenvinphos (technical grade, source: Organika-Azoty, Jaworzno, Poland; batch no, purity not specified) in olive oil was administered by IP injection to male IMP-Dak Wistar rats (outbred) in two experiments. In a test to determine ChE activity, chlorfenvinphos was administered at 0, 1 or 3 mg/kg bw/day, 5 days/week for two weeks, using 24 rats/treatment group and 4 rats as controls. In a second study to determine behavioural changes following chlorfenvinphos administration, chlorfenvinphos was administered at 0, 1 or 3 mg/kg bw/day, 5 days/week for two weeks using

5/group. Rats in this study were housed individually in cages with rotating wheels. The wheels had a circumference of 70 cm and a runway width of 10 cm. The low point was 7 cm above the floor, and the wheels were monitored with the generation of an electrical pulse for every 90 degree turn. After two weeks of dosing, both groups were untreated for two weeks, then a challenge dose of 3 mg/kg bw was administered by IP injection to all rats.

In the rats used for measurement of ChE activity, the activity in the blood and brain were determined spectrophotometrically 3 and 24 h after the first injection, 3 h after the fifth injection, 3 h and 7 days after the tenth injection and 3 h after the last injection, using 4 or 5 animals for the determination at each time period. The activity of rats in the running wheel was measured over the active (dark) period each day, for a nominated period.

There was no notable change in body weights over the administration period. In the behavioural trial, activity decreased in high-dose rats on days 1 to 5; no decrease in activity was noted in low-dose rats. Following a challenge dose of 3 mg/kg bw, the activity level of low-dose and control rats was significantly decreased, however there was no effect on the behaviour of rats previously given the high dose.

Plasma and erythrocyte ChE activity was significantly inhibited at 1 mg/kg bw, with inhibition of 45% to 65% in plasma ChE activity and inhibition of 45% in erythrocyte ChE activity. Following the recovery phase, plasma ChE was normal in all rats, while erythrocyte ChE activity was 30% inhibited in low-dose rats and 50% inhibited in high-dose rats. Following the 3 mg/kg bw challenge injection, low-dose rats showed a 30% inhibition of plasma ChE activity, while high-dose rats showed a 35% inhibition. Erythrocyte ChE activity was inhibited 45% and 65% respectively.

Brain ChE activity was approximately 30% inhibited in the low-dose group 3 h after the first injection, and remained at approximately the same level during the treatment period. Following the recovery period, the inhibition had decreased to 10% - 15%, and following the challenge dose the inhibition was between 35% and 50%, depending on the brain area tested. The cerebellum was less affected than the brainstem, with the diencephalon most severely affected. In the high-dose rats, 80% inhibition was seen 3 h after the first injection, with inhibition of approximately 70% seen throughout the treatment period. Following the recovery phase, inhibition in the cerebellum was approximately 20%, while other brain areas showed 30% - 50% inhibition. After the challenge injection, the inhibition in the cerebellum increased to 35% while inhibition in the other brain areas did not markedly increase.

This study indicated that acquired tolerance to the high dose (both in biochemical and behavioural terms) lasted longer than 7 days, however repeated exposure to the lower dose did not protect against the effects of high doses. The brain ChE activity in low-dose and high-dose rats was approximately the same after the challenge dose, but the low dose rats showed a decrease in locomotor ability not seen in the high dose rats.

5. SUBCHRONIC STUDIES

5.1 Rat

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl)-2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (purity 96%, source not specified) was administered in the diet to Wistar rats (Albino Farms, Red Bank NJ) at levels of 0, 3, 10, 30, 100 or 1000 ppm (equivalent to 0, 0.15, 0.5, 1.5, 5 or 50 mg/kg bw/day) for 12 weeks, using 10 rats/sex/group. Prior to dosing rats underwent a 5-week acclimation period in the laboratory during which baseline plasma and erythrocyte ChE activity was determined. At weeks 1, 2, 4, 6, 8, 10 and 12 plasma and erythrocyte ChE activity was determined for 5 rats/sex/group. Rats were weighed weekly, and food consumption was determined in weeks 4 and 12.

After 12 weeks, 5 rats/sex/group were maintained on control diet for 5 weeks; the remaining rats were euthanised. Plasma and erythrocyte ChE activity was determined after 1 and 4 weeks maintenance on control diets; after 5 weeks recovery, all rats were euthanised. Organ to body weight ratio was determined for the liver, kidneys, heart, spleen and testes of all rats euthanised after 12 weeks treatment and for those maintained on the control diet for 5 weeks. Histopathological examinations were performed on the heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonads, pituitary and brain of 5 female and 3 male control rats, and 2 female and 3 males on 100 ppm after 12 weeks treatment.

No individual animal data or detailed summary information was supplied. No effects on mortality or food consumption were reported. It was stated that growth was significantly depressed in both sexes at 1000 ppm. Some recovery was seen in those animals maintained on the control diet for 4 weeks. Plasma and erythrocyte ChE activity was said to be significantly depressed for rats on 30, 100 or 1000 ppm in the diet: no information on the degree of depression seen was provided. There were sporadic decreases in ChE activity seen at 10 ppm, while no depression was seen at 3 ppm. Plasma ChE activity had returned to normal after 1 week recovery except in females previously on 1000 ppm; these animals had recovered after 4 weeks maintenance. Erythrocyte ChE activity had returned to normal within 4 weeks, except for males previously maintained on 100 or 1000 ppm.

There were no significant changes in the organ to body weight ratio of all organs for male rats. In females, the spleen and kidney weight of animals at 30 ppm, and the spleen weight at 100 ppm were decreased after 12 weeks treatment, but these changes resolved after 4 weeks recovery. There were no gross or microscopic findings reported, although again no individual animal results were provided.

As there were no individual animal results or detailed summaries provided, it was not considered suitable for regulatory purposes. Based on the sporadic plasma and erythrocyte ChE inhibition seen at 10 ppm, the NOEL was 3 ppm in the diet (equivalent to 0.15 mg/kg bw/day).

Anon (1963b) Cholinesterase inhibition studies on GC-4072 in rats. Twelve week feeding period followed by four weeks withdrawal. Medical College of Virginia, Virginia

Chlorfenvinphos (source, batch no, purity not specified) was administered in the diet to Wistar rats at doses of 0, 3, 10, 30, 100 or 1000 ppm (equivalent to 0, 0.15, 0.5, 1.5, 5 or 50 mg/kg bw/day) for 12 weeks using 10/sex/group. Prior to commencement of dosing rats were maintained under laboratory conditions for 5 weeks. During this period, plasma and erythrocyte ChE activity was determined once weekly in 5 rats/sex/group, with the same rats used where possible. During the trial, body weight was determined weekly, and food consumption was measured during weeks 4

and 12. Plasma and erythrocyte ChE activity was determined at 1, 2, 4, 6, 8, 10 and 12 weeks. At the end of the 12-week feeding period, 5 rats/sex/group were maintained on control diet for a 4-week recovery period, with the remaining animals euthanised. A histopathological examination of control and high-dose rats was done at the end of the recovery stage, with 5 female and 3 male controls, and 2 female and 3 male high-dose animals examined. The heart, lung, liver, kidney, bladder, spleen, GI tract, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonad, pituitary and brain were examined. Plasma and erythrocyte ChE activity of the animals maintained on the control diet were determined after week 1 and week 4. All rats were euthanised at the end of this 4-week period.

Abnormal clinical signs were seen in rats in the high-dose group, including muscle fasciculations, tremors and bloody discharge from the nose and eyes. Body weight was statistically significantly ($p<0.05$) decreased in all animals on 1000 ppm throughout the trial, with decreases of 17% - 20% in females and 12% - 22% in males. In males on 100 ppm, there was also significant decreases of 10% - 12%. Food consumption was not significantly different from controls. In the high-dose rats body weight did not recover to normal levels during the 4-week recovery period. Mean plasma ChE activity was inhibited at all time periods in females on 10 ppm, with inhibition from 35 - 62%, and in almost all time periods in males, with inhibition from 10% to 45%. Erythrocyte ChE activity was consistently inhibited at 30 ppm (inhibition from 25% - 39%), with inhibition also observed in females at 10 ppm. ChE activity had completely recovered during the 4 weeks recovery period. Histopathological examination of a limited group of animals revealed one instance of testicular atrophy in a high-dose animal.

No individual animal data was presented for this experiment. Very limited histopathological examinations were done. Based on the inhibition of plasma and erythrocyte ChE activity seen at 10 ppm, the NOEL was 3 ppm (equivalent to 0.15 mg/kg bw/day).

Anon (1963c) Toxicologic study on the effect of adding 0, 1 and 3 ppm GC-4072 to the diet of albino rats. Medical College of Virginia

Chlorfenvinphos (batch no, purity, source not specified) was fed in the diet to Wistar rats (source not specified) at 0, 1 or 3 ppm (equivalent to 0, 0.05 or 0.15 mg/kg bw/day) for a 12-week period using 35 rats/sex/group. Details of animal housing and observations were limited. Body weight was determined weekly throughout the study, with food consumption measured in weeks 4 and 12. Plasma and erythrocyte ChE activity was determined in weeks 1, 2, 4, 6, 8, 10 and 12 in 5 rats/sex/group. Haematological examinations (Hct, Hb and total and differential white cell counts) were conducted at week 12.

No individual animal results were reported for this study. There were no treatment related effects on mortality, body weight or food consumption. There were no abnormal clinical signs reported. Plasma ChE activity was decreased in both males and females on 3 ppm throughout the trial, with inhibition ranging from 11% to 39% (mean inhibition 22% in females and 24% in males). There was no inhibition of plasma ChE at 1 ppm, or of erythrocyte ChE at either dose. There were no abnormal findings on haematological examination.

Based on the plasma ChE inhibition seen at 3 ppm, the NOEL for this study was 1 ppm (equivalent to 0.05 mg/kg bw/day)

5.2 Dog

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR. (1970) Toxicologic studies on diethyl-1-(2,4-dichlorophenyl)-2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (purity 96%, source not specified) was administered in the diet to mongrel dogs at doses of 1, 10, 100 or 1000 ppm (equivalent to 0.025, 0.25, 2.5 or 25 mg/kg bw/day) for 12 weeks, using 2/sex/group. Prior to the commencement of treatment, dogs were maintained on standard food, and the plasma and erythrocyte ChE levels determined. At weeks 1, 2, 4, 6, 8, 10 and 12 plasma and erythrocyte ChE activity was determined. Dogs were weighed weekly, and food consumption was measured daily.

At week 12, 1 dog/sex/group was euthanised, while the other was maintained on control diet for 8 weeks recovery period. During the recovery phase, plasma and erythrocyte ChE activities were determined on weeks 1, 2, 4 and 8. At the end of the recovery phase, all dogs were euthanised. Histopathological examination of the heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonads, pituitary and brain was done for all dogs.

No individual animals results or detailed summaries were presented. There were no reported effects on mortality, food consumption or weight gain. Statistically significant depression of plasma ChE activity occurred at all dose levels. Erythrocyte ChE activity was sporadically depressed. In the recovery phase, plasma ChE values returned to normal, however erythrocyte ChE activity did not. There were no abnormal histopathological findings. No NOEL could be established for this study, based on the plasma ChE depression seen at all dose levels, however given the lack of information supplied, the study was not suitable for regulatory purposes. .

Walker AIT (1965) The subacute oral toxicity of the halophenyl vinyl phosphate insecticide chlorfenvinphos (SUPONA, BIRLANE) to dogs. R(T)-11-65 Shell Research Limited, Sittingbourne

Chlorfenvinphos (batch FC 1339, purity 92%, source: Shell Chemical Co. NY) was administered in the diet at levels of 0, 0.5, 1 or 3 ppm (equivalent to 0, 0.0125, 0.025 or 0.075 mg/kg bw/day) to Beagle dogs (Tunstall Laboratories) for 16 weeks. There were 5 dogs/sex in control, 3/sex at 0.5 ppm, 4 males and 2 females at 1 ppm and 1/sex at 3 ppm. Dogs were maintained in individual cages, with daily observations for health and behaviour. Feeding occurred in the afternoon, with uneaten food removed in the morning. Food consumption was measured daily, and body weight was measured weekly. A detailed clinical examination of all animals, including auscultation of the heart and examination of the mucous membranes was done at intervals throughout the study. Blood samples for hematological examination were taken before dosing commenced, and during weeks 4, 13 and 16. Total and differential white cell counts, erythrocyte counts, Hb, platelet count and prothrombin time were determined. Total serum protein and BUN were also determined at these times. Plasma and erythrocyte ChE activities were determined pre-test and at weeks 1, 2, 4, 8, 13 and 16. A liver function test, using bromosulfophthalein (BSP) clearance, was done at weeks 7 and 13.

All animals were euthanised after 16 weeks. Brain ChE activity was determined, and major visceral organs were weighed. A detailed histopathological examination of the brain, spinal cord,

thyroid, parathyroid, heart, lungs, spleen, liver, kidney, stomach, small and large intestine, lymph nodes, bone marrow, pancreas, eyes, testicles, prostate, ovaries, Fallopian tubes, uterus, urinary bladder and skeletal muscle of all animals was performed.

No abnormal clinical signs were reported throughout the study. Bodyweight data were not reported in detail, but were stated to be normal. There was no notable change in organ weight between treatment groups. Haematological examination did not reveal any abnormalities. There were no treatment-related histopathological effects observed in this study.

Plasma and erythrocyte ChE activity was intermittently inhibited at 3 ppm in females, with no inhibition seen in males. The mean inhibition was minimal (up to 24% in plasma ChE, and 21% in erythrocyte ChE), and was not considered to be of biological significance. No significant inhibition of brain ChE was observed. The NOEL for ChE inhibition was 3 ppm, equivalent to 0.075 mg/kg bw/day.

6. CHRONIC STUDIES

6.1 Mice

Schmid H, Probst D & Luetkemeier H et al (July 1993). Oncogenicity (feeding) study with chlorfenvinphos in the mouse. RCC project 265926. Research and Consulting Company Ltd, Itingen, Switzerland. GLP (OECD, USEPA, Japan MAFF, Switzerland) (Study duration: 8/3/90–18/3/92)

Groups (74/sex/group) of mice (NMRI, Hannover-derived, outbred, spf; BRL Biological Research Laboratories Ltd, Switzerland) were dosed with technical grade chlorfenvinphos (Batch F890355; 92.8% purity) at 0, 1, 25, or 625 ppm in the diet for 90 weeks for females, and 104 weeks for males. This diet was equal to average dose levels of 0, 0.15, 3.7 and 93.1 mg/kg bw/day for males and 0, 0.2, 4.9 and 119.2 mg/kg bw/day for females.

The animals were divided into three subgroups: group A (50/sex/dose); group B (12/sex/dose); and group C (12/sex/group). Clinical signs (including palpable masses) were recorded weekly throughout the study; food consumption (7-day periods) and bodyweight were recorded weekly until week 16 and then monthly.

Blood samples were collected for ChE analysis (plasma and erythrocyte) from non-fasted group B mice at 13, 27, 52, 91 (both sexes) and 104 (males) weeks and all group C mice at 52 weeks. Blood smears for differential leucocyte counts and RBC morphology were taken from non-fasted group A females (89 weeks) and males (103 weeks).

All mice that were found dead or sacrificed *in extremis* were subjected to postmortem examination. Group C mice were killed at 52 weeks; group A and B females were killed at 90 weeks and group A and B males were killed at 104 weeks for postmortem examination. Macroscopic abnormalities were recorded and selected organs were weighed (adrenals, brain, pituitary, heart, lungs, liver, kidneys, spleen, thyroid and ovaries). Samples of the following organs and tissues were collected for microscopic examination: adrenals, aorta, brain, cervix, epididymides, oesophagus, eyes with optic nerve, Harderian gland, femur including joint, gall bladder, heart, kidney, larynx, lacrymal gland, liver, small and large intestine, prostate, seminal vesicles, ovaries, uterus, pituitaries, thyroid, spleen, bone marrow, thymus, lymph nodes, mammary

glands and skin. Whole brain tissue was collected from all group B and C animals for assay of brain ChE activity.

Stability, homogeneity and concentration in feed were determined. Chlorfenvinphos was stable in feed for at least 21 days at room temperature.

Feeding of chlorfenvinphos did not increase mortality rates. In the case of females, survival was increased after chlorfenvinphos treatment (statistically significant for low-dose females). There were no clinical signs or palpable masses that could be attributed to chlorfenvinphos treatment. There was no effect on bodyweight gain in either sex. There was no effect on food consumption for males but food consumption was significantly lower in all chlorfenvinphos-fed females from weeks 8 to 52 with the largest effect seen at 625 ppm (7% - 17% reduction for weeks 10–31). However, as there was no effect on bodyweight gain, these results were not considered to be biologically significant.

Haematology findings were similar in control and treated groups, with the exception of ChE activity. The mean ChE inhibition for plasma, erythrocytes and brain at each dose, averaged over all time points is given below.

Table 17: ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
1	7	1	0	0	0	0
25	45**	47**	4	0	0	0
625	92**	95**	52**	44	25*	36**

* $p<0.05$, ** $p<0.01$

The results show that there was a treatment-related dose-dependent inhibition of plasma ChE, which was biologically and statistically significant (>20%, $p<0.01$) for the two highest doses at all time points. Erythrocyte ChE was significantly ($p<0.01$) reduced for high-dose males (52% - 58%) and females (37% - 66%) after 13, 27 and 52 weeks. At 78 weeks inhibition was only 25% ($p<0.05$) and 36% (not statistically significant) for high-dose males and females, respectively. At 90/104 weeks it was 18% for high-dose females (not significant) but 63% for high-dose males ($p<0.01$). Brain ChE was inhibited by approximately 25% and 27% for high-dose males and females at 52 weeks and by 26% (males) and 46% (females) at 104/90 weeks ($p<0.01$).

Chlorfenvinphos had no effect on the appearance of any organ or tissue; and there was no effect on organ weights at either 52 weeks (interim kill) or 90/104 weeks (terminal kill). No detailed pathology report was supplied, however the summary information supplied stated that at the interim (52-week) postmortem examinations, there were no findings that could be attributed to administration of chlorfenvinphos. At 104 weeks, the only significant treatment-related changes recorded were seen in the adrenal cortex of the males on 625 ppm, including increased incidence and severity of ceroid pigment (38/122, 66/121 for controls and high-dose males, respectively), increased incidence of focal adrenal hypertrophy (25/122 and 40/121 for controls and high-dose males) and increased severity of nodular hyperplasia of the adrenals at 625 ppm. There was no increase in neoplastic lesions attributable to chlorfenvinphos treatment.

Overall, the only biologically significant findings were decreases in plasma ChE, at 25 and 625 ppm. Some histopathological effects were also seen in the adrenal cortex of males fed 625 ppm. Based on these findings, the NOEL for this study was 1 ppm for both males and females (equal to 0.15 mg/kg bw/day). It should be noted that the individual animal data and detailed pathology report were not provided for this study.

6.2 Rat

Larson PS & Ambrose AM (1965) Toxicologic study on the effect of adding GC-4072 to the diet of rats for 103 weeks. Department of Pharmacology, Medical College of Virginia, VA;
and
Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR. (1970) Toxicologic studies on diethyl-1-(2,4-dichlorophenyl)-2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (purity 96%, source not specified) was administered in the diet to Wistar rats (Albino Farms, Red Bank, NJ) at doses of 0, 10, 30, 100 or 300 ppm (equivalent to 0, 0.5, 1.5, 5 or 15 mg/kg bw/day) for 104 weeks, using 30 rats/sex/group. Rats were housed individually. Body weight was determined weekly; food consumption was determined over 3-day periods after 1, 3, 6 and 12 months. Haematological examinations, including Hct, Hb and total and differential white cell counts were performed on 5 rats/sex/group every 3 months. Urinalysis for protein was made at the same time. Plasma and erythrocyte ChE activity was determined on 5 rats/sex/group at weeks 1, 4, 8 and 12, and then every 3 months.

Gross and histopathological examinations were done on all animals dying during the study as well as those euthanised at scheduled sacrifices. At 13 weeks, either 4 or 5 rats/sex/group were euthanised for histopathological study. Survivors were euthanised during week 104 for histopathological examination, except for males on 300 ppm which were sacrificed during week 95. No explanation was given for the early sacrifice of these animals. The weight of the heart, spleen, liver, kidney and testes were determined on all animals, except for those dying during the study. The heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonad, pituitary and brain were examined histopathologically.

No individual animal results were presented. Mortalities were increased on 300 ppm in males late in the study, however similar mortalities were seen at 10 ppm, and the significance of these results is questionable. Body weight was decreased in female rats at doses of 100 and 300 ppm from week 26, with decreases in the range 12% - 19%. There were no significant decreases in body weights of male rats at any stage. There were no differences in haematological or urinary findings between control and treated animals.

Mean plasma and erythrocyte ChE values were presented. In males at 10 ppm, plasma ChE activity was significantly inhibited ($p<0.05$, >20%inhibition) for the first 12 months, then intermittently inhibited after this. In females from 10 ppm, plasma ChE activity was significantly (>20% inhibition, $p<0.05$) at all time periods. Erythrocyte ChE activity was sporadically inhibited in males from 10 ppm throughout the trial; in females, there was significant inhibition of erythrocyte

ChE (>20% inhibition, $p<0.05$) from week 26 to week 78. Mean ChE inhibition, averaged over all time periods is presented below.

Table 18: Mean percentage ChE inhibition, compared with concurrent controls

Dose (ppm)	Plasma ChE		Erythrocyte ChE	
	Males	Females	Males	Females
10	33	51	11	18
30	61	71	24	33
100	66	81	29	37
300	74	85	30	41

Values are expressed as percentage inhibition, averaged over all time periods measured.

There were no treatment related findings on gross pathological examination. On histopathological examination there were incidences of bronchiectasis, murine pneumonia and disseminated granulomatous inflammatory processes in both treated and control animals. There were no treatment related findings.

Based on the plasma ChE inhibition in both sexes and the erythrocyte ChE inhibition in females at 10 ppm (the lowest dose tested), no NOEL could be established. The LOEL was 10 ppm (equivalent to 0.5 mg/kg bw/day).

Pickering RG, McAusland HE, Crabtree AN, Martin JG, Hunt PF, Peristianis GC & Gellatly JBM (1980) Toxicity studies on the insecticide chlorfenvinphos: a two year feeding study in rats. TLGR.80.021 Shell Research Institute, Sittingbourne

Chlorfenvinphos (purity 90.5%, batch 61001, source: Shell Nederland BV, Rotterdam) was administered in the diet to Wistar rats (Shell Toxicology Laboratory Breeding Unit) for 2 years at doses of 0, 0.3, 1, 3 or 30 ppm (equivalent to 0, 0.015, 0.05, 0.15 or 1.5 mg/kg bw/day). The study had four groups of animals maintained on each of these doses, with interim kills at 6, 12 and 18 months (12 rats/sex/dose; 24/sex/dose in control group) and a terminal kill at 24 months (50 rats/sex/dose; 100/sex/dose in control group). Food and water were available *ad libitum* throughout the study.

Rats were examined daily for mortality and general physical appearance. All rats were weighed weekly for the first 13 weeks, then monthly for the rest of the trial. A detailed physical examination of each animal was done at the time of weighing. All rats which died or were euthanised throughout the study were sent for a detailed macro- and microscopic necropsy examination. Prior to the end of the study, overnight pooled urine samples were collected from 8 rats/sex/group and examined. The colour and volume were assessed, and the glucose, ketones, pH, urobilinogen, bilirubin and blood levels determined. Prior to euthanasia, blood samples were collected from each rat for haematological and clinical chemistry examination. Haematological examination included total and differential white cell count, Hb, Hct, MCV and erythrocyte count. Clinical chemistry examination included plasma protein, urea, chloride, AP, AST, ALT, sodium and potassium levels. Plasma, erythrocyte and brain ChE activity was also determined.

A full macroscopic examination of all animals dying during the study and at terminal sacrifice was performed, with the external surfaces, orifices, body cavities and organs all examined. The weights of the brain, heart, liver and kidney of all animals, and the weight of the testes in males were determined. Histopathological examination of the adrenals, brain, eyes with lacrimal gland, heart, intestine, kidneys, liver, lungs, lymph nodes, mammary gland, ovaries and testes, pancreas, pituitary, salivary gland, sciatic nerve, spleen, stomach, thyroids with oesophagus/trachea, thymus, urinary bladder and uterus or seminal vesicle/prostate was performed. The tissues from animals in the control, 3.0 and 30.0 ppm groups were examined histopathologically at the interim kills, and tissues from animals in all groups were examined at the terminal kill.

There were no treatment-related clinical signs observed, with the main incidental signs being sore hocks, abscesses, head tilts and paralysis. Survival was higher in males (64% - 70%) than in females (23% - 42%) with no obvious compound-related effects. There were no treatment-related effects on body weight or food consumption throughout the study.

No dose-related effects were seen in organ weights, apart from a tendency (not statistically significant) for an increase in liver weight. There were no treatment-related effects on haematological or clinical chemistry parameters (with the exception of ChE activity). Significant decreases ($p<0.01$) in plasma, erythrocyte and brain ChE were seen at 30 ppm compared to controls at each of the sacrifice intervals. In males at this dose, plasma ChE activity was inhibited by 26% - 47%, erythrocyte ChE activity by 38% - 58% and brain ChE by 23% - 40%. In females at 30 ppm, plasma ChE activity was inhibited by 66% - 76%, erythrocyte ChE activity by 37% - 82% and brain ChE activity by 42% - 54%. At 3 ppm, plasma ChE activity was significantly inhibited in females at 6 months (32%, $p<0.01$), 12 months (16%, $p<0.01$) and 18 months (15%, $p<0.05$). Plasma ChE activity was also inhibited in males at 6 months (6%, $p<0.05$). Erythrocyte ChE activity was inhibited in females at 12 months at 1 ppm (15%, $p<0.01$) and 3 ppm (10%, $p<0.01$) and also at 2 years (9%, $p<0.01$). The NOEL for plasma ChE inhibition was 1 ppm in the diet (equivalent to 0.05 mg/kg bw/day), based on the statistically significant inhibition seen in females at 6, 12 and 18 months. The NOEL for erythrocyte and brain ChE activity was 3 ppm in the diet (equivalent to 0.15 mg/kg bw/day) based on the inhibition seen at 30 ppm.

On macroscopic examination there were no findings related to treatment. On histopathological examination, there was a slight increase in the incidence of pituitary adenoma. At 12 months in females, the incidences were 0/24, 3/11 and 4/12 (0, 3 and 30 ppm), while at 18 months they were 31/48, 22/24 and 20/24. By 24 months the incidences were 85/100, 44/50, 43/49, 38/48 and 39/49 at doses of 0, 0.3, 1, 3 or 30 ppm. There was no clear dose relationship, and the incidence in control animals was often higher than in treated groups at 18 and 24 months, so this finding was not considered of biological significance.

The sciatic nerve was examined for fibre degeneration under light microscopy at the interim and terminal kills. The incidences are presented in the tables below.

Table 19: Incidence and severity of sciatic nerve fibre degeneration in male rats

	0 ppm	0.3 ppm	1 ppm	3 ppm	30 ppm
6 months	1/24 trace			0/12	0/12
12 months	4/24 trace 1/24 mild			1/12 trace	3/12 trace
18 months	26/48 trace 0/48 mild			12/24 trace 2/24 mild	10/24 trace 2/24 mild
24 months	36/100 trace 34/100 mild 20/100 mod. 2/100 severe	17/50 trace 16/50 mild 12/50 mod	12/48 trace 20/48 mild 9/48 mod	19/50 trace 21/50 mild 1/50 mod	20/49 trace 16/49 mild 7/49 mod

Table 20: Incidence and severity of sciatic nerve fibre degeneration in female rats

	0 ppm	0.3 ppm	1 ppm	3 ppm	30 ppm
12 months	0/24 trace			0/12 trace	1/12 trace
18 months	16/48 trace 1/48 mild			12/24 trace	8/24 trace
24 months	39/100 trace 12/100 mild 2/100 mod	18/50 trace 8/50 mild	20/49 trace 4/49 mild	16/49 trace 7/49 mild 1/49 mod	22/50 trace 6/50 mild

No fibre degeneration was detected in females at 6 months. There was no increase in the severity of fibre degeneration, or evidence of early onset of degeneration associated with chlorfenvinphos treatment. There was also no increase in the incidence of neoplastic findings related to the feeding of chlorfenvinphos.

Based on the inhibition of plasma ChE activity in females at 3 ppm, the NOEL was 1 ppm in the diet (equivalent to 0.05 mg/kg bw/day).

6.3 Dog

Larson PS & Ambrose AM (1965) Toxicologic study on the effect of adding GC-4072 to the diet of beagle dogs for two years. Department of Pharmacology, Medical College of Virginia, VA USA
and
Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR. (1970) Toxicologic studies on diethyl-1-(2,4-dichlorophenyl)-2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (purity 96%, source not specified) was administered in the diet to purebred Beagle dogs (source not specified) at doses of 0, 30, 200 or 1000 ppm (equivalent to 0, 0.75, 5 or 25 mg/kg bw/day) for 2 years, using 2/sex/group. Food consumption was measured daily and body weight determined weekly. Urinalysis for protein content, and haematological examination, including Hct, Hb and total and differential white cell counts were done at the start of the trial and

then every 3 months. Plasma and erythrocyte ChE activity was determined at weeks 0, 1, 4, 8 and 12, and at 3 month intervals after this. Clinical chemistry examination was done at 2 years, with determination of AST, AP and BUN levels, as well as bromosulfalein (BSP) clearance.

At the end of the study, dogs were euthanised and a gross post-mortem examination done. Organ weights were obtained for heart, spleen, kidneys, liver and testes. Histopathological examination of the heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonad, pituitary and brain of all animals was conducted.

One control male was euthanised in week 97; all other animals survived until the end of the study. There were no effects on body weight or food consumption, and all haematology and urinalysis results were normal. Liver function tests (BSP clearance, AST and AP) and BUN levels were normal.

Summaries of the plasma and erythrocyte ChE activities were presented, however these were not provided separately for males and females. During the first 39 weeks, plasma ChE activity was significantly inhibited (>20% inhibition, $p<0.05$) at all doses; in the second year, there was significant inhibition of plasma ChE activity at the top 2 doses. Erythrocyte ChE was significantly inhibited at the highest dose intermittently throughout the study.

There were no abnormal gross or histopathological findings seen. Overall, given the significant inhibition of plasma ChE activity at the lowest dose, no NOEL can be set. The LOEL was 30 ppm in the diet, equivalent to 0.75 mg/kg bw/day.

7. REPRODUCTION STUDIES

7.1 Three-Generation Rat Study

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl) -2-chlorovinylphosphate. Toxicol Appl Pharmacol 17: 323 - 336

Chlorfenvinphos (purity 96%, source not specified) was fed in the diet to Wistar rats (Albino Farms, Red Bank, NJ) at doses of 0, 30, 100 or 300 ppm (equivalent to 0, 1.5, 5 or 15 mg/kg bw/d) for 3 generations with 2 litters per generation. Rats (30/sex/group) were housed individually, and food and water were available *ad libitum*. Body weights were recorded weekly throughout the study, except during mating.

Twenty rats/sex/group of the F0 generation were mated at 15 weeks of age, with each female housed with a male from the same treatment group for 7 days. This was repeated twice with different males from the same treatment group (total of 20 days mating). Records were maintained of matings, number of pregnancies, litters, pups in litters at 1, 5 and 21 days (weaning), and the total weight of litters at weaning. Litters with more than 10 pups were culled to 10 on day 5. F1a weanlings were euthanised and autopsied. All females were remated 10 days after weaning of the last F1a litters. Thirty rats/sex/group of the F1b litter were raised on the parental diet for 11 weeks, following which 20 rats/sex/group were selected for breeding. F2a and F2b litters were produced in a similar manner to the first generation, with F2b adults mated to produce F3a and F3b litters.

Plasma and erythrocyte ChE activity was determined for F2b adults after weaning of the F3b litters, and for F3b weanlings fed 0 or 30 ppm for 3 weeks after weaning. At least 10 rats/sex/group were used for these determinations, except that for F2b adults on 100 ppm only 6 females and 3 males were available. Tissues from F3b adults were examined histopathologically, including heart, lungs, liver, kidney, urinary bladder, spleen, stomach, small and large intestine, skeletal muscle, skin, bone marrow, pancreas, thyroid, adrenal, gonad, pituitary and brain.

No individual animal results or detailed summaries were presented in the study report. Body weights of treated females were reported as being lower than controls; at 300 ppm the decrease was 11% in the F0 generation and 19% in the F1 generation. Male rats showed a similar decrease. Plasma and erythrocyte ChE activities were reduced in rats at 30 and 100 ppm, however the reductions were not quantified.

The fertility indices (pregnancies/matings x 100) for each generation are presented in the table below.

Table 21: Fertility indices

Generation	0 ppm	30 ppm	100 ppm	300 ppm
F0 - F1a	85	90	100	85
F0 - F1b	79	100	100	85
F1b - F2a	70	60	36	29
F1b - F2b	80	79	50	33
F2b - F3a	90	45	14	
F2b - F3b	70	30	14	

The fertility index was greatly reduced at all doses by the third generation, with effects seen at the two highest doses in the second generation. There was insufficient pup survival at the highest dose in the second generation to allow a third generation to be bred. As an additional test, F2b animals at 30 ppm were cross-mated with controls. Female controls mated with treated males had a fertility index of 42%, while male controls mated with treated females had a fertility index of 25%.

The viability of pups (pups surviving 5 days/pups born alive x 100) was also decreased following treatment with chlorfenvinphos, with details presented in the following table.

Table 22: Viability indices

Generation	0 ppm	30 ppm	100 ppm	300 ppm
F0 - F1a	97	95	80	64
F0 - F1b	98	96	33	33
F1b - F2a	80	93	77	77
F1b - F2b	94	93	41	0

F2b - F3a	93	97	100	
F2b - F3b	94	91	0	

There were effects on the viability of pups at the two highest doses in the first generation. There was no notable effect on pup viability seen at the lowest dose. The second litter in each generation was more affected than the first. This may be related to the increased length of exposure to the chemical.

There were no gross abnormalities noted in weanlings in any generation. Surviving F2b adults examined histopathologically showed follicle formulation, corpora lutea and ova formation in the females. Testes also showed normal development; there was therefore no explanation for the decreased fertility seen in cross-mated animals.

No NOEL could be established, given the decreased fertility index and plasma and erythrocyte ChE inhibition at the lowest dose tested. The LOEL was 30 ppm, equivalent to 1.5 mg/kg bw/d, however at this dose there were notable effects on fertility.

Eisenlord G, Loquvam GS & Nemenzo J (1967). Results of reproduction study of rats fed diets containing Compound 4072 over three generations. Report No. 5, Hine Laboratory, San Francisco

Chlorfenvinphos (batch no, source, purity not specified) was fed in the diet to Long-Evans rats (Simonsen Laboratories) at doses of 0, 1, 5 or 15 ppm (equivalent to 0.05, 0.25 or 0.75 mg/kg bw/day) for 3 generations. Each group started with 10 male and 20 female rats. Food and water was available *ad libitum*. Except during mating, males were housed in groups of 5 and females were housed individually.

Rats were mated after 79 days on the diet, at 100 days old. Each female was mated for a 2-week period, with males rotated once during that time. Pups from the F1a litter were discarded at weaning, and rats were mated again 10 days later. Randomly selected pups from the F1b litter were maintained on the appropriate diets and mated when 100 days old. This process was repeated to produce F2a, F2b, F3a and F3b litters. The number of pups per litter in each generation was counted on the day of birth and on day 5. Litters greater than 10 were reduced to 10 pups on day 5. On day 21, weanlings were counted and weighed and either sacrificed or maintained on the diet to produce the next generation. Adults were sacrificed and examined for gross abnormalities when no longer required. In the F3b litter, 10/sex in control and low dose group, and 5/sex in mid and high dose groups were selected for autopsy. Body weights were recorded, and the brain, liver and kidney weights were recorded. The brain, heart, lung, liver, spleen, kidney and testes were preserved in formalin for histopathological examination. There was no determination of ChE activity in this study.

On clinical examination, there were no changes in appearance or behaviour in treated groups in comparison to controls. There was no effect on litter size or mean survival of litters from the rats which became pregnant. The fertility indices are presented in the table below.

Table 23: Fertility indices

Generation	0 ppm	1 ppm	5 ppm	15 ppm
F1a	100	95	95	85
F1b	100	100	100	95
F2a	90	95	90	90
F2b	100	100	90	95
F3a	100	100	85	95
F3b	100	100	100	95

The mean fertility index over the three generations was 98%, 98%, 93% and 92%, and thus there appears to be an effect on the two highest doses. When the fertility indices for each generation was examined, there was no decrease in fertility in later generations, and the effects were not statistically significant.

The mean survival of pups was not affected by treatment, nor was the mean weight of weanlings. The viability indices (pups surviving 5 days/pups born alive x 100) are presented in the table below.

Table 24: Viability indices

Generation	0 ppm	1 ppm	5 ppm	15 ppm
F1a	99	99	98	98
F1b	98	92	92	94
F2a	97	89	98	92
F2b	92	95	95	97
F3a	92	80	90	89
F3b	95	91	94	96

There were no significant differences between treatment groups in pups surviving in this study.

There were no gross or histopathological abnormalities detected on examination of the adults or weanling rats.

There was no determination of ChE inhibition in this study. Based on the absence of effects on reproduction, the NOEL was 15 ppm, equivalent to 0.75 mg/kg bw/day.

7.2 One-generation reproduction study

Dotti A, Kinder J, Leutkemeier H and Biedermann K (May 1993). Preliminary study to the two-generation reproduction study in the rat. RRC Project 271822 (CH0-430-002), Research and Consulting Company Ltd, Itingen, Switzerland. GLP (USEPA, OECD, Switzerland) (Study duration: 22/6/90–14/9/90)

Groups (10/sex/group) of rats (spf Wistar Han: WIST; Biological Research Laboratories Ltd, Switzerland) were given technical grade chlorfenvinphos (Birlane, Batch F890355; purity 92.8%) at dietary concentrations of 0, 5, 25 and 125 ppm for three weeks before mating, during mating (males and females), and during gestation and lactation for the mated females (F0 generation). The doses were equal to approximately 0, 0.4, 1.9 and 10 mg/kg bw/day for males; 0, 0.41, 2.2 and 11 mg/kg/day for females during premating, mating and gestation; and 0, 0.9, 4.2 and 22.1 mg/kg bw/day for females during lactation. F1 litters were randomly adjusted to 8 pups per litter (4 males; 4 females) on day 4 post-partum. Surplus pups were killed for macroscopic examination. After weaning on day 21 post-partum, the F0 generation males and females were killed and the pups were fed at the same dietary concentrations as their parents for a further 7 days before being killed.

All animals were checked daily for clinical signs; towards the end of gestation females were checked twice daily, and the length of gestation was recorded. Bodyweights and food consumption were recorded weekly. Litters were examined for litter size, weight, sex ratio, live/stillbirths and gross anomalies. At termination, all F0 and F1 rats were subjected to gross pathological examination, including ovaries, uterus and uterine contents. ChE levels in plasma, erythrocytes and brain were determined at termination for all F0 generation rats.

The mean concentration of chlorfenvinphos in the feed was 93–99.4% of the nominal concentrations. Homogeneity and storage stability data were acceptable. No test article-related clinical signs were noted in any group; there were no deaths. The bodyweight gain of high-dose males was slightly reduced (13%) during premating (with statistically significantly lower bodyweights between days 8 and 21 of the premating period) and for high-dose females (12%, but bodyweights were not significantly reduced). A slight reduction in bodyweight gain was also seen in females at the two highest doses during gestation (13% and 9%, respectively) but bodyweights were not significantly reduced. There was no significant effect on food consumption at any dose but relative food consumption was significantly increased for high-dose males (due to lower mean bodyweight). The NOEL for parental toxicity was 25 ppm, based on the decreased bodyweight gain seen in both sexes during premating at 125 ppm.

The effects on some reproductive parameters are detailed in the table below.

Table 25: Reproductive effects

Parameter	**0 ppm**	**5 ppm**	**25 ppm**	**125 ppm**
Mean precoital time (days)	2.9	2.3	2.2	3.1
Fertility index ((pregnancies/matings) x 100)	100	90	90	90
Mean implantations	12.1	11	9.8	10.7
Post implantation losses (%)	9.9	9.1	5.1	6.5
Mean pup number	11	11	10.3	11.2
Birth index ((pups born alive/implantations) x 100)	90.9	90.9	94.9	93.4
Viability index ((pups alive day 4/pups born alive) x 100)	97.3	98	98.9	95

Chlorfenvinphos treatment did not statistically significantly affect any mating parameters (mating index, time to conception, conception rate, or gestation indices), although the fertility index (pregnancies/matings) was decreased by 10% in all treated groups. This was not considered significant as there were only 10 animals/group. All pregnant females gave birth to and reared their pups until the end of the lactation period. Due to an unusually low number of implantation sites in one female of the mid-dose group, the mean number of implantation sites was reduced for this group compared to controls. Other groups showed no effect of chlorfenvinphos treatment, although the implantation numbers in all treated groups in comparison to controls were decreased when the non-pregnant animals were considered as having no implantations. There was no effect on post-implantation loss and the birth index was similar for all groups. A slightly reduced mean pup number was recorded for the mid-dose group due to a low number of pups for one female (see above); other groups were unaffected. Pups found dead at first litter check and postnatal losses were sporadic and not related to treatment. External examination of pups revealed no chlorfenvinphos-related findings and no dose-related difference in sex ratio. Initial mean bodyweight and bodyweight gain during lactation were reduced for high-dose male and female pups (statistically significant on days 14 and 21 post-partum). After weaning, the bodyweight of high-dose pups remained significantly lower than controls and food consumption was slightly reduced but bodyweight gain was comparable with controls.

Inhibition of cholinesterase activity was recorded for F0 generation rats as set out below.

Table 26: ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
5	0	9	0	36*	0	12*****
25	0	59*****	57*****	73*	11*****	21*
125	9	81*	83*	85*	26*	33*

* $p<0.01$

There was significant plasma ChE inhibition in females from 25 ppm, while no inhibition of plasma ChE in males was seen at any dose. Erythrocyte ChE was inhibited in males from 25 ppm, and in females at all doses ($p<0.01$). Brain ChE activity was inhibited in males from 125 ppm and in females from 25 ppm, while the inhibition of brain ChE in females at 5 ppm was statistically significant ($p<0.01$). Based on the inhibition of erythrocyte ChE activity at the lowest dose tested, and the statistically significant inhibition of brain ChE in females, no NOEL could be established for ChE inhibition in this study.

There were no abnormal findings at postmortem for F0 parents or F1 pups.

Overall, no NOEL could be set as reduced erythrocyte and brain ChE activity was seen in females at all doses; the LOEL based on erythrocyte and brain ChE inhibition was 5 ppm (0.4 mg/kg/day). For pups the NOEL was 25 ppm (approximately 2.5 mg/kg bw/day), based on reduced bodyweight gain during lactation only (ChE was not measured for F1 pups).

7.3 Two-generation reproduction study

Dotti A, Kinder J, Leutkemeier H and Biedermann K (June 1993). Two-generation reproduction study with chlorfenvinphos in the rat. RRC Project 271833 (CH-430-003), Research and Consulting Company Ltd, Itingen, Switzerland and RCC (UK) Ltd. (USEPA, OECD, UK MAFF, Switzerland) (Study duration: 7/1/91–7/11/91)

Groups (25/sex/group) of rats (spf Wistar Han Ibm:WIST; Biological Research Laboratories Ltd, Switzerland) were given technical grade chlorfenvinphos (Batch F890355; purity 92.8%) at dietary concentrations of 0, 1, 10 or 100 ppm (equivalent to 0.05, 0.5 or 5 mg/kg bw/day) for 70 days before mating and during mating (males and females); and during gestation and lactation for the mated females (F0 generation). On day 4 post-partum, F1 pups were culled to give 8 pups per litter (4 male/4 female). After weaning on day 21 post-partum, F1 pups were selected to form groups of F1 parents (25/sex/group) and fed at the same dietary concentrations as their parents for 121 days before mating, during mating and during gestation and lactation for females, as before. On day 4 post-partum the F2 litters were culled to 4 male and 4 females per litter and reared until weaning after which all F2 pups and F1 parents were killed and examined macroscopically.

All animals were checked daily for clinical signs; towards the end of gestation females were checked twice daily, and the length of gestation was recorded. Bodyweight and food consumption were recorded weekly. Litters were examined for litter size, weight, sex ratio, live/stillbirths and gross anomalies. At termination, all F0, F1 and F2 rats, including those that died or were culled, were subjected to gross pathological examination. Selected organs and tissues (gross lesions, ovaries, uterus and cervix, vagina, pituitary gland, prostate, seminal vesicles with coagulating blood, testes and epididymides) were collected from F0 and F1 animals for histopathological examination. Plasma, erythrocyte and brain ChE activities were determined for F0 rats (10 rats/sex/dose) at 14 days after weaning for the dams and the same time for males.

The mean concentration of chlorfenvinphos in the feed was 90–95% of the nominal concentrations. Homogeneity and storage stability data were acceptable. No test article-related clinical signs were noted in any F0 or F1 parent group; there were no treatment-related deaths. The bodyweight gain of males was slightly reduced (10%) in the high-dose F0 males during premating. Statistically significantly lower bodyweights were recorded between days 15 and 22 of the premating period for F0 males (4 to 5%) and days 1–57 for F1 males (maximum of 8%). These reductions were accompanied by slightly but significantly reduced food consumption. These changes were not considered of biological significance. Bodyweights remained slightly lower than controls after mating but bodyweight gains were similar at this stage. During premating, the bodyweights of treated F0 females showed no difference to controls. During gestation, bodyweight gain was slightly lower for high-dose females (8%) and bodyweights were significantly lower during the first 2 weeks of lactation (decrease of 5 - 7%). Gestational body weights included the weight of the gravid uterus. Food consumption was significantly reduced during the whole of gestation for the high-dose group, during the first week of gestation for the mid-dose group and during the second week of lactation for the high-dose group, however maximum decreases were only 9%, and this is not considered of biological significance. For F1 females, the initial bodyweight of the high-dose parents was lower than controls but the bodyweight gain during premating was similar to controls (see below); food consumption was significantly lower from day 57–121 for the high-dose (up to 14%) and in the last two weeks before mating for the mid-dose group (10%). During gestation, bodyweight gain was slightly less than controls for the high-dose group (13%) and food

consumption was significantly reduced throughout gestation at the high-dose. Food consumption was comparable to controls during lactation. In summary, the only effects of statistical and toxicological significance are the decrease in food consumption seen in the mid- and high-dose F1 females in the premating period.

Table 27: Reproductive performance

Observation	Gen.	0 ppm	1 ppm	10 ppm	100 ppm
Mean pre-coital interval (days)	F1 F2	2.5 2.9	3 3	3.2 3.3	2.5 4.4
Fertility index ((pregnancies/matings) x 100)	F1 F2	100 88	100 100	100 92	92 84
Birth index ((pups born/implantations) x100)	F1 F2	91.7 87.3	92.2 89.7	85.7* 87.3	86.4* 86.8
Viability index ((pups alive day 4/pups born) x 100)	F1 F2	97.1 97.3	98.5 98.4	98.4 97.6	92.4* 98.4
Weaning index ((pups alive day 21/pups alive day 4) x 100)	F1 F2	99.5 98.8	99.5 98.4	95.4** 98.4	89.9** 94.2**

* $p<0.05$, ** $p<0.01$

For the F0 generation, two mated high-dose females were not pregnant; other mating parameters (mating index, time to conception and gestation indices) were not affected. For the F1 groups, the mean time before mating (precoital interval) was slightly increased in the high-dose group. For the F0 groups, one female in each of the mid- and high-dose groups did not give birth and 1 low- and 2 high-dose females did not rear their pups to the end of lactation. For the F1 group, 3 control, 2 mid- and 4 high-dose females were not pregnant and 1 control, 2 low- and 3 high-dose females did not rear their pups to the end of lactation.

For F0 females, there was no effect on pre-implantation loss but post-implantation loss was significantly increased for the mid- and high-dose females and the birth index was therefore significantly reduced in both these groups. There was no dose-response relationship for these effects and historical control data for Wistar rats shows a range of post-implantation loss for F0 generation dams of 4.4 – 15% (mean 9.5%). For F1 females there was no effect on pre- or post-implantation loss and birth indices were comparable to controls.

For F0 dams the number of pups found dead at first litter check were higher for the mid- and high-dose groups. Significant reductions in the viability and weaning indices were noted at 100 ppm and the weaning index was also significantly decreased at 10 ppm. The only effect seen for F1 dams was a slight increase in loss after culling for the high-dose group with a significant reduction in weaning index.

External examination of F1 and F2 pups revealed no chlorfenvinphos-related findings and no dose-related difference in sex ratio. From about day 7 post-partum, a significant reduction in bodyweight was noted for male and female high-dose pups of both the F1 and F2 generation.

Inhibition of cholinesterase activity was recorded for F0 generation rats as set out below.

Table 28: ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
1	8	0	0	0	3	0
10	14**	45**	19	8	4	16**
100	35**	78**	32*	63**	31**	35**

$p<0.05$, ** $p<0.01$

The results indicate a dose-related inhibition of ChE activity with biologically and statistically significant inhibition evident at 100 ppm for plasma, erythrocyte and brain ChE. At 10 ppm plasma ChE activity was statistically significantly inhibited in both sexes, while brain ChE was statistically significantly inhibited in females only.

There were no abnormal pathology findings at postmortem examination of F0 and F1 generation parents, and F1 and F2 pups.

The NOEL for maternal toxicity was 1 ppm (0.05 mg/kg bw/day), based on reduced food consumption and reduced plasma and brain ChE activity in females at 10 ppm. The NOEL for foetal toxicity and toxic effects on pups was 1 ppm (0.05 mg/kg bw/day), based on increased post-implantation losses at 100 ppm and increased postnatal losses during lactation at 10 ppm (first generation only) and 100 ppm (ChE was not measured for F1 or F2 pups).

8. DEVELOPMENTAL STUDIES

8.1 Rats

Mayfield R and John DM (April 1986). Effect of Birlane on pregnancy of the rat. HRC Report SLL 84/851593. Huntingdon Research Centre Ltd, Huntingdon, UK. GLP (USEPA, OECD, and Japan MAF,) (Study duration: 25/7/85–22/8/85)

Groups (25/dose) of mated female rats (spf Crl:COBS CD(SD) BR strain; Charles River UK, Ltd, Margate, UK) were given technical grade chlorfenvinphos (Birlane, Batch A4211 9200/9007; 91.8% purity) by gavage once per day at doses of 0, 0.3, 1 or 3 mg/kg bw/day from days 6–15 of gestation. The doses were based on a preliminary study in which doses up to 10 mg/kg bw/day were given to groups of 6 non-mated female rats. In this range-finding study, there were marked clinical signs and one death at 10 mg/kg bw/day; increased water consumption, decreased food consumption and initial bodyweight loss were also evident at this dose. No abnormal clinical signs were observed at any other dosage.

Clinical signs were observed daily, and food consumption and bodyweight was measured on days 1, 3, 6, 8, 10, 14, 17 and 20. Food and water were available *ad libitum*. The rats were killed on day 20 of gestation and examined for gross pathological changes in maternal organs. The ovaries and uteri were examined for reproductive parameters including fetal weight and abnormalities .

There were no deaths in the main study; the only consistent abnormal clinical sign was small faeces, observed at 3 mg/kg bw/day after 3–4 days of dosing. This finding in isolation was not considered to be toxicologically significant. Food consumption was not affected.

Pregnancy rate was comparable for all groups and there were no macroscopic changes at terminal postmortem that were attributable to treatment.

Mean values for pre- and post-implantation loss, litter size and foetal weight were not affected by treatment at any dosage. There was a treatment-related decrease in the percentage of male foetuses present (51.8%, 51%, 50.2% and 46.9%); this was not statistically significant. There was no evidence that treatment of the dams had any significant effect on embryonic or fetal development and there were no increases in malformations, visceral or skeletal variants.

No significant effects were seen at any dose tested in this study. The NOEL for maternotoxicity and foetal effects was 3 mg/kg bw/day.

8.2 Rabbits

Dix KM, Cassidy SL, Vilkauls J, Whitaker J, Andrews J, Rea PL, Barry M & Martin JG (1979) Toxicity of chlorfenvinphos: Teratological studies in rabbits, given chlorfenvinphos orally. TLGR.79.105. Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no 61001, source: Pernis Refinery, purity not specified) in corn oil was administered to pregnant banded Dutch rabbits (Tunstall Breeding Unit) orally by gelatin capsules. In a preliminary study using pregnant Dutch rabbits, doses of 30, 60 or 120 mg/kg bw/day from days 6 - 18 of gestation inclusively were used. There were reduced bodyweight gains in the high-dose group. Plasma and erythrocyte ChE values were reduced at all dose levels in comparison with controls. Based on these findings, doses of 0, 25, 50 or 100 mg/kg bw/day were chosen for the main study, in which there were 32 females in the control group, and either 21 or 22 in each treatment group. The rabbits were distributed between groups to ensure that females mated by the same male were included in different treatment groups. Dosing occurred on days 6 - 18 of gestation inclusive.

Rabbits were observed daily for clinical signs, and they were weighed at mating and on days 6, 9 12, 15, 18 and 28 of gestation. Food and water were available *ad libitum* throughout the study. Food intakes were measured over 6 day periods from the start of gestation. On days 0 and 18 of gestation, blood samples were taken to determine plasma and erythrocyte ChE activity. On day 28 of gestation the female rabbits were killed, and a full gross post mortem examination performed. Tissue samples were taken from any abnormalities noted. The numbers of live foetuses, dead foetuses and resorption sites in each uterus, as well as the number of corpora lutea were noted. Foetuses were weighed, measured (crown-rump length) and examined for any external abnormalities. One-third of foetuses were fixed in Bouin's solution for sectioning and examination of the brain, eyes and nasal cavities. Thoracic and abdominal viscera of these animals were also examined, including sex determination. The remaining foetuses were dissected, the viscera examined and sex determined, following which they were stained for skeletal examination.

One rabbit in the 50 mg/kg bw/day group showed salivation and tremors shortly after dosing on day 18. No other animals showed clinical signs throughout the trial. A number of animals from the control and mid-dose groups aborted foetuses. On post-mortem examination these animals

showed signs of enteric disease, which was not considered to be related to chlorfenvinphos administration. There were no treatment-related effects on survival, or on the number of pregnancies. Females in the high-dose group had slightly lower bodyweights than controls on days 9 and 12; although the decrease was statistically signification ($p<0.05$), it was not of biological significance, as there was only a 2% decrease. There was significant plasma and erythrocyte ChE inhibition in all dose groups. Plasma ChE activity was inhibited 37%, 51% and 73% in the low, mid and high doses, while erythrocyte ChE activity was decreased 55%, 60% and 75%, respectively.

There were no treatment-related effects on the number of corpora lutea, total implantations, pre-implantation losses, or resorptions. The number of early foetal deaths/litter was increased (0.04, 0.20, 0.31, 0.28). This increase was not clearly dose-related, however would appear to be related to the administration of chlorfenvinphos. There was no change in the incidence of late foetal deaths following chlorfenvinphos treatment. There was a slight decrease in the mean number of live foetuses in the high dose group (6.9, 6.2, 7.2, 5.8) but this was not significant. The mean percentage of male foetuses was also slightly decreased with chlorfenvinphos treatment (59.3%, 54.7%, 54.8%, 48.2%); these decreases were not statistically significant. The mean weight of foetuses was not altered with treatment, however the mean length of foetuses was significantly ($p<0.001$) increased in the high dose group (8.3, 8.1, 8.2, 8.6).

There was generally a low incidence of abnormalities in foetuses. There was an increase in the incidence of hydrocephalus seen in treatment groups (0%, 3.33%, 2.08%, 5.56%) which was not dose related. This finding has previously been seen in rabbits in this laboratory, however the frequency previously seen was around 0.3%. There were no other significant abnormalities related to treatment.

No NOEL for maternal toxicity could be established in this study, given the ChE inhibition seen at the lowest dose tested (25 mg/kg bw/day). There was an increase in the incidence of hydrocephalus seen at the lowest dose. Overall the LOEL for materno- and foetotoxicity in this study may be taken as 25 mg/kg bw/day, the lowest dose used.

Dzierzawski A & Minta M (1979) Embryotoxic effect of chlorfenvinphos and bromfenvinphos in laboratory animals. Bull Vet Inst Pulawy 23 (1-2): 32 - 41

Chlorfenvinphos (source: Shell Co; batch no, purity not specified) in rape oil was administered PO to golden hamsters, Wistar rats and New Zealand rabbits (no sources specified). Hamster females were treated with either a single dose of 10, 20 or 40 mg/kg bw on day 8 of gestation, or three separate doses of 20 mg/kg bw, on days 6, 8 and 10 of gestation. Rats were given a single oral dose of 5 mg/kg bw on day 10 of gestation, or three separate doses of 3 or 5 mg/kg bw on days 8, 10 and 12 of gestation. Rabbits were given a single oral dose of 150 mg/kg bw on day 9 of gestation, or three separate doses of 50 or 100 mg/kg bw on days 8, 10 and 12. Hamsters were killed on day 14 of gestation, rats on day 20 and rabbits on day 28. The dams and foetuses were examined macroscopically. The number of implantations, live and dead foetuses and resorptions were determined. Foetuses were weighed and measured, and the gross congenital malformations were classified. Foetuses from each litter were stained to reveal skeletal defects.
In the hamsters, a single dose of 10 mg/kg bw significantly decreased foetal weight and length, as did three doses of 20 mg/kg bw. Single doses of 20 and 40 mg/kg bw appeared to have no effect, and the biological significance of the effect seen at the lower dose must be questioned. In the rat, foetal survival was decreased by single and multiple doses of 5 mg/kg bw (to 94.8% and 91.2% in

comparison to the control value of 98.9%). The weight of foetuses was reduced following a single dose of 5 mg/kg bw and three doses of 3 mg/kg bw, but not following three doses of 5 mg/kg bw. Foetal length was not affected. In rabbits, there was a dose-related affect on foetal survival with 100% survival in controls, 87.9% in three doses of 50 mg/kg bw, 80.6% in a single oral dose of 150 mg/kg bw and 75% in three oral doses of 100 mg/kg bw. Hamsters and rabbits showed individuals (numbers not specified) with hernia umbilicus, while many rat foetuses had oedema and subcutaneous haemorrhages. There were a large number of rats with underdevelopment of skull bones, which may be related to maternotoxicity.

Overall, given the dosing protocol, and the lack of detail in reported results this study is not considered suitable for regulatory purposes.

9 GENOTOXICITY STUDIES

9.1 Gene mutation

Dean BJ (1971) The mutagenic effects of organophosphate insecticides on Escherichia coli. TLGR.0034.71 Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no TSL 66/12F, purity 99%, source: WARC), along with a number of other chemicals, was tested for mutagenic activity using *Escherichia coli* B/r WP2, a tryptophan-dependent strain. Approximately 10^8 bacteria were applied to the surface of minimal media and allowed to dry. The tested chemical was applied as a micro-drop of liquid, and the plates were incubated for 2 days. There was no clear indication of the volume of chemical that was applied, or the final concentration which this would produce. No report of the toxicity of the chemicals applied was supplied. Each compound was tested in triplicate, and at the time of reading the zone of inhibition and the revertant mutants were easily discernible. Chlorfenvinphos did not produce any reverse mutation in this test.

Brooks TM (1978) Toxicity studies with chlorfenvinphos: mutagenicity studies with chlorfenvinphos in micro-organisms in vitro. TLGR.0142.78, Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no Pernis 61001, purity 90.5%, source: Chemical Toxicology Division, Tunstall Laboratories) was tested for mutagenic activity in *Escherichia coli* WP2 and WP2 uvr A, *Salmonella typhimurium* strains TA1538, TA100 and TA98, and *Saccharomyces cerevisiae*. Liver microsomal enzymes were induced in male Wistar rats by an IP injection of 500 mg/kg bw Aroclor. Rats were killed 5 days after dosing, and the S-9 fraction of the liver obtained following homogenation and centrifugation.

E.coli and *S. typhimurium* were cultured on agar plates with 0.02 mL of chlorfenvinphos, at concentrations of 0.2, 2, 20, 500 and 2000 µg/plate, both with and without 0.5 mL of the S-9 mix. Mutant colonies were counted after 3 days incubation. There was no increase in reverse mutations in any of the strains, at any of the concentration of chlorfenvinphos tested, either with or without metabolic activation.

Broth cultures of *S. cerevisiae* were cultured with 20 µL of chlorfenvinphos at a range of concentrations, both with and without metabolic activation. The broth culture was seeded onto minimal medium after a 1-h incubation period. Mutant colonies were counted after 4 days

incubation. Initially, the culture was exposed to 0.01, 0.1, 0.5, 1.0 or 5.0 mg/mL. There was a threefold increase in gene conversion at 0.5 mg/mL with no metabolic activation. Increased toxicity was noted with metabolic activation. In a second experiment, the rate of gene conversion was increased at 0.1 and 1.0 mg/mL, but not at 0.5 mg/mL. Therefore there was no consistent or dose-related increase in gene conversion, and the effects are considered to be incidental to treatment. Chlorfenvinphos was considered to be negative for mutagenic effects under the conditions of this trial.

Arni P and Muller D (February 1979). Salmonella/mammalian-microsome mutagenicity test with C8949 (test for mutagenic properties in bacteria. Experiment 78/2589. Ciba-Geigy Ltd, Basle, Switzerland. GLP (not stated)

A *Salmonella typhimurium* histidine reverse mutation test was performed with chlorfenvinphos (C8949; batch and purity not stated) at concentrations of 25 - 2025 µg/0.1 mL (dissolved in DMSO) in the presence or absence of a rat liver microsomal S-9 metabolic activation mixture. *S. typhimurium* histidine auxotrophic strains TA1535, TA1537, TA98, and TA100 were tested. The positive controls were N-methyl-N'-nitro-N-nitrosoguanidine, 9(5) aminoacidine, daunblastin, and 4-nitroquinolinine-N-oxide (for the above strains, respectively, without rat liver S-9 metabolic activation mix); and cyclophosphamide for TA1535 with S-9. DMSO was the negative (solvent) control.

Chlorfenvinphos showed no mutagenic activity in the assay either with or without S-9. All the positive control substances gave strongly mutagenic reactions. However, the chlorfenvinphos concentrations tested did not cause any toxicity for any of the strains; therefore mutagenic activity cannot be fully ruled out on the basis of this test.

Shirasu Y, Moriya M, Kato K, Furuhashi A and Kada T (1976). Mutagenicity screening of pesticides in the microbial system. Mutat Res, 40:19–30. (Published)

This paper describes a review of the mutation induction capacity of 166 pesticides. An initial screen was carried out using a rec-assay procedure with H17 rec^+ and M45 rec– strains of *Bacillus subtilis*. Chemicals that gave a positive result in this assay were then tested in Salmonella and *E.coli* reverse mutation assays. Chlorfenvinphos did not appear to have been positive in the rec-assay and was not tested in the other systems.

Clive D (1979) Mutagenicity testing of BW0024Z61 (Supona) in Salmonella. Doc Ref TTEP/79/0021 Burroughs Wellcome Report USA

Chlorfenvinphos (source: Burroughs Wellcome; batch no 8E8018; purity not specified) was tested for mutagenicity using five test strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) with and without metabolic activation by rat liver microsomes, using a modified Ames protocol with plate incorporation and pre-incubation mutagenicity trials.

Dosing was carried out at 0.003 to 10 µL/plate, with toxicity seen at doses of 0.1 µL/plate and higher. Positive controls were run with each assay. 2-Aminoanthracine was plated at 0.4 µg/plate with metabolic activation for strains TA98, TA100 and TA 1538. Propane sultone was plated at 0.02 µL/plate without activation using TA 1535, and 9-aminoacridine was plated at 75 µg/plate without activation using TA 1537.

There was an increase in the reversion index in TA 100 in the pre-incubation trial. The number of revertants per plate peaked at 2.5 µL/plate without metabolic activation and at 5 µL/plate with metabolic activation There was also a slight increase in the number of revertants/plate in TA98 without activation at 2.5 µL/plate, however this was not significant. The increase in revertants was seen at doses above those producing 35% toxicity (0.1 µL/plate). There was also a single incidence of an increase in the number of revertants/plate using TA1535, however this was not seen in repeat trials.

Overall, the results were equivocal as positive effects were only seen at doses which produced significant toxicity in these bacteria.

9.2 Chromosomal Effects

Strasser F (December 1988) Chromosome studies on human lymphocytes in vitro. Test 871201. Ciba-Geigy Ltd, Basle, Switzerland. GLP (FIFRA Federal Register Part IV, EPA 1983)

Chlorfenvinphos (C8949 technical; Batch P 510559, purity 93.1%) was tested for mutagenic effects on human lymphocytes *in vitro*. White blood cells were prepared from heparinised human blood and stimulated to divide *in vitro* with PHA. After 46 h of culture, the test substance (dissolved in DMSO) was added at concentrations of 19 to 300 µg/mL, with or without S-9 metabolic activation (rat liver microsomal preparation), arrested in metaphase and examined microscopically for the presence of structural chromosome aberrations (100 metaphases for each of two cultures per concentration were scored). DMSO also served as the negative control. Positive controls were mitomycin C (without S-9) and cyclophosphamide (with S-9). The chlorfenvinphos concentrations used were based on a preliminary cytotoxicity study in which the mitotic index was reduced to approximately 60% of controls at 250 µg/mL, with no live cells at 500 µg/mL.

At the chlorfenvinphos concentrations used, there were no significant increases in chromosome aberrations with or without S-9. The positive control substances gave a high incidence of specific chromosome aberrations. Under the conditions of this study, chlorfenvinphos was negative for genotoxicity.

Dean BJ (1978) Toxicity studies with chlorfenvinphos: Chromosome studies on bone marrow cells of Chinese hamsters after two daily oral doses of chlorfenvinphos. TLGR.0087.78 Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no 61001, purity 90.5%, source: Shell Biosciences Laboratory, Sittingbourne) in carboxymethyl cellulose was administered PO to Chinese hamsters (Tunstall Laboratory, Sittingbourne) at doses of 0, 25 or 50 mg/kg bw, using 8/sex/group on two consecutive days. A positive control, cyclophosphamide, was administered at 100 mg/kg bw (8/sex) on two days. Each animals was given 10 mL/kg of 0.04% colchicine solution by IP injection 90 min before the end of the experiment. Four animals/group were killed 6 or 24 h after the second dosing. The femurs were removed, and the bone marrow prepared for chromosome analysis. At least 100 cells/animal were examined, and any deviations from normal chromosome morphology were recorded.

There were no abnormal clinical signs following dosing with chlorfenvinphos or cyclophosphamide. At 6 h after the second dose of chlorfenvinphos, males showed an increase in the incidence of polyploidy. This was not seen in the other groups examined. There was no increase in any other abnormalities, including chromatid gaps and chromatid breaks. There was no evidence that chlorfenvinphos at 50 mg/kg bw increased the incidence of chromosomal damage in comparison to controls.

Dean BJ & Hend RW (1978) Toxicity studies with chlorfenvinphos: Dominant lethal assay in male mice after single oral doses of chlorfenvinphos. TLGR.0063.78 Shell Research Institute, Sittingbourne

Chlorfenvinphos (batch no 61001, purity 90.5%, source: Shell Biosciences Laboratory, Sittingbourne) in DMSO was administered orally to CD1 (Charles River) male mice (Tunstall Breeding Unit, Sittingbourne) at doses of 0, 10, 20 or 40 mg/kg bw, using 12/group. Additionally, 12 males were dosed with cyclophosphamide at 200 mg/kg bw. Following dosing, each male was housed with 3 females for 7 days, then housed with new groups of 3 females for 7-day periods over 8 weeks. Mating was presumed to have occurred by mid-week, and females were killed 13 days after the presumed mating. The uterus of each female was removed and examined; non-pregnant animals were noted, and the number of early foetal deaths, live foetuses and late foetal deaths were recorded.

Ten males died during the study; two from the low-dose group, one from the mid-dose group and three from the high-dose group. Four positive control animals died. The percentage of females pregnant in the 8 weeks following dosing ranged from 69% to 91% in the negative control animals. Dosing with chlorfenvinphos did not affect the percentage of animals pregnant, while the positive control decreased the percentage pregnant from a mean of 84.8% in negative controls to 71.5%. The mean number of foetal implants in the negative control over the 8-week period was 12.5. The means in the high and low-dose groups were significantly ($p<0.05$) decreased to 12 and 12.1 respectively. These findings were not dose related, and are not considered of biological significance. The mean number of foetal implants in the positive control group was 11.0, significantly decreased in comparison to negative controls. Early foetal deaths were not affected by dosing with chlorfenvinphos. In this study, there was no evidence of dominant lethality related to dosing with chlorfenvinphos.

10. SPECIAL STUDIES

10.1 Hens

Ambrose AM, Larson PS, Borzelleca JF & Hennigar GR (1970) Toxicologic studies on diethyl-1- (2,4-dichlorophenyl) -2-chlorovinylphosphate. Toxicol Appl Pharmacol 17:323 - 336

Chlorfenvinphos (96% purity) in 20% ethanol - 80% propylene glycol was administered by IP injection to White Leghorn hens at doses of 0 (5 hens), 100 (6 hens), 150 (3 hens), 200 (4 hens) or 300 mg/kg bw/day (2 hens). Additionally, 3 hens each received 100 or 200 mg/kg bw/day chlorfenvinphos along with 1 mg/kg bw atropine. Hens were dosed daily for 10 days and were then observed for a further 20 days for signs of neurotoxicity. At the end of this period, hens were euthanised and samples of brain, spinal cord and sciatic nerve removed for histopathological examination.

Immediately after injection hens showed signs of acute intoxication, including an inability to stand, salivation and retching. Atropine did not fully protect from these signs. Deaths occurred at all doses, with only 8 treated birds (5 at 100 mg/kg bw/day, 2 at 150 mg/kg bw/day and 1 at 200 mg/kg bw/day) surviving the 10 days of treatment. None of these surviving animals showed neurotoxic signs. Histopathological examination of the brain, spinal cord and sciatic nerve did not reveal any demyelination or other signs of neural toxicity.

Redgrave VA and Cameron DM (October 1996) An acute delayed neurotoxicity study of CL 58,085 in adult domestic laying hen (*Gallus Gallus domesticus*). AID 12/961938. Huntingdon Life Sciences Ltd, Cambridgeshire, UK. GLP (UK DHSS, EC [directive 87/18], OECD, USEPA[Pesticide Assessment Guidelines, Subdivision F, Addendum 10: Neurotoxicty Series 81-7], Japan MAFF)

Female hens (24) were each given a single oral dose (capsule) of technical grade chlorfenvinphos (CL 58,085; Batch Ac 9934-86; 93.2% purity) at 40 mg/kg bw (sublethal dose, based on a range-finding study). A further 16 birds received empty capsules and 16 received tri-ortho-cresyl phosphate (TOCP) at 1000 mg/kg as positive controls. The birds were observed daily for 21 days for signs of behavioural abnormalities, locomotor ataxia and paralysis. At 24 and 48 h after dosing, 3 birds from each group were killed and brain and spinal cord tissues were examined for ChE and neuropathic target esterase (NTE) activities. At 21 days after dosing, a further 6 birds per group were killed and brain and spinal cord nerves were processed for histopathological examination.

There were no signs of delayed locomotor ataxia in any birds treated with chlorfenvinphos. Six birds in the positive control group developed clinical ataxia. At short time points (24 and 48 h), brain and spinal cord ChE activities were markedly reduced in birds treated with chlorfenvinphos. NTE was unaffected after chlorfenvinphos but was markedly reduced in TOCP-fed birds.

Mean bodyweight gains were seen for chlorfenvinphos-treated birds; TOCP-treated birds lost weight. At post-mortem examination, there were no macroscopic changes or histological evidence of delayed neurotoxicity and no other treatment related changes to the CNS or peripheral nervous system from birds dosed with chlorfenvinphos.

In conclusion, there was no histological evidence of acute delayed neurotoxicity in the hen following a dose of 40 mg/kg bw chlorfenvinphos. There were no other treatment-related changes.

Wallwork LM & Malone JC (1974b) Nankor, Supona neurotoxicity in hens. Doc Code HIBG 74-2 Lab Ref No TL 3-74 Research and Development (V & A), The Wellcome Foundation Ltd

Chlorfenvinphos (source, batch no, purity not specified), fenchlorphos and a mixture of both were tested for neurotoxic effects in mature hens. Initially, groups of 4 hens were dosed orally with the chemicals to determine the acute oral LD50. Following this, groups of 7 hens were dosed at the LD50 on two occasions, 21 days apart. Atropine (10 mg/kg bw) and P-2-S (50 mg/kg bw) were administered IM as required, with all birds receiving chlorfenvinphos also dosed prophylactically with antidotes due to the rapid onset of clinical signs. Hens were observed for 21 days after both injections, and were killed 21 days after the second dose. They were perfused with normal saline

and formalin, and the sciatic nerve and spinal cord removed and stored for possible histopathological examination.

The acute oral LD50 of chlorfenvinphos was 160 mg/kg bw, of fenchlorphos >3000 mg/kg bw, and of the mixture of the two, 280 mg/kg bw. Following treatment with chlorfenvinphos at the LD50, 2 birds died 2 days after the first dose. Severe cholinergic signs were seen in all birds; all had recovered by 21 days after treatment. There were no deaths after the second dose, although severe cholinergic signs were seen. There were progressive signs of ataxia, and all birds had recovered by 4 days after treatment.

Fenchlorphos produced cholinergic signs in 4 birds after the first dose; all had recovered by 3 days after treatment. After the second dose, one bird died on day 2, and two on day 5. All other treated birds recovered, and there were no signs of ataxia. Following dosing with the mixture of chlorfenvinphos and fenchlorphos three birds died on the first day, while all showed severe cholinergic signs. There were no deaths immediately following the second dose, but one bird died on day 17 after dosing. In no cases were there persistent gait abnormalities following treatment. No positive controls were used, and histopathology was not reported.

Gaines TB (1969) Acute toxicity of pesticides. Toxicol Appl Pharmacol 14:515 - 534

Chlorfenvinphos (technical grade: batch no, source, purity not specified) in peanut oil was administered PO to White Leghorn chickens premedicated with atropine sulphate at 15 mg/kg bw PO. The doses of chlorfenvinphos used were not specified. It was stated that no paralysis was observed.

10.2 Rat

Osumi Y, Fujiwara H, Oishi R & Takaori S (1975) Central cholinergic activation by chlorfenvinphos, an organophosphate, in the rat. Jap J Pharmacol 25:47-54

The central action of chlorfenvinphos (source: Shell Kagaku, purity, batch no not specified) was investigated in male Wistar rats (source not specified). The ChE activity in the brain and the levels of noradrenaline were determined at intervals of up to 72 h following PO administration of 1, 2 or 4 mg/kg bw chlorfenvinphos. ChE activity in erythrocytes was also determined. Oral doses of 2 and 4 mg/kg bw decreased brain ChE activity by a maximum of 38% and 82% respectively. Maximum inhibition was seen 3 h after administration of the compound, followed by a slow recovery. At 72 h post-dosing, inhibition was still 33% in comparison to controls. Erythrocyte ChE was inhibited by 4 mg/kg bw, with 80% inhibition seen 3 h after administration. No significant change in noradrenaline levels were seen following treatment.

Rats were prepared by locating electrodes on the surface of the motor cortex. An EEG recording was made from these, and an EMG recording was made from the dorsal neck muscles. Animals were allowed at least 7 days recovery from the surgery prior to testing. Recording were done for 8 h/day, from 10 am to 6 pm. The sleep patterns were classified into 4 stages; wakefulness, drowsiness, slow-wave sleep and parasleep. In control animals, these stages made up 30%, 19%, 41% and 10% respectively of the 8 h recording period. Doses of up to 1 mg/kg bw of chlorfenvinphos did not affect the sleep patterns, however a dose-dependent increase in the duration of wakefulness and decrease in slow-wave and parasleep were observed at doses above 2 mg/kg bw. With doses of 4 mg/kg bw, wakefulness was more than twice as long as controls for

the 5 h period following dosing, while parasleep was not present in treated animals. Sleep patterns returned to normal on the second day, with a rebound increase in parasleep seen on day 3 and 4. Atropine treatment 2 h after chlorfenvinphos treatment resulted in an earlier return to normal sleep patterns. It is not clear whether the changes in parasleep were directly related to the cholinergic function in the sleep mechanism, or were a secondary phenomena.

Kuga T, Watanabe Y, Kono Y & Naito J (1973) Pharmacological studies of some vinylphosphate insecticides. Jap J Pharmacol 23(Suppl.):24 (abstract only)

Chlorfenvinphos administered by gavage to anaesthetised rats at unspecified doses produce a rise in blood pressure and respiratory failure, which were antagonised by toxogonin or large doses of atropine. 2-PAM did not antagonise these effects. Neuromuscular transmission in rat gastrocnemius muscle was increased by chlorfenvinphos. The contractile response of guinea-pig ileum to acetylcholine or electrical stimulation were increased by low doses of chlorfenvinphos. This article was only submitted in abstract and there were no details of dosing or methods; therefore it is unsuitable for regulatory purposes.

Takahashi H, Kojima T, Ikeda, T, Tsuda S and Shirasu, Y (1991). Differences in the mode of lethality produced through intravenous and oral administration of organophosphorus insecticides in rats. Fundamental and Applied Toxicology, 16:459–68

This study was undertaken to investigate the possibility that mechanisms other than AChE inhibition account for the acute toxicity of organophosphorus pesticides. Organophosphorus insecticides are classified into two categories:
P=O type — direct AChE inhibitors (eg chlorfenvinphos, dichlorvos)
P=S type — indirect ChE inhibitors (eg diazinon and fenthion), requiring metabolic activation.

Rats treated with lethal IV and PO doses of chlorfenvinphos or dichlorvos (P=O type) and lethal oral doses of diazinon or fenthion (P=S type), exhibited typical signs of AChE inhibition, along with marked inhibition of brain and erythrocyte AChE activity. Rats given lethal IV doses of P=S type insecticides, however, exhibited tonic convulsions and arching (opisthotonos), with only slight inhibition of AChE activities.

Differences were also observed in the cardiorespiratory and EEG responses to diazinon (P=S type) when given IV to anaesthetised and conscious rats (with and without atropine), compared to IV or oral doses of chlorfenvinphos (P=O type). It was concluded that lethality following P=S type insecticides may be independent of AChE inhibition.

Maxwell JC & Le Quesne PM (1982) Neuromuscular effects of chronic administration of two organophosphorus insecticides to rats. Neurotoxicology 3:1-10

Chlorfenvinphos (source, purity, batch no not specified) was administered in the diet to Sprague Dawley rats (source, number not specified) at 0 or 150 ppm (equivalent to 0 or 4.5 mg/kg bw/day) for 12 months. Muscle action-potential amplitude was measured in treated and control animals prior to commencement of treatment, and 3-monthly after this. Muscle potentials were measured in anaesthetised rats kept in thermostatically controlled conditions. The nerve was stimulated with a needle inserted beside the posterior tibial nerve at the ankle. Blood and plasma ChE activity was determined at 3 and 6 months after commencement of dosing.

No abnormal signs were seen in treated rats during the experiment. Rats treated with chlorfenvinphos had decreased body weight (approximately 10%) in comparison to controls. Blood and plasma ChE activity was decreased to less than 50% of control values in rats treated with chlorfenvinphos. There were no changes in muscle action potentials related to treatment with chlorfenvinphos. There were abnormalities related to late action potentials, including prolonged negative potentials, and repetitive activity. The size and duration of the prolonged negative potential, and the amount of repetitive activity increased over the dosing period. Chlorfenvinphos-treated animals showed a reduction in repetitive activity following a double stimulus. Therefore it appears that there are clear neuromuscular effects following chlorfenvinphos to rats, and that these changes can be detected using EMG monitoring.

Gorny D & Miszcak M (1981) Brain level and synthesis of acetylcholine during exposure to vibration and organic-phosphorus and phenol-derived pesticides. Acta Physiol Pol 32: 469-476

The effect of the simultaneous exposure of rats to vibration, noise and chlorfenvinphos on the synthesis and content of ChE in brain neurons was investigated. Chlorfenvinphos was administered IP in oil solution at 1.66 mg/kg bw, with control animals receiving an equivalent dose of olive oil IP. Vibration was produced by a shaker producing oscillations in the vertical plane, with a 2 mm amplitude and 50 Hz frequency. The noise produced by this shaker was 80 decibels, and animals were exposed for 2 h. Animals were exposed to chlorfenvinphos alone, chlorfenvinphos and noise, and chlorfenvinphos, shaking and noise, using 5 animals per group. An untreated control, as well as animals exposed to noise alone and noise and vibration were also used. Immediately after the end of exposure, animals were euthanised, and the acetylcholine extracted from the cerebral cortex and brain stem.

Exposure to vibration and noise decreased the levels of acetylcholine present to less than 50% of those in untreated controls, while the rate of synthesis of acetylcholine increased to 120% of controls ($p<0.001$). Noise alone did not significantly affect either the levels or the rate of synthesis of acetylcholine in the rat brain. Exposure to chlorfenvinphos alone significantly ($p<0.001$) increased the level of acetylcholine to 180% of controls, and decreased the rate of synthesis to approximately 50% of controls. Exposure to noise or to noise and vibration resulted in the levels of acetylcholine and the rate of synthesis being not significantly different from levels seen in controls. Thus it appears that noise and vibration may counter some of the biochemical effects seen following the administration of chlorfenvinphos by altering the process of acetylcholine production.

Osicka-Koprowska A, Lipska M & Wysocka-Pamszewska B (1984) Effects of chlorfenvinphos on plasma corticosterone and aldosterone levels in rats. Arch Toxicol 55: 68-69

Chlorfenvinphos (purity 98%, source, batch no not specified) was administered to male Wistar rats (source not specified) at 6.2 mg/kg bw by stomach tube. Control animals were dosed with a similar amount of oil. Rats were killed by decapitation at 1, 2, 3, 6, 12 and 24 h after treatment, this being determined as the method least likely to affect plasma steroid concentration. Corticosterone levels in plasma were assayed by the fluorimetric method, while aldosterone levels were determined by radioimmunoassay. Whole blood ChE activity was determined by a colorimetric method, while brain ChE levels were determined using the pH method.

At 1 h after treatment plasma aldosterone levels were approximately 300% of those in controls. These levels decreased over the next 24 h to approach control values. Corticosterone levels were approximately 180% of control levels 1 h after treatment, reducing to comparable with controls between 4 and 6 h after treatment. Brain ChE activity was not significantly different from control values 1 h after treatment, but had significantly decreased to approximately 10% (estimated from graph) at 2 h after treatment. Brain levels were still less that 50% of control values 24 h after treatment. Blood ChE activity was significantly decreased, to less that 50% of control values by 1 h after treatment, and remained at this level for the 24 h observation period. The mechanism of action of the elevation of corticosteroid levels was not well defined, as to whether it was related to a central action on ACTH secretion or to other mechanisms.

Kisielinski T, Gajewski D, Gidynska T & Owczarcyk H. (1980) Antiesterase activity of chlorfenvinphos and phospholine in certain rat tissues after administration by different routes. Acta Physiol Pol 31: 279-288

Chlorfenvinphos (source, batch no, purity not specified) was administered to female Wistar rats in an initial test to determine the LD50, and in a subsequent test to investigate ChE inhibition in a variety of organs. The oral LD50 was 14.2 mg/kg bw, and the dermal LD50 was 33.6 mg/kg bw.

Chlorfenvinphos was then administered at doses of 3/4 the LD50 either orally or dermally. Rats were killed 15 and 30 min and 3, 24 and 72 h after dosing. Blood samples were taken for determination of erythrocyte ChE activity. The pontomedullary area, the hypothalamus and the cerebral cortex of the brain, as well as the caudate lobe of the liver and right tibialis muscle were homogenised and the ChE activity determined.

Following oral administration of chlorfenvinphos, there was marked inhibition (>20%) of ChE in the hypothalamus, cerebral cortex, erythrocytes, liver and muscle within 15 min. Inhibition in the pontomedullary area was seen after 30 min. Inhibition was still >20% in the brain at 72 h, while no inhibition was seen in the erythrocytes, liver or muscle. Following dermal administration of chlorfenvinphos, there was marked inhibition (>20%) of all tissues examined within 15 min, with inhibition persisting in all tissues except erythrocytes to 72 h. Liver ChE inhibition was less marked following dermal exposure than following oral (47% inhibition cf. 81% inhibition). Liver metabolism of chlorfenvinphos following oral administration may have produced the difference seen in inhibition patterns.

Socko R, Gralewicz S & Gorny R (1989) Neurotoxicity of chlorphenvinphos an organophosphorus pesticide: Effects on blood and brain cholinesterase activity, open field behavior and response-to-change in a "T" maze in rats. Pol J Occup Med 2(3): 294 - 308

Chlorfenvinphos (purity 92%, source: Organika-Azot, Poland; batch no not specified) in sterile olive oil was administered by IP injection to male Wistar rats (imp-DaK, outbred, source not specified) at doses of 0, 1 or 3 mg/kg bw. The doses were calculated to be approximately 1/3 or 1/10 of the LD50 for the rat. Rats from each group (3 - 5 rats/group) were killed by decapitation 3, 24 or 48 h and 7 or 14 days after exposure. Blood was collected for determination of plasma and erythrocyte ChE. The brain was removed, placed on ice and dissected. ChE activity in the anterior hemisphere, brain stem, hippocampus, cerebellum and diencephalon was determined.

Behavioural studies were also carried out, using the same doses and 10 - 14 rats/group. Rats were handled for 10 min/day for 4 days prior to the commencement of the trial. Animals were tested in an open field trial every second day for 2 weeks prior to injection, 3 h after the injection and for 2 weeks after the injection. The variables measured were the latency of entry into the open arena, the number of squares crossed (locomotor activity), the number of rearings (exploratory behaviour) and the number of faecal boluses left in the arena. In the last 3 sessions, a novel object was placed in the observation area, and the investigative behaviour of the rats was also assessed. Behaviour was also assessed using a T maze. Rats were exposed to the maze once daily for 4 days, during which time the preferred direction of exploration for each rat was determined. Rats were then tested 24 h after injection, and every 2nd day for 4 trials (interchanging with the open field trial). Each session of exposure had 2 trials, a presentation trial and a challenge trial. In the presentation trial, the rat was placed in the maze facing the cross-section, and allowed to explore for 1 minute. One of the T arms was white and the other black in this trial. The rat was then transferred to a plastic box for 1 min, while the arms were changed so they were both the same colour. The rat was then returned to the maze, and the trial ended when it made a choice of arms (entered with all 4 paws), or had remained in the shaft area for 3 min. The measured variables were: the type of choice (the previously preferred arm, or the changed arm) and the choice latency.

Plasma ChE was inhibited for 3 h after an injection of 1 mg/kg bw, and for 48 h after the 3 mg/kg bw injection; following this, values were within a normal range. Erythrocyte ChE was inhibited for the same period after a 1 mg/kg bw injection, however remained inhibited for 14 days after an injection of 3 mg/kg bw. Brain ChE activity in all areas of the brain was inhibited for 24 h after the low dose, with little difference in the degree of inhibition between different brain areas. Following the 3 mg/kg bw injection, the cerebellum had recovered to normal activity within 7 days, while the other brain areas were still inhibited. All brain ChE activity had returned to normal 14 days after the 3 mg/kg bw injection.

There were changes in the general behaviour of high dose rats, including lacrimation, frequent urination and defecation, tremor and immobility. While most of these symptoms disappeared within several hours, there was a slowing of movement, compared with control animals, up to the third day after treatment. In the open field trial, the locomotor and exploratory activity were significantly ($p<0.05$) decreased in high dose rats in the first 3 trials after treatment. There were no differences noted in the latency of entry to the arena, or in the number of faecal boluses between treated and control animals. In the last 3 session, when a new object was introduced into the field, the control animals increased their activity but chlorfenvinphos-treated rats (both high- and low-dose) did not show increased activity. It was suggested that there may be an increased fear response to the new object. In the T maze trials, high dose animals differed from controls mainly in their unwillingness to make a choice, with 43% of animals not moving from the shaft area in the high dose, compared with none in the control group. This difference was not seen in the low dose group. From 3 days after injection, there were no differences in activity observed.

From the behavioural tests performed it appears that chlorfenvinphos induced changes in the rat's behaviour, the majority of which may be related to the acute effects of the pesticide. The only notable behavioural effect observed at the low-dose was a failure to increase exploratory behaviour when presented with a novel object; this may have been related to a change in the fear response. This occurred after peripheral and brain ChE activity had returned to normal.

Gralewicz S & Socko R (1990) Effects of a single exposure to chlorphenvinphos, an organophosphate insecticide, on hot-plate behaviour in rats. Pol J Occup Med 2(2): 215 - 220

Chlorfenvinphos (purity 92%, source: Organika-Azot, Poland; batch no not specified) in sterile olive oil was administered to male Wistar rats by IP injection at doses of 1 or 3 mg/kg bw, with control rats given a similar volume of olive oil. Rats were tested in a hot-plate trial on the 18th and 19th days after treatment, by which time it was assumed from previous trials that ChE activity would have returned to normal or near normal. Rats were placed on a hot plate in a plastic enclosure. After a response (licking the foot) was seen, or after 1 min, the rat was transferred to the shock chamber, where it received footshocks every 5 sec for 2 min. Immediately after shocking, the rat was placed on the hot-plate again. The latency of the paw-lick response before and after shocking was measured.

Abnormal clinical signs were observed in the high-dose group, with decreased motor activity, tremor and lacrimation. Signs were visible from 15 to 30 min after treatment. Most signs had disappeared within 6 h, however the decreased motor activity persisted for 3 days. On the first day of testing, there was no difference in the latency to paw-licking prior to shocks between treated and control groups. After the shock, the latency period in the high-dose group had increased significantly ($p<0.05$) in comparison to controls, being 50% longer than in control groups. On the second day of treatment, the high-dose groups had a longer period before paw-licking both before shocking (25.6 sec compared with 10.3 in controls) and after shocking (37.1 sec compared with 17.7 sec in controls). While the time until the foot licking behaviour was increased in the animals on the lower dose, this was not significant at any time. These results suggested that stress-induced (foot shock) analgesia may have increased in the rats exposed to a high dose of chlorfenvinphos, with a slow dissipation of stress effects, with some residual effects present 24 h later.

Gralewicz S, Tomas T, Gorny R, Kowalczyk W & Socko R (1991) Changes in brain bioelectrical activity (EEG) after repetitive exposure to an organophosphate anticholinesterase. II Rat. Pol J Occup Med Env Hlth 4(2): 183 - 196

Male imp-DaK rats were given chlorfenvinphos (technical grade; source: Organika-Azot, Poland: purity, batch no not specified) in olive oil by IP injection at 0, 0.5 or 1 mg/kg bw 5 days/week for 2 weeks (10 injections in total). Rats from the low-dose group were killed 3 h, or 4, 7 or 14 days after treatment. High-dose rats were killed after 21, 35, 41 or 49 days. Blood was collected, and the brain was removed and dissected. ChE activity was determined in plasma, erythrocytes, and in the cerebellum, lower brain stem, diencephalon, hippocampus and in the anterior cerebral hemispheres. Rats were also prepared for electrophysiological studies by the implantation of bipolar electrodes bilaterally into the anterior neocortex and the dorsal hippocampus. Around one month before treatment, extensive recordings of hippocampal and neocortical activity was made on each rat, with recordings made for 2 h three times at 7-day intervals. Hippocampal and neocortical EEG was also measured before and after a sporadic stimulus on days 39 - 41 after exposure, using 15 min sessions on each day. The spontaneous EEG was recorded for 5 min, followed by an acoustic stimulus at 1 min intervals. In the 2nd session, the stimulus was associated with an electric shock, while the 3rd session did not use any electric shocks.

Plasma ChE activity was inhibited by both doses at 3 h after treatment, but had recovered by 4 days. Erythrocyte ChE activity was inhibited by 3 h at both doses, with recovery by 14 days.

There was little inhibition of brain ChE activity by the low dose; at 1 mg/kg bw, all areas of the brain were inhibited at 3 h, with recovery in the cerebellum at 4 days, in the brain stem at 21 days and in all other areas by 35 days. In rats treated with chlorfenvinphos, an acoustic stimulus associated with pain produced a stronger hippocampal theta response than that seen in non-treated rats. High-dose rats could be generally described as less aroused generally, but more responsive to threatening stimuli than either low-dose or control rats. This was seen in time periods when ChE activity had returned to normal.

Gralewicz S, Tomas T and Socko R (1989). Effects of single exposure to chlorphenvinpos, an organophosphate insecticide, on electrical activity (EEG) of the rat brain. Polish Journal of Occupational Medicine, 2(3)

The effect of a single exposure to chlorphenvinphos on neocortical seizure activity induced or promoted by cardiazol, and on hippocampal and neocortical EEG was studied in rats. It was found that chlorfenvinphos, given intraperitoneally at doses of 1.0 and 3.0 mg/kg bw did not result in any changes in the number and in the duration of epileptic bursts occurring spontaneously, or in the hippocampal theta rhythm. The effect of cardiazol (12.5 mg/kg IP) was slightly diminished if the drug was given 3 h, but not 14 days, after the injection of chlorfenvinphos. IP injection of 1.0 mg/kg of a carbamate cholinesterase inhibitor, physostigmine, resulted in a dramatic increase of the theta content in the hippocampal EEG, and in the total disappearance of the spontaneous seizures. Determination of ChE activity in blood and in the brain in a separate group of rats showed that after injection of physostigmine (1.0 mg/kg), ChE inhibition did not exceed the inhibition after injecting chlorfenvinphos at the doses used. It has been suggested that the differences between chlorfenvinphos and physostigmine in their potential to reduce spontaneous epileptic activity and to induce the hippocampal theta rhythm may be due to an antagonistic action of chlorfenvinphos on cholinergic postsynaptic receptors.

Brzezinski J and Wysocka-Paruszewska B (1980) Neurochemical alterations in rat brain as a test for studying the neurotoxicity of organophosphorus insecticides. Further Studies in the Assessment of Toxic Actions, Archives of Toxicology, Supplement ;, 475–478

This study examined the effects of acute and chronic administration of organophosphate insecticides on the brain noradrenaline (NA) level in relation to ChE inhibition. Groups of 6 male Wistar rats were given a single oral dose of chlorfenvinphos, dichlorvos or fenitrothion (batch details and purity not given) at 0.5 times the LD50 in soya bean oil (acute study). A further 10 - 12 males per group were given the same pesticides at dosages equal to 0.05 times the LD50 as soya bean oil solution, every day for 12 weeks (short-term study). Control animals received an equal volume of oil.

After 15, 30, 60, 120 and 180 minutes in the acute study and every 2 weeks in the short-term study, rats were killed (numbers not specified) and whole brains were analysed for NA content and ChE activity.

The effect of chlorfenvinphos on the rate of disappearance of brain NA was also measured following IP administration of 400 mg/kg disulfiram (a potent inhibitor of dopamine -hydroxylase) at 30 minutes before administration of chlorfenvinphos.

After acute oral administration, chlorfenvinphos caused maximal inhibition of ChE activity at 2–3 h. This was accompanied by a significant reduction of brain NA within 15 min. No consistent reduction of NA was observed at the remaining time points. Fenitrothion and dichlorvos depressed the ChE activity to a lesser degree but their action was long-lasting and persisted for the remainder of the time points. Both these insecticides reduced brain NA concentration at all the time points, with maximum effect for dichlorvos after 30 min and for fenitrothion after 2 or 3 h. Brain ChE inhibition was reduced by only 20 - 40% compared to controls during short-term dosing with chlorfenvinphos and dichlorvos. However, fenitrothion induced highly significant inhibition (approx 80%) of ChE activity, shown by an accumulation of toxic effects. Simultaneously, the NA content in the rat brain was significantly decreased by all three insecticides. Chlorfenvinphos and dichlorvos caused this effect over the total 12-week period, with maximum depletion at 8 weeks for chlorfenvinphos and at both 8 and 12 weeks for dichlorvos. Fenitrothion showed a delayed effect on the brain NA level: between 2 and 6 weeks, moderate increases were observed and at 8 - 12 weeks inhibition occurred, which was significant on week 10. A single oral dose (0.5 times the LD50) of chlorfenvinphos following pretreatment with disulfiram increased the rate of disappearance of NA in brain and simultaneously decreased the turnover time.

The mode of organophosphate toxicity is conventionally linked to their central and peripheral cholinergic action. However, numerous overt signs of organophosphate poisoning (such as increase in blood pressure and hyperglycemia) could be related to an adrenergic effect. The disruption of the central noradrenergic system following organophosphate intoxication may be characterised by the alteration of brain NA content.

The results showed that organophosphates produce significant changes in the brain NA level separate from their cholinergic action. The changes of whole brain NA level in both the acute and short-term experiments correlated more precisely with the structure and toxicodynamics of the pesticide tested rather than the degree of ChE inhibition. The observed NA changes might have resulted from releasing properties of acetylcholine accumulated during the course of organophosphate poisoning. However, the experiment demonstrated that ChE inhibition did not parallel the decrease in NA content. The study of the rate of NA disappearance after disulfiram indicated that another mechanism was involved.

10.3 Other Species

Tomas T & Gralewicz S (1992) A comparison of changes in spontaneous (EEG) and evoked brain activity induced by chlorphenvinphos and physostigmine in rats and rabbits. Pol J Occup Med & Env Hlth 5(1): 55 - 69

The effects of single IP injections of two cholinesterase inhibitors, chlorfenvinphos (source: Organika Chemical Plant, Poland) and physostigmine sulphate (Sigma Co) on hippocampal and cortical EEG and flash evoked potentials in occipital cortex were compared in male New Zealand White rabbits and male IMP-DAK outbred rats. A spectral analysis method was used for evaluation of changes in EEG. In rabbits receiving 14 mg/kg bw chlorfenvinphos, there was a decrease in cortical activity in some individuals. A decrease in hippocampal activity was also observed in treated rabbits. A prolonged decrease in either cortical or hippocampal activity was observed in the rabbits treated with 50 mg/kg bw chlorfenvinphos. Decreases in cortical activity were seen in rabbits receiving physostigmine and 0.2 or 0.4 mg/kg bw. In rats receiving 1 or 3 mg/kg bw, changes were less evident, while physostigmine at 1 mg/kg bw produced notable changes. Changes were observed in the flash evoked potentials in rabbits receiving either dose of

chlorfenvinphos, and in rabbits receiving physostigmine. Changes in the flash evoked potentials were also evident in rats in the 3 h following treatment. Changes observed following chlorfenvinphos administration in this trial only persisted for a short period of time, often disappearing within 3 h. Not all individuals were affected in a similar fashion.

Chambers PL & Reiff B (1965) The physiological and pharmacological effects of the chlorovinyl phosphate insecticide SUPONA (SD 7859). PPR TL/3/65, Shell Research Institute, Sittingbourne, UK

The physiological changes produced in mammals by an intravenous injection of chlorfenvinphos (purity 92.6%, source: Shell Development Company, Modesto) were investigated using rats, rabbits and a cat. Electrodes were fitted into the skull of the animals under anaesthesia, and the animals allowed a recovery period. Pretreatment readings were taken prior to the injection of chlorfenvinphos.

Rats were anaesthetised with urethane prior to the IV injection of 25 mg/kg bw chlorfenvinphos. This injection produced a notable rise in blood pressure 15 min after injection (80 - 100 mm Hg). Increased heart rate, dyspnoea, salivation, lacrimation, defecation and muscle fasciculations were also seen. After 10 - 15 min, respiratory failure and cardiac collapse were observed. All symptoms were abolished by an IV injection of 5 - 10 mg/kg bw atropine sulphate. The increase in blood pressure was not an expected result, and additional investigations were undertaken to determine the site of action. An adrenergic blocking agent injected prior to chlorfenvinphos prevented the increase in blood pressure. Removal of the adrenal gland did not affect the signs of poisoning, indicating that circulating adrenaline is not a major factor in the signs seen. When the spinal cord was sectioned at C1 there was no increase in blood pressure following dosing with chlorfenvinphos. The heart rate also remained normal. There was no effect seen following sectioning of the vagus nerve. It was concluded that the symptoms resulted from peripheral sympathetic nerve supply, originating from the central nervous system above the spinal cord. Electroencephalographic recordings were made after an IV injection of 50 mg/kg bw. After 5 to 8 min, there was a decrease in the amplitude of the trace, accompanied by an increase in the frequency. At 20 to 30 min, high voltage waves and wave complexes were seen.

Anaesthetised rabbits were injected IV with 50 to 150 mg/kg bw chlorfenvinphos via the ear vein. An increase in blood pressure and heart rate were seen, as was dyspnoea which culminated in respiratory failure. Rabbits were observed to lose blood through the nose and mouth. This was found to come from the lungs, which were congested at autopsy. Treatment with atropine or antihistamines did not prevent the lung haemorrhage. EEG examination following an IV injection of chlorfenvinphos at 100 mg/kg bw into the ear vein revealed similar changes to those seen in the rat. When chlorfenvinphos was administered by intracarotid injection all central nervous system activity was depressed.

An anaesthetised cat was treated with an IV injection of 5 mg/kg bw chlorfenvinphos. No abnormal signs were observed for the first 30 min, after which there was a marked decrease in blood pressure. Miosis, lacrimation, salivation, defecation, muscle fasciculations and slowing of the heart rate were also observed. Respiratory rate decreased, and ceased 35 min after dosing. At this point, 10 mg/kg bw atropine sulphate was administered by IV injection. This reestablished normal respiration and abolished all abnormal clinical signs.

Overall, chlorfenvinphos appears to have centrally-acting effects on the cardiovascular and respiratory systems. The cat appears to be very sensitive to the effects of the compound, while guinea-pigs tolerated a much higher dose.

Takeda Y, Tsukahara I & Takaori S (1976) Effects of chlorfenvinphos, an organophosphate insecticide, on afferent transmission in the central visual system. Jap J Ophthalmol 20: 195-203

Chlorfenvinphos (batch no, purity not specified, source: Shell Kagaku) in peanut oil was administered IP in cumulative doses at 1 h intervals to healthy cats. Glutathione, atropine sulphate and PAM were administered 30 min after injection of chlorfenvinphos to determine their efficiency as antidotes. Cats were prepared by fixing the head in a stereotaxic instrument, and stainless steel electrodes inserted in the optic tract and lateral geniculate nucleus on the left side of the brain. Another electrode was placed on the frontal cranium. Wound edges and pressure points were anaesthetised with lidocaine, and the cats were immobilised with gallamine triethiodide. Respiration was maintained artificially, and animals were kept in a dark, sound-proof room. The right eye was dilated with Mydrin-P (tropicamide and phenylephrine).

The right eye was stimulated with light flashes every 2 seconds. The ERG and EEG readings were recorded, and the blood pressure in the femoral artery were monitored. ChE activity in erythrocytes were determined, as were the ChE activities in the retina, lateral geniculate nucleus and the visual cortex.

The a-wave of the ERG was significantly increased ($p<0.01$) by doses of 1 to 16 mg/kg bw chlorfenvinphos. The b-wave of the ERG was significantly increased by doses of 4 - 64 mg/kg bw chlorfenvinphos. The N-wave in the optic tract was significantly decreased by all doses of chlorfenvinphos. The N wave and P wave in the lateral geniculate nucleus were also decreased, while the N and P waves in the visual cortex were not affected. The erythrocyte ChE activity was significantly decreased by 1 h after injection with 4 mg/kg bw chlorfenvinphos, while there was only marginal inhibition of ChE activity in retina, lateral geniculate nucleus and visual cortex. The antagonists glutathione, atropine and PAM administered individually reversed the effects of chlorfenvinphos on the ERG, optic tract waves, and those detected in the lateral geniculate nucleus and visual cortex. The effects on the electrical activity in the brain were seen at doses without significant effects on the ChE levels in some areas of the brain.

Takeda, Y, Tsukahara I & Takaori S (1977) Effects of chlorfenvinphos, an organophosphate, on E.R.G. and afferent transmission in the central visual pathway (Japanese). Nippon Ganka Gakkai Zasshi (Acta Soc. Opthalmol) 79: 1345 - 1352 (1975); abstract cited in: Pest Abstr 10: 134,77-0621

The IP injection of 4 mg/kg bw of chlorfenvinphos increased the electroretinogram amplitude, decreased the photo-responsiveness of the optic tract and lateral geniculate nuclei, and slightly increased the photoresponsiveness of the visual cortex in cats. This dose also caused an approximately 80% decrease of ChE activity in the retina. The effects were slowed by treatment with atropine and 2-PAM. This article was only available in abstract, thus no details of treatment regime or methods were available.

Gralewicz S, Tomas T & Socko R (1995) Interaction of Chlorfenvinphos with Cholinergic Receptors in the Rabbit Hypothalamus. Neurotox & Teratol 17(3): 289 - 295

The purpose of this study was to find out whether chlorfenvinphos interacts with muscarinic cholinergic receptors in the central nervous system. The effects of intrahypothalamic injections of oxotremorine, a muscarinic agent, and physostigmine, a carbamate anticholinesterase, were compared with those produced by intrahypothalamic injections of chlorfenvinphos in rabbits. It was found that the infusion of oxotremorine (20 µg) as well as physostigmine (200 µg) into the anterior hypothalamus led to an increase in the 4-7 Hz theta rhythm in the hippocampus and to the appearance of behavioural symptoms suggestive of a threat response. In the case of oxotremorine, the effects could be prevented by injections of 20 µg scopolamine, a muscarinic antagonist. Pretreatment of the hypothalamus with 100 µg hemicholinium did not prevent the effects of physostigmine injected 3 h later, (hemicholinium prevents the resynthesis of acetylcholine by blocking choline uptake, leading to a gradual depletion of acetylcholine stores and to an inhibition of the cholinergic transmission). It suggested that physostigmine directly activated postsynaptic muscarinic receptors. Intra-hypothalamic injections of chlorfenvinphos in doses of up to 1360 µg produced no overt changes in behaviour nor in the hippocampal EEG of the rabbit, and did not prevent the effect of subsequent injections of oxotremorine. This suggested that chlorfenvinphos was neither an agonist nor antagonist of muscarinic receptors in the rabbit hypothalamus.

Gralewicz S, Tomas T, Gorny R, Kowalczyk W & Socko R (1988) Changes in physiological and electrophysiological parameters in rabbits after single exposure to chlorphenvinphos. Pol J Occup Med 2(1): 3 - 14

Male New Zealand rabbits (source not specified) were fitted with intra-brain bipolar stainless steel teflon-coated electrodes about 2 weeks prior to dosing. Chlorfenvinphos (purity 92%, source: Organika-Azot, Poland; batch no not specified) in olive oil was administered IP at doses of 22, 33, 50, 75, 112, 167 or 250 mg/kg bw. Each rabbit was dosed twice, at the same dose, at an interval of 80 - 90 days (providing the animal survived the first injection). ChE activity in plasma and erythrocytes, body temperature, and EEG readings were monitored, with each measure made at least 3 times prior to the first injection, then 30 and 90 min, 3 and 6 h, 1, 2, 4, 6, 8, 17 and 24 days and 1 and 2 months after injection. Following the second injection, observations were made for 4 days after the injection.

Plasma ChE inhibition was seen at all doses tested, with inhibition seen from 30 min in all but the lowest dose; inhibition at the lowest dose was seen from 90 min, and persisted until 96 h (last time interval reported in detail). Following the second dose (90 days after the first), inhibition was seen from 30 min at all doses tested, persisting until the end of the observation period. Erythrocyte ChE activity showed a similar pattern of inhibition.

In the group receiving the lowest dose, there were no changes in general behaviour. In all other dose groups, panting, weakness, salivation, mucoid nasal discharge, urination and defecation were seen from 30 min after injection. The animals in general remained immobile, with partial normalisation beginning 6 h after injection. By the day after treatment, animals had virtually returned to normal, with diarrhoea being the only persistent sign. There was no evidence of induced neuropathy, although no details of testing methods employed were provided. Following the repeat dose, there were no behavioural changes seen in most doses, with mild changes seen at 112 mg/kg bw (the highest dose tested by the second injection).

Body temperature was rapidly decreased by the first injection of chlorfenvinphos, with decreases between 0.7^{o}C at 22 mg/kg bw and 3.5^{o}C at 250 mg/kg bw. Decreases were seen within 90 min

of injection, with body temperatures starting to return to normal 6 h after injection. After 24 h, all body temperatures had returned to normal. After the second injection, 2 animals showed a decrease in body temperature (at 22 and 112 mg/kg bw). One animal showed hyperthermia, while the rest showed no change in temperature.

Changes in the hippocampal EEG mainly consisted of an increase in the immobility-related rhythmic slow activity (I-RSA), with a concurrent decrease in the large irregular activity and the movement related rhythmic slow activity. This may have been related to the behavioural changes seen. In the 2 highest doses, the changes progressed to include abnormalities in the frequency and amplitude of I-RSA, progressing to tonic-clonic seizure activity. Following the second injection of chlorfenvinphos, the changes in hippocampal EEG were less pronounced and less consistent. This suggested a degree of adaptation in the hippocampus to the effect of chlorfenvinphos.

Gralewicz S, Kowalczyk W, Gorny R & Socko R (1990) Brain electrical activity (EEG) after repetitive exposure to chlorophenvinphos, an organophosphate anticholinesterase: I. Rabbit. Pol J Occup Med 3(1): 51 - 67

Chlorfenvinphos (purity 92%, source: Organika-azot, Poland) in olive oil was administered to male New Zealand rabbits (source not specified) by IP injection at 14 mg/kg bw 5 days/week for 2 weeks. Control animals received IP injections of olive oil. Animals were used either for determination of ChE activity or in electrophysiological studies. In the ChE studies there were 5 treated and 9 control rabbits. Plasma and erythrocyte ChE activity was determined prior to injections, 3 h after the first injection, 10 min prior to the last injection, then 3, 24, 49 and 72 h and 7 days after the last injection. ChE activity was then determined weekly until levels returned to 90% of control values. At this stage, rabbits were killed and the brains removed and dissected into the cerebellum, medulla oblongata, midbrain, diencephalon, hippocampus, entorhinal cortex, temporoparietal cortex and anterior part of the cerebral hemisphere. Each brain area was weighed and the ChE activity determined. For the electrophysiological study, 5 treated and 5 control rats with intrabrain chronic bipolar electrodes implanted in the dorsal hippocampus, anterior neocortex and posterior neocortex were used. Measurement of the hippocampal and neocortical EEG with no sporadic stimuli was done weekly pretest and 1, 7 and 14 days after the last injection. Hippocampal arousal following strong acoustic stimulus was measured once pre-exposure and 8 weeks after the last injection. Hippocampal and neocortical EEG was also measured before and after a sporadic stimulus on days 49 - 51 after exposure, using 15 min sessions on each day. The spontaneous EEG was recorded for 5 min, followed by an acoustic stimulus at 1 min intervals. In the 2nd session, the stimulus was associated with an electric shock, while the 3rd session did not use any electric shocks.

Chlorfenvinphos treatment resulted in a very slight decrease in bodyweight which recovered within 1 week of the end of exposure. There was a decrease in rectal temperature within 90 to 120 min, which resolved within 24 h. The effect disappeared with the development of tolerance. Plasma ChE activity showed a 40% inhibition prior to the last injection, with 50% inhibition after the last injection. Levels returned to within normal limits by 2 days after the final injection. Erythrocyte ChE activity was inhibited 60% in comparison to controls just prior to the 10th injection, with no change immediately after the injection, but with a slight increase to 65% inhibition 24 h later. Erythrocyte ChE levels did not return to normal until 3 weeks after the end of treatment.

Brain ChE activity, determined after plasma and erythrocyte ChE levels had returned to normal, was significantly inhibited in most areas. Inhibition of 16% ($p<0.05$) in the midbrain and 26%

($p<0.05$) in the temporo-occipital lobe was seen. Inhibition ($p<0.001$) was seen in the diencephalon (14%), hippocampus (23%) and pyriform lobe (27%). Therefore in this trial significant brain ChE inhibition persisted after plasma and erythrocyte activity had returned to normal.

There was no change in passive hippocampal activity between treated and control animals. In both groups, habituation decreased the response to the acoustic stimulation, although the magnitude of the response increased in rabbits exposed to chlorfenvinphos. This effect was more marked when the acoustic stimulus was associated with a foot shock. These altered responses were seen around 50 days after treatment, by which time the brain ChE would be assumed to have returned to normal. Therefore there was some evidence of functional changes in the brain which outlast ChE depression.

11 HUMAN STUDIES

11.1 Toxicity Studies

Hunter CG, Robinson J, Bedford CT & Lawson JM (1972) Exposure to chlorfenvinphos by determination of a urinary metabolite. J Occup Med 14: 119-122

Male volunteers were given daily oral doses of 3 mg chlorfenvinphos (purity 85%, batch no, source not specified) for 53 days. Mean weights of the volunteers were not supplied, however assuming an average weight of 70 kg, this dose was approximately 0.04 mg/kg bw/day. Analysis of 24-h urine samples were made on days 10, 24, 38 and 52 during exposure. The average urinary excretion of desethyl chlorfenvinphos was 4.7% of the administered daily dose. There were no reports of any abnormal clinical signs following the administration of this dose of chlorfenvinphos. No monitoring of ChE levels was reported.

Brown VKH (1966) The effect of chlorfenvinphos (SD 7859) on two human volunteers. IRR TL/18/66. Shell Research Institute, Sittingbourne

Chlorfenvinphos (purity, batch no, source not specified) was administered to male human volunteers, aged between 35 and 40 years in an oral and a dermal study (1/study). The first volunteer swallowed a gelatine capsule containing chlorfenvinphos to give a dose of 1 mg/kg bw. Blood samples were taken at 1.5, 3, 6, 24, 48 and 120 h, and 9, 19, 26 and 54 days after exposure. The plasma and erythrocyte ChE activity, and the concentration of chlorfenvinphos in the blood were determined. An EEG pattern was analysed before exposure and 4 h after dosing. Urinalysis was also done at intervals following dosing. There were no abnormal clinical signs observed following exposure. The EEG pattern did not change. Urinalysis revealed glycosuria for some weeks following exposure; the abnormality had not previously been detected in this individual. Plasma ChE was significantly inhibited (>50% inhibition) for 19 days following exposure. Erythrocyte ChE was inhibited (>40% inhibition) for 6 h after dosing. Chlorfenvinphos was detected in the blood for 6 h after dosing.

In the second trial, chlorfenvinphos, as a 24% emulsifiable concentrate formulated with xylene, was applied to the forearm of a volunteer at 5 mg/kg bw. After partial air drying, the area was covered with aluminium foil and a bandage. After 6 h the occlusive dressing was removed and the skin washed with water. A total dose of 380 mg was applied, and 260 mg was recovered from the foil. The total absorbed dose was therefore no more than 120 mg. Blood samples were

collected 3, 6, 24 and 96 h and 8 and 18 days after dosing. There were no abnormal clinical signs observed during this period. Plasma ChE activity was inhibited by 45% at 96 h, and had returned to normal at 18 days. Erythrocyte ChE activity was not inhibited.

The results of this study indicated that there was a depression of ChE activity following a 1 mg/kg bw oral dose and a 5 mg/kg bw dermal dose (of which no more than approximately 1.6 mg/kg bw was absorbed) in human volunteers.

Hunter CG (1969) Dermal toxicity of chlorfenvinphos (CFVP). Ind Med 38: 49 - 51

Chlorfenvinphos was formulated as an 80% emulsifiable concentrate (EC), a 24% EC and a 25% wettable powder formulation. Nine healthy males, with no previous exposure to anticholinesterase agents participated. Basal measurement of creatinine clearance and phosphate reabsorption indices were made in each subject. Cardio-respiratory functions and EEG readings were measured during exposures. The activity of plasma and erythrocyte ChE were measured. The chemical was applied to the left forearm of each volunteer, and the area covered with an occlusive dressing. After exposure, the dressing was removed and excess chlorfenvinphos was removed from the skin. The chemical remaining on the foil and on the swabs was quantified, and the absorbed dose determined. The doses and length of exposures varied between subjects.

The formulations applied, absorbed dose and inhibition of plasma and erythrocyte ChE activity are presented below.

Table 29

Formulation	Dose of Chlorfenvinphos Applied (mg/kg bw)	Time (h)	Absorbed Dose (mg/kg bw)	ChE Inhibition at 24 Hours	
				Plasma	Erythrocyte
80% w/v EC	4	4.0	1.43	<5%	None
	5	3.7	1.81	None	None
	10	3.8	0.06	7%	None
	10	4.0	.032	27%	None
24% w/v EC	5	4.0	0.18	25%	None
	5	3.8	0.12	17%	None
	10	4.0	0.20	76%	None
	10	4.0	0.08	53%	9%
25% WP	5	4.2	0.32	17%	None

There was considerable variability in the absorbed dose between volunteers. The plasma ChE activity was also inhibited to varying degrees. There were no effects seen on the cardiovascular system, and no EEG changes were recorded. No glycosuria was observed. Where the applied chemical remained liquid, rather than evaporating, an irritant dermatitis was noted. It was not specified in which applications this occurred.

For the 80% EC formulation (approximately 4 mg/kg bw of undiluted chlorfenvinphos), as no significant plasma or erythrocyte ChE inhibition was seen at 5 mg/kg bw. No information on doses or formulations producing irritant dermatitis were supplied.

Stevenson DE & Pickering RG (1965) Technical Memorandum TOX 13/65 The effect of dermal exposure to BIRLANE on the blood cholinesterases of human volunteers. Shell Research Institute, Sittingbourne

A 0.05% solution of chlorfenvinphos (no other formulation details supplied, source, batch no not specified) was used to determine the effects of a worst-case occupational exposure when dipping cabbage roots into a chlorfenvinphos solutions. Two healthy male volunteers (approximately 40 years old) participated in the study. A pre-test blood sample was taken, then each volunteer immersed his hands, wrists and forearms in approximately 6 L of 0.05% chlorfenvinphos solution. The volunteers immersed their hands every 2 - 5 minutes from 9.30 am to 1 pm and 2.15 pm to 5 pm. The hands were washed with soap and water at 9 pm. Blood samples were taken at 1 and 5 pm on the day of exposure, and on the next day at 9 am and 2 pm and plasma and erythrocyte ChE activities determined. Plasma ChE activity was approximately 50% inhibited in both subjects by 5 pm on the day of treatment, but had returned to normal by 2.15 pm on the day following treatment. No significant erythrocyte ChE inhibition was seen in either volunteer. Neither volunteer showed any abnormal clinical signs. There was no attempt to determine the quantity of chlorfenvinphos which was absorbed in this trial.

11.2 Field Studies

Blok AC, Mann AH & Robinson J (1977) Organophosphorus insecticide exposure of sprayers under field conditions on rice in India. I BIRLANE (chlorfenvinphos). The Hague, SIRM, Toxicol Div., Tox 77-005

Chlorfenvinphos was applied to paddy rice under normal conditions, using 3 groups of 5 volunteers. The first group applied a dilution of a 24% EC formulation; the material was applied at 0.11% on the first day, and at 0.06% for 5 consecutive days. Workers applied approximately 110 L in a working day; on most days this was 66 g of chlorfenvinphos (121 g of chlorfenvinphos on the first day). The second group of workers applied a 10% chlorfenvinphos granular preparation. This was spread by hand, with each worker applying 1.5 - 3 kg/day. The third group did not apply any pesticide. Workers wore short-sleeved cotton shirts, cotton shorts and a cotton cloth covering the head, with bare arms, legs and feet. They made efforts to avoid direct contact with formulation during mixing, filling and spraying, and did not spray against the wind or upwind of other workers. No other protective equipment was supplied or used.

Blood samples were taken morning and night on the 2 days prior to the trial. Blood samples were taken each morning during spraying, and were also taken on the 2 days after spraying stopped. There were no abnormal signs observed during the spraying period. The pretest blood samples indicated that there were differences between the groups, and comparison between the groups was therefore not useful. The pretest samples were used as the control values for each worker. Plasma ChE activity in both groups applying chlorfenvinphos was decreased on the first measurement in the evening of the first day of spraying (81% inhibition in the liquid group, 51% inhibition in the granule group). The group applying the liquid showed inhibition of plasma ChE until the end of day 3, while the granule group did not have any inhibition after the first day. There was no significant inhibition of erythrocyte ChE in either group at any of the observation times.

Ottevanger CF (1976) An epidemiological and toxicological study of occupational exposure to an organophosphorus pesticide. University of Amsterdam, M.D. thesis. Rotterdam, Phoenix and den Oudsten

An epidemiological study of workers exposed to chlorfenvinphos was performed, and a summary of the results were presented. Workers exposed for more than 5 years were compared with workers exposed for 2 years and workers who had not been exposed to determine if there were any identifiable effects from exposure to chlorfenvinphos; if there were, how frequently did they occur, and if there was any evidence of any harmful effects on health. Analysis of ChE activity, haematological parameters (unspecified) and EMG voltages were done. There was no significant difference in ChE activity between groups. No specific results were presented, however it was determined that there was a lower EMG voltage (details of measurement not provided) in workers exposed for 5 years in comparison to those working for 2 years or controls. This lowering of voltage was reversible and an improvement in industrial hygiene or removing the worker from the workplace for a number of weeks returned the values to normal. A lowering of EMG voltage was also seen in another study in which 10 workers were assessed over 8 years. These findings were suggestive of a reversible neurological effect from contact with chlorfenvinphos at relatively low levels (no significant ChE inhibition) over a long period of time.

Roberts DV (1976) E.M.G. voltage and motor nerve conduction velocity in organophosphorus pesticide factory workers. Int Arch Occup Environ Health 36: 267-274

Pesticide factory workers, exposed to a range of pesticides including chlorfenvinphos, were examined electromyographically, and a relation between work with organophosphorus compounds and low voltage EMG following supra-maximal ulnar nerve stimulation was demonstrated. This effect was reversible, as a 3-week break from exposure increased the voltage measured. It was also found that workers with low voltage EMG also had low conduction velocities in both fastest and slowest motor nerve fibres. A comparison of the maximum conduction velocities of motor nerve fibres in control and organophosphorus exposed workers showed exposed workers had approximately a 10% decrease in conduction velocity.

Anon (1988) Organophosphate Toxicity Associated with Flea-Dip Products - California. MMWR: 27 (21): 229 - 337

Pet groomers were surveyed in California. One person, out of 24 surveyed, complained of symptoms of headache, dizziness, nausea and fatigue following the use of chlorfenvinphos. Most of the groomers surveyed reported that they did not use protective clothing, and did not use pesticides in accordance with label directions. Often the undiluted concentrates were applied with bare hands, and their skin and eyes were frequently exposed.

11.3 Accidental poisoning

Jay WM, Marcus RW & Jay MS (1982) Primary position upbeat nystagmus with organophosphate poisoning. J Pediatr Ophthal & Strabismus 19: 318-319

A case report of a 21-month old child who swallowed 60-100 mL of a chlorfenvinphos formulation (as Dermaton - no formulation details supplied) was reported. Symptoms of poisoning on admission to hospital were: laboured ventilation (requiring intubation and ventilatory assistance),

unreactive and pinpoint pupils, upbeating jerk nystagmus, hypotonia and frequent muscle twitches, hyporeflexia and occasional myotonic spasm. Erythrocyte ChE levels prior to treatment were 1530 U/L (normal 3590 - 6000 U/L) and plasma ChE activity was 150 U/L (normal 2436 - 4872 U/L). Antidote treatment with atropine and pralidoxime chloride was successful. Improvement was rapid, with extubation occurring at 18 hours and discharge from hospital at 4 days.

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