Section 4
EVALUATION OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS

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ABBREVIATIONS AND ACRONYMS

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 body weight/day
IV	Intravenous	**ppb**	Parts per billion
PO	Oral	**ppm**	Parts per million

ADI	Acceptable Daily Intake
A/G	Albumin/globulin ratio
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
ALT	Alanine aminotransferase (SGPT)
BUN	Blood urea nitrogen
CCl_4	Carbon tetrachloride
ChE	Cholinesterase
CPK	Creatinine phosphokinase
DDM	4,4'-Diaminodiphenylmethane
DMSO	Dimethyl sulfoxide
EUP	End Use Product
GLP	Good Laboratory Practice
Hb	Haemoglobin
Hct	Haematocrit
LAP	Leucine aminopeptidase
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
OP	Organophosphorus pesticide
2-PAM	Pyridine-2-aldoxime methiodide
TGAC	Technical Grade Active Constituent
WBC	White blood cell/leucocyte
ACPH	Advisory Committee on Pesticides and Health
NHMRC	National Health and Medical Research Council
NDPSC	National Drugs and Poisons Scheduling Committee

SUMMARY

Background

Fenitrothion, an organophosphorothioate (or organophosphorothionate) with insecticidal activity, was independently synthesized by Bayer AG (company code, Bayer 41831) and Sumitomo Chemical Co (S-1102-A) in 1959. Its development was prompted by a desire to identify a pesticide with reduced acute toxicity in mammals relative to parathion-methyl and yet maintain comparable efficacy. Hence, it is structurally related to parathion-methyl in that it differs only by the presence of a methyl group in the 4-nitrophenyl ring (see chemistry in section 1.3). Fenitrothion is used to control insect pests in a wide range of horticultural and agricultural crops and has been registered for use in Australia for nearly 30 years.

The current Australian ADI for fenitrothion is 0.003 mg/kg bw/day based on an NOEL of 0.3 mg/kg bw/day for plasma ChE inhibition seen in a rat dietary study. Fenitrothion is in poisons schedule S6 of the Standard for Uniform Scheduling of Drugs and Poisons (SUSDP).

IBT Involvement

Following discovery of fraud by Industrial BIO-TEST (IBT) laboratories in 1976/77 it was recognised that many of the toxicological studies submitted to establish the ADI set by the the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) were performed by this organisation. Some of the submitted studies have since been validated and only those studies have been incorporated in this review. Following several revisions the current JMPR ADI is set at 0.005 mg/kg bw/day.

Kinetics and Metabolism

An overview of data obtained from a number of studies indicate that fenitrothion is relatively well absorbed from the intestinal tract in most species. Activation to the active metabolite, fenitrooxon, occurs in the liver, followed by relatively rapid degradation to non-active metabolites which are then excreted. Additionally, there is a direct degradation pathway seen in rats. Fenitrothion metabolites are mainly excreted in the urine, in both conjugated and unconjugated forms. A lesser amount of excretion occurs via the faeces and, in lactating animals, by the milk. There is no evidence of bioaccumulation in body tissues following longer term feeding (6 months in the rabbit).

In mice, rats, rabbits and dogs, fenitrothion was readily absorbed from the gastrointestinal tract, and rapidly distributed to a number of organs, including the liver, kidney and fat. Fenitrothion appeared to be relatively rapidly activated to the major active metabolite, fenitrooxon, which was then inactivated by metabolism to other metabolites. Excretion of fenitrothion and its metabolites occurred mainly in the urine; this was most rapid in the rabbit and mouse, with dogs excreting metabolites over the longest period. In all species tested, more than 80% of the radioactivity was excreted in the urine within 24 h after oral administration. No labelled carbon dioxide was detected in the expired air of rats. After 2 days, the recovered radioactivity from mice was 92%; rats 91-92%; rabbits 94%, and male dogs 88%. Very little fenitrothion was

excreted unchanged in the faeces. The main urinary metabolites were desmethylfenitrothion and desmethylfenitrooxon. 3-methyl-4-nitrophenol (both free and conjugated with sulfate or -glucuronic acid) was another major metabolite group. Repeated administration or increased dosing did not appear to change the excretion pattern, and there was little tissue accumulation following prolonged administration (6 months in the rabbit) (Miyamoto et al, 1976).

A direct degradation pathway for fenitrothion involves an initial reduction followed by hydrolytic cleavage to produce O,O-dimethyl phosphorothioate. While this metabolite can be produced directly from fenitrothion in plants, insects and soil, the kinetic data suggests an intermediate metabolite in rats. A process of oxidative desulfuration follows (Kumar et al, 1993).

Following oral administration of fenitrothion to mice and SC administration to guinea pigs and rats, there was quite rapid clearance, with 90% of the administered dose being recovered within 3 days. Mice were able to rapidly metabolise fenitrothion to fenitrooxon, 3-methyl-4-nitrophenol and desmethylated products (Douch et al, 1968).

The metabolism of a variety of dose levels of fenitrothion and fenitrooxon by mouse tissue homogenates was investigated, and the liver was found to be most efficient compared with blood, brain, kidney and small intestine. The soluble fraction of the liver tissue homogenate was the most active of the liver tissue fractions investigated (Kawamura et al**,** 1981, 1982).

The ChE inhibitory action of S-methyl fenitrothion (an isomer of fenitrothion present in TGACs as an impurity) was investigated in *in vitro* studies using horse serum, human serum and fly heads, and was found to be 200-300 times as active as fenitrothion. A mixture with 1% S-methyl fenitrothion was 10 times more active than fenitrothion alone (Kovacicova et al, 1973).

Radiolabelled fenitrothion administered daily to goats in the diet for 7 days was detectable at high levels in the gastrointestinal tract and lower levels in the tissues on day 8, and negligible by day 25. Fenitrothion was excreted both in the urine and in the faeces. Milk levels of fenitrothion were relatively low, and decreased rapidly when fenitrothion was withdrawn from the diet (Mihara et al, 1978).

In an *in vitro* study to ascertain why fenitrothion has reduced mammalian toxicity relative to parathion-methyl, even though they apparently have comparable insecticidal efficacy, both compounds were incubated with mouse liver slices or liver microsomes. After incubation it was found that the degree of bovine erythrocyte or fly head ChE inhibition was increased for parathion-methyl and slightly reduced for fenitrothion relative to the respective parent compounds. Testing for the presence of metabolites arising from metabolism by liver supernatant revealed that the conversion to inactive metabolites was approximately 3-times faster for fenitrothion than for parathion-methyl. Therefore, it was suggested that the lower mammalian toxicity of fenitrothion in comparison to parathion-methyl is due to the increased metabolism of fenitrothion and its metabolites that occurs in the mammalian liver, in comparison to the metabolism of parathion-methyl. Following incubation of with cockroach fat there was an increase in ChE inhibitory potency for both compounds (Vardanis & Crawford, 1964).

The metabolism and excretion of parathion-methyl and fenitrothion were compared in mice, at the same doses and at doses producing similar clinical signs. The urinary metabolite profile of fenitrothion changed depending on the administered dose, with dimethyl phosphate being the predominant metabolite at a low dose (3 mg/kg bw) whereas desmethyl phosphorothioate was predominant at a high toxic dose (850 mg/kg bw). The measurement of excreted radioactivity in urine over 72 h indicated that fenitrothion was more rapidly and completely excreted relative to parathion-methyl (Hollingworth et al, 1967).

The influence of oral malathion administration on fenitrothion concentration in the liver of the rat was investigated. Liver fenitrothion concentration was lower with malathion relative to controls. At 24 h after dosing, there were no significant differences in the levels seen with or without malathion administration (Hladka et al, 1974).

Fenitrothion, prothiophos, profenofos or RH 218 (an unspecified pesticide) were investigated using adult white rats. The *in vivo* effects of sublethal doses of the insecticides were tested, as well as *in vitro* bioassay testing of detoxification. Homogenates of liver and brain tissue from untreated rats were used for *in vitro* assessments. The ChE inhibition seen with fenitrothion was increased from 37% to 60% by incubation with liver tissue. The effect of administration of 1/10 of the LD50 of the insecticide to be tested on blood urea and blood sugar was investigated. Fenitrothion produced a transitory lowering of blood glucose levels, and an increase in blood urea levels, however this was of limited significance, given the small number of animals tested and the variability of results (El-Sebae et al, 1981)

The penetration of fenitrothion and parathion-methyl into brain tissue, and the effects on brain ChE were investigated. It was found that the levels of both compounds in the brain peaked 5 min after administration. Brain ChE inhibition was more rapid and significant following treatment with parathion-methyl than with fenitrothion (Miyamoto, 1964).

Dermal absorption study

The dermal absorption of fenitrothion was examined in ITRC rats by applying a low dose (0.73 g/kg bw), a high dose (3.69 g/kg bw) or repeated doses to shaved skin. After a single application, maximum plasma levels were obtained 8 h after application. Whole blood ChE inhibition correlated with the fenitrothion concentration in plasma. Approximately 55% of the low dose applied remained unabsorbed (in and on the skin) after 24 h exposure. No absorption data for the high dose were supplied. The high dose of fenitrothion produced significant clinical signs, and plasma levels approximately 10 times that observed at 0.73 g/kg bw fenitrothion at 48 h. Multiple applications resulted in increasing plasma levels of fenitrothion and increasing ChE inhibition (Kohli et al, 1971).

Effects on ChE and on hepatic enzymes

Fenitrothion was administered by gavage to young male Wistar rats at 50 mg/kg bw/day for 5 consecutive days. Animals were euthanised 2, 4, 6 or 16 days after the last dose. Moderate signs of toxicity were seen within 1 h of the 3rd dose. Severe signs, including generalised tremors and clonic convulsions were seen after 5 doses. Clinical signs had resolved within 4 days of the final dose. Plasma and erythrocyte ChE levels were inhibited from after the 3rd dose. Plasma ChE levels were normal 6 days after the final dose, while erythrocyte ChE levels returned to normal after 17 days. Additionally, fenitrothion was administered by gavage to young male Wistar rats at 50 mg/kg bw/day for 1, 2 or 3 consecutive days, with animals killed for examination 24 h after the last dose. Significant brain, plasma and erythrocyte ChE inhibition was seen 24 h after a single dose of fenitrothion, with the degree of inhibition increasing following the administration of a second dose. The activity of aniline hydroxylase and *p*-nitroanisole O-demethylase were decreased by 3 doses, as were the liver glycogen levels. The pentobarbitone sleeping time was increased in these animals. Wistar rats were also dosed at 300 mg/kg bw, and killed at hourly intervals up to 8 h after dosing. The levels of fenitrothion in the tissues were determined. Peak levels in liver and plasma were obtained 2 h after dosing. Clinical signs were seen within 30 min of administration, with greatest severity seen at 2 h, and recovering by 3-4 h post administration (Ecobichon & Zelt, 1979).

The effects on liver enzymes following administration of a number of insecticides was investigated. Fenitrothion decreased the mitochondrial respiratory rate, the ATPase activity and cytochrome P-450 content. Aniline hydroxylase activity and aminopyrine N-demethylase activity were also decreased. Diazinon decreased cytochrome P-450 content, aniline hydroxylase activity and aminopyrine N-demethylase activity. Parathion-methyl decreased ATPase activity and cytochrome P-450 content. There therefore appeared to be marginally greater effects on liver systems following fenitrothion treatment than treatment with diazinon or parathion-methyl (Mihara et al, 1981).

Fenitrothion appears to affect cytochrome P-450 enzyme activity of the liver and the testes. In the short term (ie less than 72 h), fenitrothion (240 mg/kg bw) caused a decrease in activity resulting in reduced levels of serum testosterone (25% of normal levels). This returned to normal within 5 days (Clos et al, 1994, Gradowska-Olszewska et al, 1984).

Reflecting the importance of conversion of fenitrothion to the biologically active fenitrooxon, hepatic lesions induced by CCl_4, low protein high fat diets or DDM, resulted in reduced toxicity of fenitrothion as indicated by an increased LD50 and minimum toxic dose. The blood fenitrothion levels peaked later in animals with concurrent liver damage. The *in vitro* metabolism of fenitrothion using liver homogenates of animals pretreated with hepatotoxins was investigated. This indicated that the metabolism of fenitrothion and fenitrooxon was reduced, with the conversion rate of fenitrothion to fenitrooxon being markedly reduced (Miyamoto et al, 1977a, 1977b).

Interaction with other pesticides

Fenitrothion was administered with a range of insecticides known to have anticholinesterase effects (including parathion-ethyl, parathion-methyl, malathion and diazinon) to female Wistar rats. No potentiation with any of the other compounds was seen (Dubois & Kinoshita, 1973).

Effects on hormones

A published study reported the effect of fenitrothion administered orally to male Wistar rats at doses of 0, 7.25 or 14.5 mg/kg bw/day on adrenal weight and plasma corticosterone and glucose concentration. Plasma corticosterone and glucose concentration increased 2.5-fold ($p<0.05$) and 30% (not significant) respectively by week 1 and preceded a significant increase in adrenal weight (35%; $p<0.05$) by week 2 at 14.5 mg/kg bw/day. However, these changes were transient with all returning to control levels at the end of treatment. A similar trend was observed at the lower dose but the changes did not achieve significance (Yamatomo et al, 1982).

Acute Toxicity

A large number of acute toxicity studies have been performed on fenitrothion, its metabolites and some product formulations (end-use products, or EUPs). Fenitrothion technical is of low to moderate oral, intraperitoneal and subcutaneous toxicity, low inhalational and low to moderate dermal toxicity and possibly may be a slight eye and skin irritant. Fenitrothion does not appear to a dermal sensitiser. Clinical signs of intoxication are the same as for other OP insecticides and are due primarily to the presence of the major active metabolite, fenitrooxon. Main signs are salivation, tremor, diarrhoea, lacrimation, exophthalmos, urinary incontinence, piloerection, muscle fasiculations, convulsions and dyspnoea.

The lowest oral LD50 for technical fenitrothion was 235 mg/kg bw in female Holtzman rats (mean of 4 experiments with different batches). In male dd mice the lowest oral LD50 was 775 mg/kg bw (female mice 901mg/kg bw). The lowest intraperitoneal LD50 value was 110 mg/kg bw in female CF mice (males 115 mg/kg bw) and 110 mg/kg bw in male guinea pigs (strain not stated). The lowest subcutaneous LD50 for fenitrothion (in a Tween 80 vehicle) was 840 mg/kg bw in male Sprague-Dawley rats (male dd mice, 1350 mg/kg bw).

The lowest dermal LD50 (intact skin) in rats was 1002 mg/kg bw in females and 890 mg/kg bw in males. In female rabbits, the dermal LD50 was in excess of 2000 mg/kg bw whereas in males it was 3290 mg/kg bw (Hixson, 1982b). The inhalational toxicity of fenitrothion in kerosene:xylene was in excess of the highest dose tested namely, 186 mg/m^3 after 8 h of whole body exposure (Khoda & Kadota, 1979). Even with whole body exposure to 2210 mg/m^3 for 4 h, no median lethal dose was established for either males or females but there was significant plasma, erythrocyte and brain ChE inhibition 24 h after exposure (Kohda et al, 1986). Direct intratracheal administration of fenitrothion yielded an LD50 of 950 mg/kg bw in male SD rats (Chevalier et al, 1982). There was no evidence of any irreversible pulmonary pathology after either whole body inhalational exposure at 2210 mg/m^3 (Kohda et al, 1986) or after intratracheal administration at doses up to 200 mg/kg bw (Chevalier et al, 1982).

Diluted fenitrothion (24%, w/v) was neither an eye nor skin irritant in rabbits but was a mild skin irritant in rabbits with abraded skin (Hixson, 1982c). Undiluted fenitrothion was not a skin sensitiser in guinea pigs (Kohda et al, 1972).

The toxicities of fenitrothion metabolites and the S-methyl isomer (TGAC impurity) are also dependent on the vehicle and species used. In rats the acute toxicity of fenitrooxon, a metabolite of fenitrothion, was greater than the parent compound with the lowest oral LD50 being 3.3 mg/kg bw in rats (strain and sex unknown) which was 100-fold lower than the lowest fenitrothion LD50 (235 mg/kg bw) in the same species.

In male Wistar rats the lowest reported oral LD50 for the S-methyl isomer of fenitrothion was 315 mg/kg bw. In the same study it was shown that although the oral LD50 for pure fenitrothion was 700 mg/kg bw, the presence of the isomer in a 96% pure TGAC reduced the LD50 to 490 mg/kg bw (Rosival et al, 1976). The lowest oral LD50 values for bis-fenitrothion (manufacturing by-product) and 3-methyl-4-nitrophenol (a metabolite excreted in urine) were 612 mg/kg bw (male ICR mice) and 250 mg/kg bw (dd mice) respectively. Comparing the acute oral LD50 of fenitrothion (range 775-1416 mg/kg bw) and the oral LD50 for 3-methyl-4-nitrophenol in mice (250 mg/kg bw, with males only) with that in rats (fenitrothion LD50 in 9 studies, ranged from 235 mg/kg bw to 1050 mg/kg bw with a tenth study reporting a value of up to 3344 mg/kg bw; for 3-methyl-4-nitrophenol the range was between 1200-2300 mg/kg bw) indicates that whereas the 3-methyl-4-nitrophenol metabolite is orally lethal at doses approximately 6-fold higher than fenitrothion in rats, it is 4-fold *lower* in mice. This result suggests that the metabolism in mice is different from rats. Similarly for the S-methyl isomer, rats have approximately equal oral sensitivity to both parent and isomer (S-isomer, 315-540 mg/kg bw) whereas mice are approximately 3-fold less sensitive (S-isomer, 420-550 mg/kg bw) to fenitrothion.

To assess changes in fenitrothion lethality after sunlight-induced degradation, a 50% (v/v) EUP (designated MEP emulsion) at 3 different pH levels was exposed to sunlight until the amount of unchanged fenitrothion was reduced to 10%. The LD50 of the degraded MEP administered IP to ICR mice ranged from 60 to 120 mg/kg bw depending on pH (dose calculated relative to fenitrothion pre-exposure concentration); the greatest increase in toxicity was associated with degradation that occurred at pH 14. However, all degradation solutions were substantially more toxic than the non-sunlight irradiated solution (which had an LD50 of 410 mg/kg bw, irrespective of gender) (Hiraoka et al, 1989).

Expressed as concentration of the active ingredient the lowest oral LD50 for a 50% emulsifiable concentrate of fenitrothion was 508 mg/kg bw in female Wistar rats (males 517 mg/kg bw). The lowest oral LD50 in ICR mice was 410 mg/kg bw in males and females.

A dermal LD50 for a 50% emulsifiable concentrate of fenitrothion was found to be in excess of 2500 mg/kg bw (calculated as active ingredient). Inhalational lethality (nose only) of a 50% EUP was assessed at two exposure durations. For a 1-h exposure the LC50 in male and female rats was greater than 1103 mg/m^3, and with a 4-h exposure it was found to be 1261 mg/m^3 in males and in excess of 1261 mg/m^3 for females (Flucke & Thyssen, 1980).

A 50% emulsifiable concentrate formulation of fenitrothion (E5560) was a moderate eye irritant and a strong skin irritant, whereas a 0.2% dilution was found to be a non-irritant to both eyes and skin.

Atropine, 2-PAM, scopolamine, and biperiden administered IP (following IP or PO administration of fenitrothion) either individually or in various combinations (atropine and 2-PAM, scopolamine and 2-PAM or biperiden and 2-PAM) were able to ameliorate clinical signs and increase survival in rats and mice (Valecha, et al, 1990, Kimmerle, 1962, Kadota et al, 1974, Matsubara & Horikshi, 1983 & 1884); and also when administered by the IV and intramedullary routes in rats (Uehara et al, 1993). Additionally, some improvements in plasma, erythrocyte and brain ChE activity were observed following 2-PAM IP or IV in rats and rabbits (Matsubara & Horikoshi, 1983).

Short-Term Repeat-Dose Studies

In a published study to investigate whether animals treated with sublethal doses of fenitrothion could develop tolerance to the compound, technical fenitrothion was administered to male Wistar rats PO at doses of 0, 7.25, 14.5 or 29 mg/kg bw/day for 28 consecutive days. Additionally, in a second experiment the effects of pretreatment with fenitrothion PO at doses of 0, 7.25 or 14.5 mg/kg bw/day for 5 days, before, PO administration of fenitrothion at 58 mg/kg bw/day for 10 days was investigated. Clinical signs of tremors and ataxia and reduction in body weights were observed during treatment with both dosing regimens, however, gradual recovery occurred suggesting that rats could exhibit tolerance to repeated daily doses of fenitrothion. However, the limited numbers of rats used in each treatment group meant that this conclusion could not be reached with any certainty (Yamatomo et al, 1983).

In a published study fenitrothion was administered PO at 0, 2.5, 5, 10 or 20 mg/kg bw/day for 30 days to male CD rats. At 20 mg/kg bw/day 8/36 rats died during the first week of treatment. Clinical signs of toxicity preceding death were salivation, piloerection, diarrhoea, chromodacryorrhoea, excitability, ataxia, muscle fasciculations, generalised tremors and convulsions. During treatment at 20 mg/kg bw a significant reduction in body weight was observed. Significant reductions in plasma, erythrocyte and brain ChE activities, and liver and renal carboxylesterases were observed in rats dosed from 5-20 mg/kg bw/day, however, these levels returned to comparable control levels between 8-30 days post termination of treatment. The NOEL for the study was 2.5 mg/kg bw/day, based on reductions in plasma and erythrocyte ChE in male rats at the next higher dose (Trottier et al, 1980).

In a published study to assess short-term toxicity, fenitrothion technical in ethanol:propylene or as a formulation (with 1.5% (v/v) Atlox 3409F, 1.5% (v/v) Dowanol TPM and the same vehicle) were administered PO separately at 0, 1, 5, 10 or 20 mg/kg bw/day for 4 weeks to male and female Sprague-Dawley rats. No deaths were observed in any of the treatment groups. Clinical signs were only apparent in rats dosed at 20 mg/kg bw/day (both preparations) and consisted of piloerection, salivation, hyperexcitability, and chromodacryorrhoea for both sexes with females additionally having muscle fasciculations. Slight non-significant reductions in body weight were observed during the treatment period in males and females, but recovery to control levels in males occurred by 8 weeks after treatment. Reductions in erythrocyte and brain

ChE and liver and renal carboxylesterase activities were observed at doses ranging from 5-10 mg/kg bw/day in males and 5-20 mg/kg bw/day in females (although there did not appear to be a dose-related trend). Overall, there was little difference in toxicity between the technical and formulated product. The NOEL for the study was 1 mg/kg bw/day based on reductions in erythrocyte ChE in male and female rats at the next higher dose (Durham et al, 1982).

Using 3 different dosing regimens a fenitrothion aerosol was administered by nose-only exposure to Sprague-Dawley rats or ICR mice at; 0, 15 or 62 mg/m^3/day (2 h/day, 6 days/week for 4 weeks; protocol 1); 0, 7, 15 or 62 mg/m^3/day (2 h/day for 5 days; protocol 2) and 0, 2 or 7 mg/m^3/day (2 h/day, 5 days/week for 4 weeks; protocol 3). No deaths occurred for any study but rats at 62 mg/m^3 had salivation and urinary incontinence. Non-significant reductions in body weight were observed at all doses in mice, and at 62 mg/m^3/day in rats. Although there were some significant changes in some haematological parameters, ie WBC, Hb, Hct and thrombocyte counts in mice they are unlikely to have any biological significance as there was no underlying histopathology or any apparent dose relationship. Similarly, the same conclusion applied for the clinical chemistry changes observed in A/G ratio, ALT and AST concentrations.

Significant reductions in plasma, erythrocyte and brain ChE levels were observed at 62 mg/m^3/day in protocol 1 in male and female rats, with recovery of plasma ChE only (brain and erythrocyte levels remained significantly reduced) by day 14 after the final exposure. At 15 mg/m^3/day plasma ChE was significantly reduced in males and females and erythrocyte and brain ChE in females only, however, activities were comparable to control levels by day 7 after the final exposure (day 14 for brain ChE). In protocol 2, rats had no significant reductions in any of the ChEs at 7 mg/m^3, however, significant reductions in plasma ChE occurred at 15 mg/m^3/day, and in plasma, erythrocyte and brain ChE at 62 mg/m^3/day. With protocol 3, no significant reduction in any of the ChE's were observed in rats at 2 mg/m^3/day, whereas, at 7 mg/m^3/day in rats significant reductions were observed in plasma ChE (females only). In mice (protocol 1) significant reductions in plasma and brain ChE levels were observed at 62 mg/m^3 in male and females, with recovery of plasma ChE only by day 14 after the final exposure. At 15 mg/m^3, plasma ChE was significantly reduced (males only) and brain ChE (females only), however, activities were relatively comparable to control levels by day 14 after the final exposure (slight persistence of reduced brain ChE). In protocol 3, no significant changes was observed in plasma, erythrocyte or brain ChE at a dose of 2 or 7 mg/m^3/day in male or female mice. The NOEL for the overall study was 2 mg/m^3/day, based on reductions in plasma ChE in female rats at the next highest dose (Kohda & Kadota, 1979b).

In a published study to assess short-term nose-only inhalational toxicity, fenitrothion formulation aerosols were administered at 0, 5.2, 16.5 or 57 µg/L/day (0, 5.2, 16.5 or 57 mg/m^3/day) to Sprague-Dawley rats for 2 h/day for 4 weeks. No treatment-related deaths were observed. At the highest dose muscular fasciculations, convulsions, hunched posture, extension of limbs, uncoordinated movements and a clear secretion from the eyes were observed in most rats. No changes in body weight, haematological, biochemical or urinalysis parameters were observed but plasma ChE levels were significantly reduced at 5.2, 16.5 and 57 µg/L/day (up to 90%). Erythrocyte ChE levels were significantly reduced (up to 65%) only at the highest dose of 57 µg/L/day whereas significant ChE activity reductions (up to 65%) were achieved in the brain at all doses tested. Recovery of these enzyme activities was complete and

by day 60 was comparable with control levels. The NOEL for this study could not be established because significant inhibition of plasma and brain ChE activity was observed at the lowest dose tested, namely 5.2 µg/L/day or 5.2 mg/m^3/day (measured as fenitrothion concentration) (Breckenridge et al, 1982).

An undated report by Miyamoto describes the effects on Sprague-Dawley rats after 5 days of whole body exposure (2 h/day) to a vapour of fenitrothion (7, 15 or 62 mg/m^3/day) in a mixture of 85% kerosene and 15% xylene. A second series of experiments involved rats and mice (strain not reported) being exposed to 15 or 62 mg/m^3/day for 6 days/week for 4 weeks. Although clinical signs were not reported, rats had significantly suppressed plasma ChE levels (>20%). Plasma ChE activity was reduced on day 1 at 15 mg/m^3/day and on day 3 at 62 mg/m^3/day in the 5-day study. In the 4-week study, activity was reduced (>20%) at both doses tested during the first 2 weeks of exposure. For erythrocyte and brain ChE activity a significant reduction (>20%) was observed on days 3 and 5 at the highest dose in the 5-day study whereas in the 4-week study only males at 62 mg/m^3/day and females at 15 and 62 mg/m^3/day had significant inhibition. In mice, both sexes had reduced plasma and brain ChE activity at 62 mg/m^3/day but only males had reduced plasma ChE activity at 15 mg/m^3/day. Rats did not lose body weight after a 5-day exposure but male mice lost around 10% during their 4-week exposure. Rats at 62 mg/m^3/day had a significant reduction in the concentration of AST and ALT which was associated with an increased liver weight that under histopathological examination was due to a fatty change in parenchymal cells. Thus, based on the effects on plasma ChE seen at 15 mg/m^3/day, the NOEL for the 5-day study was 7 mg/m^3/day. However, because of effects seen at the lowest dose, no NOEL could be established in either mice or rats in the 4-week study.

A published study compared changes in rat and pigeon myelin composition following fenitrothion treatment. Fenitrothion administered PO at doses of 0, 5 (in pigeons) or 50 mg/kg bw/day (in rats) for 1, 3 or 5 days gave rise to significant changes in the constituents of the myelin membrane (decreases in total protein, cerebroside, sulphatide, Wolfgram and proteolipid protein; and increases in cholesterol and myelin basic protein) in pigeons, but rats appeared relatively unaffected (Nag & Ghosh, 1985).

In a GLP study, fenitrothion technical was applied to a shaved area between the shoulder and rump of New Zealand White rabbits at 0, 3, 10, 50 or 250 mg/kg bw/day for 3 weeks. At 250 mg/kg bw/day all males (5/5) and 2/5 females either died or were sacrificed moribund during the treatment period. Prior to death, rabbits had hypoactivity, muscular hypotonia, tremor, bradypnea, hypothermia, salivation, clonic convulsions, loose or mucous stools, diarrhoea and a soiled periproctal region. Survivors at this dose had significant body weight loss. No treatment-related haematological or biochemical changes were observed at any dose. Significant reductions in erythrocyte ChE was observed in males and females at 10, 50 or 250 mg/kg bw/day, and in plasma and brain ChE in males at 50 mg/kg bw/day and females at 10, 50 or 250 mg/kg bw/day. Dermal irritation was observed and scored and consisted of very slight to well defined erythema and very slight to slight oedema at 3, 10 or 50 mg/kg bw/day. Dermal reactions were more severe at 250 mg/kg bw/day. Histopathological examination of the skin in the treated groups found thickening of the epidermis, hyperkeratosis and inflammatory infiltrates in the corium in males and females at all doses; and, haemorrhage in the corium at 10, 50 or 250 mg/kg bw/day. At 250 mg/kg bw/day, 1 rabbit/sex had slight necrosis in the liver and

some showed changes in the digestive tract and kidneys that were considered to be the cause of deterioration in general condition. The NOEL for this study was 3 mg/kg bw/day, based on the inhibition of serum and brain ChE activity at higher doses (Suetake et al, 1991).

Subchronic Toxicity

Fenitrothion was administered by IP injection at 10, 25, 50 or 100 mg/kg bw/day to female Sprague-Dawley rats for 60 days. All rats receiving 25, 50 or 100 mg/kg bw/day died within the first 20 days. All rats receiving 10 mg/kg bw/day survived. Body weight was decreased in comparison to controls. Brain and plasma ChE was decreased to less than 50% of control values from 3 days after the first dose, up to day 25 (Dubois & Puchala, 1960b)

Sprague-Dawley rats were fed fenitrothion in the feed at concentrations of 0, 6, 20, 60 or 200 ppm for 13 weeks. Food consumption was significantly ($p<0.01$) lower in both males (7%) and females (14%) during the first week of treatment at 200 ppm but was then significantly increased during weeks 5, 6, 7, 8 and 13 (average 12%) in females only. Bodyweights were significantly reduced ($p<0.01$-$p<0.05$) in males (4% average) and females (13% average) for the first 3 weeks of treatment at 200 ppm and this continued in females until week 8. No treatment-related changes in either a quantitative or qualitative functional observation battery (FOB) were observed. Similarly, no treatment-related neuropathology was observed in six levels of the brain, namely forebrain (through septum), cerebrum (through hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and 3 levels of the spinal cord (cervical, thoracic and lumbar) under light microscopy or in the sciatic nerve (at mid-thigh region; cross-section, and sciatic notch; longitudinal and cross-section), sural nerve (at knee; cross-section) and tibial nerve (at knee; longitudinal and cross section) under electron microscopy. Additionally, various levels of the spinal cord (left Gasserian ganglion; cross-section, lumbar dorsal root ganglion (L4); cross-section, lumbar dorsal root (L4); cross-section, lumbar ventral root (L4); longitudinal and cross-section, cervical dorsal root ganglion (C5); cross-section, cervical dorsal root (C5); cross-section, cervical ventral root (C5); cross-section) examined by electron microscopy did not reveal any treatment-attributable effects.

Glial fibrillary acidic protein (GFAP) concentrations in 6 regions of the brain, namely cerebellum, cerebral cortex, hippocampus, striatum, thalamus/hypothalamus and the rest, were similar among all treatment groups. After 13 weeks of treatment, significant inhibition of erythrocyte and brain ChE was observed at 60 and 200 ppm whereas in plasma the inhibition was 52% in females at the lowest concentration tested (6 ppm). The degree of plasma ChE inhibition measured after 4 and 8 weeks of treatment at 6 ppm together with a clear dose-related inhibition at each sampling time confirmed the biological significance of the 13-week observation. Thus, no NOEL for plasma ChE inhibition could be established in this study, given the substantial inhibition observed in females at the lowest dose tested (equal to 0.46 mg/kg bw/day) (Beyrouty et al, 1993).

Fenitrothion was administered in the diet to male Wistar rats at 0, 32, 63, 125, 250 or 500 ppm for 90 days. In addition, 2 groups of rats were maintained on 0 or 500 ppm fenitrothion in the diet, and were euthanised when clinical signs were maximal (11 days after commencement of dosing). Clinical signs were seen at 250 and 500 ppm, mainly involving muscle fasciculations

and lacrimation. There was a decrease in body weight and food intake for rats on 500 ppm. Plasma ChE inhibition was seen at 125 ppm, while erythrocyte ChE inhibition was seen at 63 ppm. Brain ChE inhibition was only seen at 500 ppm. The NOEL, based on effects on erythrocyte ChE inhibition, was therefore 32 ppm in the diet (equivalent to 3.2 mg/kg bw/day) (Misu et al, 1966)

Fenitrothion (150 ppm), fenitrooxon (50 ppm) and 3-methyl-4-nitrophenyl (1500 ppm) was administered in the diet to Wistar rats for 90 days. At the end of this period, rats were maintained on a control diet for 3 days prior to euthanasia. There were slight changes in body weight seen with fenitrothion at 150 ppm, and fenitrooxon at 50 ppm. Liver weight was not significantly changed by treatment. There were no significant effects on the liver enzymes systems following treatment with any of these compounds (Hosokawa & Miyamoto, 1975).

Fenitrothion (10, 30, or 150 ppm), fenitrooxon (5, 15, or 50 ppm) and 3-methyl-4-nitrophenyl (150, 500 or 1500 ppm) were administered in the diet to Wistar rats for 6 months. There were no clinical signs of toxicity. There were no differences between treated groups for final body weight, or any treatment related change in food or water consumption. Haematology, clinical chemistry and urinalysis did not reveal any treatment related changes. Plasma ChE inhibition was seen in females on fenitrothion at 10 ppm, and in males at 150 ppm. Erythrocyte and brain ChE inhibition was seen in females on fenitrothion at 30 ppm, and in males on 150 ppm. No NOEL could be established for the study, given the signs seen in plasma ChE at 10 ppm, the lowest dose tested (equal to 0.6 mg/kg/day) (Kadota et al, 1975)

Fenitrothion was administered at 0, 10, 30 and 150 ppm in the diet to Wistar rats for 6 months. No treatment related clinical signs or deaths were observed, and there was no difference in body weights between groups. Plasma, erythrocyte and brain ChE inhibition was seen at 30 ppm in females and 150 ppm in males. No histopathological changes were seen. Thus, the NOEL can be set at 10 ppm (equal to 0.6 mg/kg bw/day) based on ChE inhibition at 30 ppm (Sumitomo Chem Co, 1980).

Fenitrothion was administered by inhalation in a whole body exposure to Crl(W1)BR rats for 90 days at 0.2, 1 or 10 µg/L/day (mg/m^3/day). There were no treatment related deaths during the study, and no changes in body weight or food consumption. Haematology and clinical chemistry did not reveal any notable findings. Plasma ChE inhibition was seen at all tested doses in females and at the highest dose in males. Erythrocyte ChE inhibition was seen at the highest dose in both males and females, while brain ChE inhibition was seen in females at all doses tested. There were no significant findings on macroscopic or histopathological examination. No NOEL could be established for the study, given the effects on both brain and plasma ChE seen at the lowest dose tested in females (0.2 µg/L/day or 0.2 mg/m^3/day) (Coombs et at, 1988).

Fenitrothion was administered orally to Holstein cattle at 3 mg/kg bw/day for 90 days. No abnormal clinical signs were seen. Plasma ChE activity was decreased at day 1, but had returned to normal at 30 days. Fenitrothion was also administered to 2 sheep at 3 mg/kg bw/day for 60 days. Plasma ChE activity was decreased on day 7 of treatment, however had returned to normal levels by day 30. Fenitrothion was administered at 31 mg/kg bw to a male pig. The

animal had diarrhoea for 20 h, while at 30 h after dosing it showed paralysis in the hind legs, convulsions and ataxia. At 48 h after dosing, these signs had resolved (Namba et al, 1966).

Chronic Toxicity

Fenitrothion was administered at 0, 30, 100 or 200 ppm in the diet to ICR Swiss mice for 78 weeks. There were no significant changes detected in mortality, clinical signs, body weight or food consumption during the study. Ophthalmological examinations were normal for all animals. No treatment-related abnormalities were detected on gross postmortem or histopathological examination. No clinical chemistry, haematology or urinalysis examinations performed. ChE levels were not determined. The NOEL, based on the absence of any abnormalities was 200 ppm, equal to 21 mg/kg bw/day (Kudzins, 1975)

Feeding fenitrothion in the diet at concentrations of 0, 3, 10, 100 or 1000 ppm to B6C3F1 mice for 2 years resulted in significant bodyweight loss (up to 19%) with attendant reductions in food and water consumption among males and females at 1000 ppm. Females at 1000 ppm had a markedly lower incidence of alopecia that appeared to correlate with reduced hair follicle atrophy (ie. 1/43 and 17/36 in controls). There were no consistent treatment-related changes in any of the haematology or urinalysis parameters measured. However, clinical chemistry revealed that plasma cholesterol concentrations were significantly elevated ($p<0.01$-0.05) at 100 ppm and 1000 ppm whereas blood glucose was significantly reduced at 1000 ppm only in both sexes. Similarly, ChE activities in plasma, erythrocyte and brain were significantly inhibited ($p<0.01$) for males and females of the 100 and 1000 ppm groups. No change in survival rate or in the background incidence of any neoplasms was observed. Both absolute and body relative weight of the brain were significantly increased ($p<0.01$), a finding that correlated with a significant reduction ($p<0.01$) in the expected calcification of the brain parenchyma in 2-year old mice. Thus, there was no evidence of carcinogenicity and the NOEL for this study can be set at 10 ppm (equal to 1.44 mg/kg bw/day in males and 1.51 mg/kg bw/day in females) based on significant inhibition of ChE and an elevated plasma cholesterol concentration in both sexes at the next higher dose of 100 ppm (Tamano et al, 1990).

In a published study, fenitrothion was administered by daily gavage to male Wistar rats at doses of 0, 0.5, 1, 5 or 10 mg/kg bw/day for 12 months. Body weight was significantly lower in rats on 10 mg/kg bw/day from week 20. These animals also showed clinical signs including hyperexcitability, piloerection, exophthalmos, fasciculations and chromodacryorrhea. In the post-treatment recovery phase, the body weight of high dose animals increased, and at 58 weeks there were no differences in body weight between groups. A reflex activity test (dropping a rat from a height of approximately 23 cm onto a padded surface) did not reveal any changes. Haematology and clinical chemistry examination did not reveal any treatment related changes. Plasma ChE activity was consistently and significantly decreased at 5 mg/kg bw/day. Erythrocyte ChE was significantly decreased by fenitrothion at 5 mg/kg bw/day. Brain ChE was also significantly decreased by the 2 highest doses. The NOEL was therefore 1 mg/kg bw/day, based on effects on ChE activity seen at 5 mg/kg bw/day. Although there were 60 rats/group and all underwent necropsy at their scheduled kill, only 2/group were used for histopathological examination that involved both light and electron microscopy. Therefore, although there were no significant treatment-related findings found on histopathological examination that included

examination of the distal sciatic nerve, the results from so few rats are insufficient to exclude neuropathy (Ecobichon, 1980).

Wistar rats were fed fenitrothion in the diet at 0, 2.5, 5 or 10 ppm for 92 weeks. There were no clinical signs of toxicity during the study. Mortalities were not increased with treatment, and there was no treatment related change in body weight or food consumption. There was no assessment of haematology, clinical chemistry or urinalysis. However, ChE inhibition was investigated. The only significant inhibition was of plasma ChE in females at 10 ppm. The NOEL for the study was 5 ppm, equal to 0.3 mg/kg bw/day, based on the effects on plasma ChE in females at 10 ppm (Kadota et al, 1980).

Fenitrothion was administered to Sprague-Dawley rats at doses of 0, 10, 30 or 100 ppm in the diet for 104 weeks. Although the rats in this study were derived from the F1a group of a 3-generation reproduction study (Rutter Jr, 1973) and randomly distributed among treatment groups, the high-dose group had a mean body weight 20% lower than controls at the commencement of the study. This difference in body weight was maintained throughout the study. No significant differences between treated and control animals were found for haematological, clinical chemistry or urinalysis examinations. Inhibition of ChE levels in plasma, erythrocytes and brain was determined, with significant inhibition of plasma ChE found in females at 10 ppm (the lowest dose tested) and males at 30 ppm. Erythrocyte ChE levels were significantly inhibited in both males and females at 30 ppm, while brain ChE was significantly inhibited in males at 30 ppm and females at 100 ppm. There were no significant treatment related abnormalities on either gross or histological post mortem examination although the extent of nerve examination was limited to an unspecified “nerve with muscle” preparation. Overall, no NOEL could be established for the study, based on the effects seen on plasma ChE in females at the lowest dose tested (Kudzins, 1974).

Purebred Beagle dogs received fenitrothion in the diet at 0, 5, 10 or 50 ppm for 12 months. No deaths occurred during the study. There were no treatment related clinical signs or changes in body weight or food consumption. There were no changes of biological significance in haematology, clinical chemistry or urinalysis examination. Plasma ChE inhibition was seen in males at 50 ppm and females at 10 ppm. Erythrocyte ChE inhibition was seen in males at 50 ppm, however no inhibition was seen in females. No inhibition of brain ChE was seen in this study. No abnormalities were detected on post mortem examination, although no histopathological examination of tissues was done. Overall, based on the plasma ChE inhibition seen in females at 10 ppm, the NOEL for the study was 5 ppm in the diet (equal to 0.2 mg/kg bw/day) (Griggs et al, 1984).

Fenitrothion was administered by gavage at 0, 0.1, 0.5 or 2 mg/kg bw/day to feral Cynomolgus monkeys for 2 years. No treatment-related deaths or clinical signs were seen during the course of the study. Body weight changes were seen in females at the highest dose tested. No significant changes were seen on haematological, clinical chemistry or urinalysis examinations. There was significant inhibition of plasma and erythrocyte ChE in both males and females at 2 mg/kg bw/day. Brain ChE was also significantly inhibited in females at this dose. There were no abnormal ophthalmological findings in this study. EMG examination showed slight abnormalities in the highest dose group, while ECG examination revealed no abnormalities.

There were no abnormal post mortem findings, either on gross or histopathological examination. Overall, the NOEL for the study was 0.5 mg/kg bw/day, based on the EMG changes and the ChE inhibition (Sumitomo Chem Co, 1978).

Reproductive Toxicity

Fenitrothion in the feed at concentrations up to 120 ppm did not cause any impairment in the reproductive performance of Sprague-Dawley rats in a 2-generation study. The NOEL for parental toxicity was 10 ppm [between 0.65 (male) to 0.74 (female) mg/kg bw/day] based on a dose-related reduction in food consumption, body weight gain and body weight of both sexes at higher doses (40 or 120 ppm). The NOEL for reproductive toxicity was 40 ppm (mean, 3.07 mg/kg bw/day for females), on the basis of reduced pup weight, viability and lactation indices at the next higher dose of 120 ppm (Hoberman, 1990).

Fenitrothion in the feed at concentrations up to 150 ppm did not cause any impairment in the reproductive performance of Sprague-Dawley rats in a 3-generation study. The NOEL for parental toxicity was 100 ppm [between 5 (male) to 10 (female) mg/kg bw/day], on the basis of reduced body weight at the next higher dose of 150 ppm. The NOEL for reproductive toxicity was 30 ppm (3.0 mg/kg bw/day for females) based on poor pup survival and an inability of pups to gain weight during lactation at higher doses. This outcome suggests fenitrothion is present in milk (Rutter Jr, 1973).

Developmental Toxicity

In a series of studies, fenitrothion administered PO to mice, rats or rabbits revealed no evidence of teratogenicity.

Fenitrothion administered by gavage at 0, 20, 70 or 200 mg/kg bw/day to ICR-JCL mice from days 7-12 of gestation caused no observed maternotoxicity or embryo/fetotoxicity at the highest dose tested. The same investigators also observed an absence of any embryo/fetotoxicity in pregnant Sprague-Dawley rats given fenitrothion by gavage during fetal organogenesis (days 9-14 of gestation) at doses of 0, 2, 7 or 20 mg/kg bw/day. However, the maternal NOEL was 7 mg/kg/bw/day based on body weight loss at 20 mg/kg bw/day (Miyamoto et al, 1975).

A published report investigated the teratogenic effects of a 50% EUP emulsion and its sunlight-induced degradation products in ICR mice (5/group). Doses equivalent to 20, 40 or 90 mg/kg bw/day were administered SC on days 3-15 after detection of a vaginal plug. There were no clinical signs reported or differences in pregnancy rate, resorption rate, live or dead fetuses per litter, and skeletal malformations observed. Fetal body weight was comparable among groups except for a significantly lower weight in the pH 8 degraded product group (but this result may be influenced by the small number of live fetuses arising from 3/4 live dams). Owing to the small number of animals in the treatment groups this study is inadequate for regulatory purposes (Hiraoka et al, 1989).

In a GLP study, fenitrothion (0, 3, 8 or 25 mg/kg bw/day) administered to female Sprague-Dawley rats by gavage on days 6-15 of pregnancy showed evidence of maternotoxicity with

body weight loss and clinical signs (tremors) at 25 mg/kg bw/day. Although reproductive parameters (pregnancy rate, number of corpora lutea, implantation scar number, resorption, live and viable fetuses) and fetus weight and size were unaffected, there was a significant increase in the incidence of skeletal variants. This included a significant increase in the incidence of litters (20%; p 0.05) and fetuses (3%; p 0.05) with 1 full and 1 rudimentary 13th rib at 25 mg/kg bw/day; and an increased non-significant litter (14%, 10%) and fetal incidence (2.5%, 2%) apparent at 3 and 8 mg/kg bw/day respectively (control, 0%). An increased litter incidence of 7th cervical rib observed at all doses (14%, 10%, 20%; control 4.2%) was not significant. The unexpected absence of any 13th rib (1 full and 1 rudimentary) in the control group contributed to the significance achieved at the highest dose tested but the attendant maternotoxicity at this dose suggests biological significance for skeletal variants. Hence, the NOEL for maternotoxicity appears to be 8 mg/kg bw/day and the NOEL for embryo/fetotoxicity can be set at the same dose (Morseth, 1987).

In a teratology study using thalidomide as a positive control, fenitrothion (0, 0.3 or 1 mg/kg bw/day) was administered in gelatin capsules PO to presumed pregnant New Zealand White rabbits on gestation days 6-18. Fertility indices (corpora lutea, implantation and resorption number), fetal body weight, viability and number of gross abnormalities were similar among treatment groups. The incidence of incompletely ossified sternum was similar among treatment groups other than at 1 mg/kg bw/day (32%; control 14.6%), the incidence of non-ossified sternum appears to be significantly elevated (though significance was not calculated by the investigators) in a dose-related trend, ie 2.7%, 39%, 12.7% and 26% for control, positive control, 0.3 and 1 mg/kg bw/day fenitrothion respectively. Thus, a NOEL for embryo/fetotoxic effects cannot be set because of a dose-related incidence of non-ossified sternums (Ladd et al, 1971).

In a GLP study, fenitrothion (0, 3, 10 or 30 mg/kg bw/day) was administered to New Zealand White rabbits by gavage on days 7-19, after artificial insemination. At 30 mg/kg bw/day, 6/16 died before day 29; all does displayed clinical signs of toxicity (reduced motor activity, ataxia, salivation, dyspnoea, tremors and lost body weight) while 3 delivered prematurely (days 22-29). No embryo/fetotoxicity was evident. Hence, the NOEL for maternotoxicity can be set at 10 mg/kg bw/day whereas the embryo/fetotoxicity NOEL is the highest dose tested, 30 mg/kg bw/day (Morseth, 1986).

Genotoxicity

The mutagenicity of fenitrothion was examined using a variety of tests including *in vitro* and *in vivo* gene mutation, DNA damage and repair, and chromosomal aberration assays. Most of the 30 studies cited were negative indicating that fenitrothion is not a mutagen. Some studies gave weak positive results [ie Ames test with *S. typhimurium* TA100 (Kawachi, 1978; Moriya et al, 1983; Hara et al, 1989), sister chromatid exchange (SCE) assay (Kawachi, 1978) and chromosomal aberration test (Kawachi, 1978)] but a recent study showed that fenitrothion is negative in gene mutation assays with *S. typhimurium* TA100 NR, a nitroreductase-deficient strain of TA100, and in mammalian cells, V79 Chinese hamster lung cells. Mutagenicity in *S. typhimurium* TA100 was considered attributable to the nitroreductase inherent to bacteria (Hara et al, 1989; as cited in IPCS, 1992). Since this type of nitroreductase is absent in mammalian systems the relevance of this observation for humans is neglegible. Other SCE assays using ICR mouse embryo cells were negative (Suzuki & Miyamoto, 1980).

A report not reviewed but cited in IPCS (1992), indicates that 3-methyl-4-nitrophenol (a major metabolite and degradation product of fenitrothion) administered IP to CFLP mice (25 mg/kg bw) once weekly for 10 weeks, significantly increased the numbers of chromosome gaps in bone marrow cells (Nehez et al, 1985).

Neurotoxicity

The effects on the behavioural development of weanlings after *in utero* exposure to fenitrothion during organogenesis in Lati CFY rats were described in a short communication report. Fenitrothion (50% EC) was administered at 0, 5, 10 or 15 mg/kg bw/day on days 7-15 of pregnancy. Dose-related effects were seen in behavioural development (reflexes, motor coordination in the rotor-rod test and open-field activity) and significance ($p<0.05$) was achieved for weanlings in the high-dose group although a significant delayed response to conditioned stimuli was also observed at mid dose. A no-effect dose level was 5 mg/kg bw/day (Lehotzky et al, 1989).

Fenitrothion was administered by gavage to male rats at 0, 12.5, 50 or 200 mg/kg bw, while females were treated at 0, 50, 200 or 800 mg/kg bw. Males at 200 mg/kg bw had significantly reduced bodyweight at day 7 and this was maintained till the end of the study on day 15. No significant differences in female bodyweights were observed. Clinical signs for males at 50 and 200 mg/kg bw were tremors of the head, body and limbs, gait changes, pupil constriction and salivation (with some having a red discolouration). For females, all at 800 mg/kg bw and some at 50 mg/kg bw had tremors. Gait changes were observed in all female treatment groups with an apparent dose relationship. At 800 mg/kg bw, 4/12 were immobile and 1/12 had severe, 6/12 had moderate and 1/12 had slight ataxic gait. At 200 mg/kg bw, 1/12 had severe, 8/12 had moderate and 2/12 had slight ataxic gait. At 50 mg/kg bw, 1/12 had moderate and 7/12 had slight ataxic gait.

Significant ($p<0.05$-0.001) qualitative differences in functional observation battery (FOB) tests between treatment and control groups occurred in both sexes only on the day of dosing (ie. 1-1.25 h and 0.75-1 h post dosing for males and females respectively). Males at 50 and 200 mg/kg

bw had reduced arousal and rearing. A quantitative assessment of leg extensor thrust revealed reduced strength in most males at 200 mg/kg bw and some at 50 mg/kg bw. Other significant changes at 50 and 200 mg/kg bw were decreased pinna reflex, reduced or absent tail and toe pinch test response, impaired visual placing test response, delayed postural passivity and increased failure at the air righting reflex test. In females, significant reductions in arousal, rearing and locomotor activity were observed at all doses whereas tail and toe pinch response and visual placing test were reduced only at 200 and 800 mg/kg bw. Other significant changes were pupil constriction, salivation, air righting reflex failure, and increased positional passivity. Pinna reflex response at 800 mg/kg bw was also reduced or absent. Significant reductions in grip strength (forelimb at 200 mg/kg bw and fore- and hindlimb at 800 mg/kg bw) were recorded at 200 and 800 mg/kg bw and motor activity and body temperature were significantly reduced at all doses.

No gross pathological or neuropathology findings were observed. Immuno-detection of glial fibrillary acidic protein (mainly associated with astrocytes) from 6 regions of the brain revealed 3/6 females at 800 mg/kg bw had significantly increased concentrations. However, given that chemical-induced injury often results in proliferation and hypertrophy of astrocytes, the biological significance of this finding in the absence of any histopathological changes is unclear.

Thus, no NOEL could be established because of reduced motor activity, body temperature and gait changes at 50 (lowest dose tested in females), 200 and 800 mg/kg bw in females. For males, the NOEL can be set at 12.5 mg/kg bw based on marked changes in FOB testing at 50 and 200 mg/kg bw (Beyrouty et al, 1992).

In a study designed to assess the potential of PO administered fenitrothion (500 mg/kg bw) to induce delayed neurotoxicity after a single dose, hens exhibited ataxia, balance loss, spontaneous motor activity loss, leg weakness and an irregular respiration rate but no leg paralysis or histopathological evidence of sciatic nerve degeneration or demyelination (Kadota et al, 1974, 1975, 1976).

Male rats took longer (up to 46%) to respond to a conditioned avoidance reflex (jumping onto a stick in response to an electrical stimulation) during the first 14 days of a 30-day PO administration of fenitrothion (0, 10 or 100 mg/kg bw/day) (No results reported for days 15-30). Rabbits that were dosed with 10 mg/kg bw/day fenitrothion PO for 8 weeks had no clinical signs although their whole blood ChE activity was inhibited by 50%. However, for the sciatic nerve, conduction velocity was progressively and significantly reduced from 26.0 to 18.2 m/sec ($p<0.01$). This change was associated with histopathology evident under both light and electron microscopy. Although the lesion incidence or number of slides examined was not reported, swollen and dislocated axoplasm were seen at low magnification and intramyelinic vacuoles containing a thin lammelae of split myelin sheaths were seen at higher magnification. Lesion reversibility was not examined. For rabbits treated with 25 mg/kg bw/day fenitrothion PO for 4 weeks classical clinical signs of OP toxicity were observed, namely excitation, salivation, mild ataxia and an abnormal gait. There was no indication of the extent of blood ChE inhibition or sciatic nerve histopathology reported but it was claimed that sciatic nerve conduction velocity

was increased and rabbits responded with extensive muscle contraction after a single electrical stimulus (Lehotzky & Ungváry, 1976).

Ocular Toxicity

Only 1 published study was reviewed but the US EPA (R.E.D.) has assessed 2 other studies examining ocular toxicity that were not submitted by the Australian registrants. The latter 2 summaries shown below were taken without amendment from the R.E.D. report.

In a published report to assess the acute effect of IP administered fenitrothion (0 or 13.8 mg/kg bw) on xenon flash derived electroretinographic parameters (amplitude and latency) in anaesthetised male rats, a transient non-significant reduction in b-wave amplitude was observed at 5 h and a significantly increased a- and b-wave amplitude after 2 days. There was no change in the latency period at either 5 h or 2 days. Three days after dosing the ChE activity in the brain was found to be significantly reduced (by 29%). ChE activity was unchanged in the retinochoroid (Yoshikawa et al, 1990).

"An acute ocular toxicity study was conducted with Sprague-Dawley (SD) (Crj:CD) rats using fenitrothion (94.5%). Doses were administered by oral gavage at the following levels: 0, 20, or 200 mg/kg for males, and 0, 40, or 400 mg/kg for females. The cholinesterase NOEL could not be determined. The cholinesterase LOEL was less than 20 and 40 mg/kg (LDT) based on erythrocyte cholinesterase inhibition in male and female rats, respectively. The ocular NOEL is greater than 200 mg/kg for males, and 400 mg/kg for females, based on lack of changes clearly related to treatment in electroretinography (ERG) and ophthalmic examination of the anterior portions of the eye. The ocular LOEL was not determined. No residual effect was observed on the electroretinograph following doses which produced signs of toxicity and were accompanied by depression of plasma and erythrocyte cholinesterase activity. No indications of ocular toxicity were observed."

"A 13-week subchronic study was conducted with Sprague-Dawley (SD) (Crj:CD) rats using fenitrothion (94.5%). Doses were administered in the feed at the following doses: 0, 2.5, 5, 10 or 30 ppm (Males: 0, 0.14, 0.282, 0.570 or 1.70 mg/kg/day; Females: 0, 0.169, 0.331, 0.648, or 1.96 mg/kg/day). The cholinesterase NOEL is 5 ppm based on a statistically significant inhibition of plasma cholinesterase in female rats to approximately 54% of the control activity levels. In addition, at 30 ppm, statistically significant inhibition of plasma, erythrocyte and brain cholinesterase was observed in female rats, and of plasma and erythrocyte cholinesterase in male rats.

No effect was observed on the ERG at the end of dosing either in comparison to pretreatment values or in comparison to concurrent control values. The high dose showed depression of plasma, erythrocyte and brain cholinesterase at the end of the study. The study showed no evidence of ocular toxicity."

Human studies

Oral administration

There were two oral studies performed in volunteers but neither produced any treatment-related signs or significant plasma/erythrocyte ChE inhibition.

Using an unusual dosing regimen, a group of 6 volunteers was given 0.1 mg/kg bw of a fenitrothion EC (an *in house* formulation, not a commercial product) by capsule then 6-7 days later had the same emulsion topically applied (0.1 mg/kg bw). Two and 3 days later, 0.5 and 0.1 mg/kg bw respectively was reapplied to the skin. A second group of 6 were given capsules containing 0.5 mg/kg bw and a repeat dose 2-3 days later (Shelanski et al, 1977). Nosál and Hladká (1968) investigated urinary excretion of the major fenitrothion metabolite, 3-methyl-4-nitrophenol, after either a single or daily PO doses (0.042-0.33 mg/kg bw) for 4 days. Only 1/24 had significant plasma ChE inhibition (by 35%) after acute dosing. No clinical signs were observed after either treatment and most of the dose (70% - 50% depending on dose) was excreted within 12-24 h.

Occupational exposure

The plasma ChE activities of 3 spraymen involved in a field spraying operation near a village in southern Nigeria were measured on the first, second, and sixth days after spraying with a 5% fenitrothion spray. ChE activities were not depressed compared with pre-spraying levels. Eighteen villagers examined one week later did not show any clinical symptoms of toxicity or plasma ChE depression (Vandekar, 1965).

During a 30-day program of indoor spraying of fenitrothion (2 g/m^2) for malaria control in Southern Iran in August 1971, a group of 28 pest control operators and 925 inhabitants were monitored for clinical signs and plasma ChE activity. Clinical investigations and ChE tests on 840 workers showed 42 cases of clinical symptoms, most of which were very slight and subsided after the workers had showered and rested (2-3 h). Of 20 spraymen, 8 showed decreased plasma ChE levels, and one individual preparing the spray mixture showed a significant depression of the enzyme activity, which was reactivated after an appropriate treatment. Among 925 inhabitants, only 15 cases of very mild complaints, namely dizziness and nausea, were reported (Motabar, 1972).

Workers attending monitoring equipment during aerial spraying of Sumbassa 75% EC (45% fenitrothion and 30% o-sec-butylphenyl N-methyl carbamate (Bassa)) from a helicopter were exposed to a maximum fenitrothion spray mist concentration of 1.64 µg/L and 1.26 µg/L of Bassa but showed no clinical signs of poisoning. However, although the spray mist concentration declined to undetectable levels 30 min after spraying, their mean plasma ChE levels declined by 13% and 21% after 1 and 3 h respectively. By contrast, erythrocyte ChE only declined by 5% and 7% respectively over the same time interval (Ueda et al, 1977).

Japanese orchard workers involved in the manual spray application of fenitrothion were monitored for inhalational exposure by measuring the inhaled quantity and the concentration in

plasma; the pooled 24 h urinary excretion of 3-methyl-4-nitrophenol (a major metabolite); and plasma ALT, AST and ChE activity. Although 0.001 mg/m^3 of fenitrothion was inhaled, no clinical signs were reported. Reflecting a demarcation of activities, operators had a lower maximum fenitrothion concentration in plasma (16 ng/mL; median 8 ng/mL; 3/5 had detectable concentrations after day 1 and 1/5 after day 3 spraying; day 2 concentrations not determined because rain suspended afternoon spraying) relative to assistants (30 ng/mL). Fenitrothion clearance from plasma appeared to be relatively rapid since only 2/7 (the other 2 in the group were not tested, for unknown reasons) had detectable quantities (Cmax, 13 ng/mL) the following morning (elapsed time between sampling times not reported). AST, ALT and ChE activity determinations revealed no treatment-related changes. A urine sample collected after spraying revealed a mean 3-methyl-4-nitrophenol concentration of 133 ng/mL for operators and 229 ng/mL for assistants, a result reflecting similar differences in plasma concentration (Usutani et al, 1978).

In the second of the series of reports translated from Japanese, the investigation examined worker exposure to fenitrothion (0.1 or 0.15% solutions of wettable powder in water) when the insecticide is distributed through an orchard via rigid piping to several ports from which a hand-held spray apparatus was connected. In addition to the parameters recorded in the first study (Usutani et al, 1978), LDH, CPK and LAP activities were measured. There was no reference to any clinical signs. The mean concentration of inhaled fenitrothion was 0.023 and 0.035 mg/m^3 at 0.1 or 0.15% respectively whereas the mean concentration in plasma was 16 and 5 ng/mL at 0.15% for the sprayers and the mixer respectively. Fenitrothion did not appear to have any affect on any of the enzyme activities, measured before and after exposure. The concentration of 3-methyl-4-nitrophenol (a major metabolite) in urine was 2.18 µg/mL (mean) for the sprayers and 0.7 µg/mL for the mixer (Nishiyama et al, 1978).

After the spraying of fenitrothion (62.5 g/L) for malaria control in the central region of Sudan, a group of pest control operators (2 supervisors, 3 group heads, 5 mixers, and 7 spraymen) had their whole blood ChE activity monitored (on days 1-5 then day 7, 8, 15, 20, 39, 40, 41, 42) and checked for signs of poisoning. Nine workers experienced 1 or more of the following toxicity symptoms during the course of the 42 day study; sweating, weakness, abdominal cramps, blurred vision, dizziness or salivation. Except on 3 occasions (day 4, 41 and 42), whole blood ChE activity was significantly inhibited (p 0.05) by between 12.5% and 50% (Fakhri, 1993).

Poisoning Incidents

A published report described the clinical outcome of 150 patients admitted to hospital after consuming varying quantities of different insecticides; 32 had consumed fenthion, 48 fenitrothion, 50 malathion, 6 carbamate and the compound was unknown in 14 cases. Of the 48 fenitrothion cases, 1 death occurred after ingestion of 3 g and paralysis was observed in 2/3 patients at 6 g, 7/20 at 3 g, 2/16 at 1.5 g and 0/9 at less than 1.5 g. For the 11 patients with paralysis, plasma ChE levels were inhibited by >80% in 7 cases, between 60-80% in 2 cases and between 40-60% in 1 case; the remaining case received no comment (Wadia et al, 1977).

“Intermediate Syndrome” experienced after OP poisoning can be distinguished from the characteristic muscarinic, nicotinic and CNS effects observed soon after exposure and the

delayed neurotoxicity effects seen 2-3 weeks later despite apparently satisfactory clinical management. Intermediate syndrome occurs 24-96 h after exposure and is characterised by muscular weakness affecting neck, proximal limb and respiratory muscles. Since only some OPs are capable of inducing this phenomenon, this study retrospectively examined 16 fenitrothion oral poisoning cases (14M & 2F; 9 attempting suicide, 7 accidental) for the occurrence of intermediate syndrome. Patients had consumed between 50-100 mL of a 50% fenitrothion solution and 6 died within 5-22 days after exposure, despite gastric lavage, atropine and oxime therapy. Fenitrothion concentrations in the plasma of these fatal cases ranged between 0.47-8.35 µg/mL. Of the remaining 10 survivors, intermediate syndrome was observed in 7. Although plasma ChE activity was not detectable in survivors that exhibited Intermediate syndrome (and 1 other) at the time of their admission, recovery time to normalisation ranged from 5 to >10 weeks whereas 2/3 symptomless patients had activities of 200 and 1200 mU/mL and all 3 had recovered within 2-4 weeks. Fenitrothion concentration in the plasma of survivors with Intermediate Syndrome ranged between 0.18 and 3.02 µg/mL while for the 3 others it ranged between 96 and 360 ng/mL (Groszek et al, 1995).

The presence of several fenitrothion metabolites including 3-methyl-4-nitrophenol, aminofenitrothion, S-methylfenitrothion and acetylaminofenitrothion were detected in the urine of 23-year old male attempting suicide by ingesting approximately 50 mL of a 50% fenitrothion emulsion. All metabolites except S-methylfenitrothion were detected up to 62 h after ingestion. The half-life of fenitrothion in plasma was calculated to be about 4.5 h and its concentration after 3 h (first recording) was 170 ng/mL. Plasma ChE activity at 3 h was approximately 13% that of the average population range and it gradually increased to 14%, 27% and 34% at day 1, 2 and 3 respectively. The patient recovered sufficiently to be discharged after 3 days (Kojima et al, 1989).

A 56-old male ingested about 60 mL of a 50% fenitrothion emulsion in an attempt to commit suicide. At admission and up until his death due to respiratory insufficiency on day 6, plasma ChE levels were <10% that of the normal mean despite combined haemoperfusion and haemodialysis treatment. Urinary excretion of the metabolite 3-methyl-4-nitrophenol was maximal at about 58 mg/day on day 3 after ingestion. Measuring the concentration of unchanged fenitrothion in the organs after death revealed that most was found in fat followed in order by pancreas, muscle and lung (Yoshida et al, 1987).

Accidental inhalational and dermal exposure to fenitrothion (EC, 7.5% v/v) resulted in a 33-year-old female technician having blurred vision, nausea, abdominal cramps, muscular weakness, mental confusion and tremors 2 days later. Plasma and erythrocyte ChE levels were inhibited by 44% and 14% respectively at the time of the clinical symptoms. Despite therapy with 1 g/day praloxime chloride (but not atropine) IV for 2 days, symptoms intensified and after 5 days plasma and erythrocyte ChE were inhibited by 64.5% and 34% respectively. Her condition improved after 7 IV doses of praloxime chloride and was discharged on day 16 after exposure (Ecobichon et al, 1977).

DISCUSSION

Acute Toxicity

The toxicological profile of fenitrothion is typical of organophosphorus anti-ChE pesticides, with clinical symptoms being similar in experimental animals and humans. Fenitrothion is synthesised by the introduction of a methyl group into the parent parathion-methyl structure. This structural modification has the effect of dramatically reducing the acute oral toxicity in rats (ie 235 mg/kg bw compared with 2.9 mg/kg bw for parathion-methyl). This change in toxicity appears to be the result of a more rapid detoxification and elimination, rather than slower formation of the biologically active oxon metabolite.

It has been claimed in some published reports examined in this review that fenitrothion has comparable insecticidal efficacy with parathion-methyl despite its lower acute toxicity. Although no efficacy data were assessed in this toxicology and public health review, it would seem prudent to confirm this assertion, since the compound could possibly provide a safer alternative to the parathions.

As expected for some OPs, the acute median lethal oral dose of the biologically active oxon metabolite is substantially less than the parent compound. For fenitrothion this difference is in the order of 7- to 14-fold in mice, 10- to 140-fold in rats and 4- to 8-fold in guinea pigs.

In rats, the acute oral median lethal dose of purified fenitrothion is approximately twice that for the S-methyl isomer. Thus, in a TGAC having 96% fenitrothion and about 0.5% of the S-methyl isomer (cf. Appendix II) as one of the impurities, the median dose lethality in rats is increased from 700 mg/kg bw for pure fenitrothion to 490 mg/kg bw. The major urinary metabolite 3-methyl-4-nitrophenol is significantly less toxic and requires approximately a 6-fold higher dose to achieve the same lethality in rats as technical fenitrothion. Mice are 3-fold more acutely sensitive to the S-methyl isomer and 4-fold more sensitive to 3-methyl-4-nitrophenol, than to technical fenitrothion.

Sunlight-induced degradation of a fenitrothion EUP resulted in the formation of uncharacterised breakdown products with a 3- to 7-fold lower median lethal dose in mice than the unchanged OP. This observation that fenitrothion becomes more toxic following UV degradation may necessitate consideration being given to setting a date limit on EUPs.

Cholinesterase Inhibition

As ChE inhibition is a primary target for fenitrothion toxicity, 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 the plasma, erythrocyte and brain ChE activity.

Summary of NOELs (mg/kg bw/day) for Cholinesterase Activity Inhibition Following Fenitrothion Administration

Species	**Rat**	**Rat**	**Rat**	**Rat**	**Rat**	**Mouse**	**Rat**	**Dog**	**Monkey**
Duration	3 mo	3 mo	6 mo	6 mo	92 weeks	2 years	2 years	1 year	2 years
Route	Diet	Diet	Diet	Diet	Diet	Diet	Diet	Diet	Gavage
Plasma ChE	6.3	NE (<0.5)	NE (<0.6)	0.6	0.3	1.44	NE (<0.5)	0.2	0.5
Erythrocyte ChE	3.2	1.6	1.8	0.6	0.5	1.44	0.5	0.3	0.5
Brain ChE	25	1.6	1.8	0.6	0.5	1.44	0.5	1.6	0.5

NE - Not established

With fenitrothion, no clear difference in binding specificity for plasma (a pseudo- or butyryl-ChE), erythrocyte or brain ChE (an acetyl- or true ChE) is evident. This conclusion can be drawn from Table above, which reveals considerable variability among studies.

Neurotoxicity

The anticipated clinical signs associated with OPs and attributable to an interaction with the muscarinic, nicotinic and central nervous system were common to all animal studies using fenitrothion. Measurements of plasma, erythrocyte and brain ChE activity in a variety of studies did not reveal a clear hierarchy of inhibition with brain ChE being generally depressed in concert with the activities in erythrocyte and plasma.

Although single-dose administration to hens or rats did not reveal any clinical or histopathological evidence for neurotoxicity, there is a paucity of information on such effects after chronic dosing. Apart from recording ChE inhibition in chronic dosing studies, there were only two repeat-dose studies of relatively short duration (ie. not exceeding 3 months) that specifically examined nerve conduction velocity or peripheral neuropathy. A 3-month feeding study in rats by Beyrouty et al (1993) did not reveal any neuropathy but a published study by Lehotzky & Ungváry (1976) provided some evidence of impaired sciatic nerve conduction velocity, slowed conditioned avoidance reflex, and axonopathy after a 4- or 8-week exposure in rats and rabbits at relatively high doses. However, the use of a single dose level for each exposure duration (in rabbits) and the judicious reporting of results, limit its value for regulatory purposes.

Given that there is a suspicion that chronic exposure to OPs at low dose produces cumulative poisoning with subclinical effects initially but with increased susceptibility to further toxic assault and thereby progressive neuropathy, it would seem prudent to initiate appropriate laboratory studies aimed at addressing this issue (Jamal, 1995). Furthermore, a clinical report cited in this review (Groszek et al, 1995), which shows that fenitrothion is able to induce "Intermediate Syndrome" with its attendant muscle paralysis, provides justification for the provision of such studies.

Ocular Effects

A single dose of 13.8 mg/kg bw fenitrothion administered to male rats caused a significant change in the a- and b-wave amplitude (without latency period change) of a electroretinograph after 2 days (Yoshikawa et al, 1990) but no treatment-related ocular toxicity, assessed by ophthalmoscopic examination and electroretinography, was observed in Cyanomolgus monkeys gavaged with up to 2 mg/kg bw/day for 2 years (Sumitomo Chem Co, 1978). In two studies reviewed by the US EPA (R.E.D.), no treatment-related changes in electroretinography were observed in rats given a single dose of up to 400 mg/kg bw or a daily repeat dose of 1.96 mg/kg bw/day for 13 weeks. Thus, based on the available data, there is little evidence that fenitrothion causes ocular toxicity.

Genotoxicity

In a large range of studies *in vitro* and *in vitro* in bacteria, *Drosophila* and mammalian cells, across all genotoxic endpoints (gene mutation, DNA damage and repair, and chromosomal aberration assays), predominantly negative results were obtained and therefore the weight of evidence suggests that fenitrothion is not genotoxic.

Reproduction and Development

No teratogenic effects were observed in two rat reproduction studies or in any of the developmental studies with mice, rats or rabbits.

Carcinogenicity

There were no carcinogenic effects related to the feeding of fenitrothion in the diet to either ICR Swiss mice at levels up to 200 ppm for 78 weeks (Kudzins, 1975) or B6C3F1 mice up to 1000 ppm for 2 years (Tamano et al, 1990). Similarly, in Wistar or Sprague-Dawley rats at oral doses up to 10 mg/kg bw/day for up to for 2 years, no treatment-related increase in neoplasia was observed (Ecobichon et al, 1980; Kudzins, 1974).

Human Toxicity

Apart from classical clinical signs of poisoning resulting from accidental or deliberate ingestion, it would appear that fenitrothion has an additional attribute not associated with all OPs, namely induction of “Intermediate Syndrome”. This effect can be distinguished from the characteristic muscarinic, nicotinic and CNS effects observed very soon after exposure by a delayed (24-96 h) onset of muscular weakness affecting neck, proximal limb and respiratory muscles (Groszek et al, 1995). This observation provides further justification for studies to specifically examine the neuropathy potential of fenitrothion, with chronic dietary feeding in laboratory animals.

NOEL considerations

To establish the lowest NOEL for fenitrothion, a summary of the NOELs determined in **those studies deemed adequate for regulatory purposes** are shown in the Table below.

Study Type NOEL (mg/kg bw/day) LOEL and Toxic Effect		
B6C3F1 mice: 2-year dietary	1.44	Brain, erythrocyte and plasma ChE inhibition observed at 12.6 mg/kg bw/day in females.
Wistar rat: 92-week dietary	0.3	Plasma ChE inhibition observed at 0.6 mg/kg bw/day in females.
Sprague-Dawley rat: 2-year dietary	Not established (<0.5)	Plasma ChE inhibition observed at 0.5 mg/kg bw/day in females. NOEL for brain and erythrocyte ChE 0.5 mg/kg bw/day.
Beagle dog: 1-year dietary	0.2	Plasma ChE inhibition observed in females at 0.3 mg/kg bw/day. LOEL for erthyrocyte ChE was 1.6 mg/kg bw/day. No brain ChE inhibition was observed.
Cynomolgus monkey: 2-year oral gavage	0.5	Body weight changes, plasma, erythrocyte and brain ChE inhibition, and EMG changes at 2 mg/kg bw/day.
Sprague-Dawley rat: 3-gen reproduction	10 [100 ppm]	Maternal body weight loss at 150 ppm.
	3 [30 ppm]	Reduced pup survival and an inability to gain weight during lactation at the next higher dose (100 ppm).
Sprague-Dawley rat: 2-gen reproduction	M, 0.65; F, 0.74 [10 ppm]	Reduced food consumption, body weight gain and body weight in both sexes at next higher dose of 40 ppm.
	3 [40 ppm]	Reduced pup weight, viability and lactation indices at the highest dose (120 ppm).
Sprague-Dawley rat: Gavage teratology	8	Maternal body weight loss and tremors at 25 mg/kg bw/day.
	8	Skeletal variants observed at the highest dose tested (25 mg/kg bw/day).
NZW rabbit: Gavage teratology	10	Maternal mortality, body weight loss and cholinomimetic signs at 30 mg/kg bw/day.
	30	No embryo/fetotoxicity effects observed at the highest dose tested (30 mg/kg bw/day).

Determination of Public Health Standards

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.003 mg/kg bw/day. This ADI was derived from a NOEL of 0.3 mg/kg bw/day, based on plasma ChE inhibition seen in a 92-week Wistar rat study.
This review has identified a 1-year Beagle dog study that had a lower NOEL (0.2 mg/kg bw/day) based on the same endpoint, namely plasma ChE inhibition. Although this NOEL is only slightly less than that in the 92-week rat study, an effect was observed at 0.3 mg/kg bw/day in the dog study. Hence, using a 100-fold safety factor (10-fold each for interspecies extrapolation and 10-fold for variability in human sensitivity), it is recommended that the ADI be changed to 0.002 mg/kg bw/day.

Considerations of the Advisory Committee for Pesticides and Health (ACPH)

A draft toxicological evaluation of fenitrothion was considered at the 14th meeting of the ACPH [Report extract (not yet ratified), see Appendix IV]. The Committee acknowledged the change to the current ADI.

Public exposure

In Australia, fenitrothion has only one product, namely an outdoor fogger for flying insect control registered for domestic use. Hence, the greatest potential for public exposure is via ingestion of fenitrothion residues in food. Fenitrothion has MRLs established in a wide range of foods, including fruits and vegetables. The current Australian MRL's are listed in the residues section of this report or can be accessed from the National Registration Authority website on: http://www.dpie.gov.au/nra/welcome.html. Fenitrothion is registered for use on stored grain, mite control in chicken runs and plague locust control.

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, recommends the use of National Theoretical Maximum Daily Intakes (NTMDI) as an initial estimate, while admitting that these can produce a gross overestimation 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 fenitrothion, using the Australian 1983 survey of average food consumption, it was calculated that a 75 kg adult male (weight as used by Australian Market Basket Survey) would possibly consume 0.036 mg/kg bw/day of fenitrothion, assuming that residues were present in all foods consumed at the MRL. This is 18 times the proposed ADI (0.002 mg/kg/day).

A more reliable estimate of fenitrothion intake may be derived from the Australian Market Basket Survey, a procedure which uses the measure of fenitrothion residues found in that year, rather than assuming that all pesticides are present at the MRL. The estimated consumption in the group with the highest consumption (toddlers aged two), based on the average energy intake, is 0.0003 mg/kg/day. This makes up 15% of the proposed ADI, giving an additional 6-fold safety factor.

Market Basket Survey

The 1994 Market Basket Survey found detectable levels of fenitrothion in a range of products, involving mainly cereal grain products. The maximum residues found were in wheat, and were 1.20 mg/kg. Residues of 0.31 mg/kg were found in wholemeal bread, and of 0.11 mg/kg in untoasted muesli. The only fruit or vegetable with detectable fenitrothion levels (0.01 mg/kg) was dried fruit in the form of fruit sticks and leathers. The Market Basket Survey estimated the daily intake of a range of pesticides based on the average energy intake. In the groups studied, the highest exposure for fenitrothion was in toddlers aged 2; estimated at 0.0003 mg/kg bw.

Acute Reference Dose

To reflect safe/acceptable exposure from a single or short exposure to fenitrothion, an acute reference dose (acute RfD) may be derived using appropriate data. One human study has been identified in the available toxicology database that provides a NOEL for ChE inhibition following a single PO dose. This study by Nosál & Hladká (1968) found that doses up to 0.33 mg/kg bw caused neither plasma nor erythrocyte ChE activity inhibition. An acute RfD, based on this study is 0.03 mg/kg bw, derived from the NOEL and a 10-fold safety factor.

An acute RfD, based on a 1-month rat study (Trottier et al, 1980) is 0.025 mg/kg bw/day derived from the NOEL of 2.5 mg/kg bw for ChE inhibition and a 100-fold safety factor.

Thus, the fenitrothion acute RfD in humans is 0.03 mg/kg bw/day and this is supported by a value of 0.025 mg/kg bw/day in rats.

Safety Directions

The current safety directions are as follows:

Fenitrothion

EC 1000g/L or less: more than 1 g/L. ULV 1280 g/L or less. DU 20 g/kg or less. **WP 12 g/kg or less.**	
Product is poisonous if	120, 130
Absorbed by skin contact, inhaled or swallowed.	131, 132, 133
Repeated minor exposure may have a cumulative poisoning effect.	190
Avoid contact with eyes and skin.	210, 211

Do not inhale spray mist.	220, 223
When opening the container and preparing the spray	279, 280, 281
wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves and full face respirator with combined gas and dust cartridge.	290, 292, 294 303
If product on skin, immediately wash area with soap and water.	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each days use, wash gloves, respirator (and if rubber wash with detergent and warm water), and contaminated clothing.	360, 361, 364, 366
Obtain an emergency supply of atropine tablets 0.6 mg	373
AE 50 g/kg or less	
Avoid contact with eyes and skin.	210, 211
Do not inhale spray or vapour. Wash hands after use	220, 222, 223, 351
EC 1g/L or less	
Avoid contact with eyes and skin.	210, 211
Wash hands after use	351

These Safety Directions are considered to be acceptable for public health considerations.

First Aid Instructions

Currently a, h. No changes to the current first aid directions are recommended

The T-value is currently 20. No change is recommended.

RECOMMENDATIONS FOR PUBLIC HEALTH STANDARDS

1. Acceptable Daily Intake

The current acceptable daily intake (ADI) for fenitrothion is 0.003 mg/kg bw/day. This ADI was derived from a NOEL of 0.3 mg/kg bw/day, based on plasma ChE inhibition seen in a 92-week Wistar rat study.

Based on a NOEL of 0.2 mg/kg bw/day and a LOEL of 0.3 mg/kg bw/day for plasma ChE inhibition in a one-year Beagle dog study, a change to the current ADI is recommended. Using a safety factor of 100, the new ADI is 0.002 mg/kg bw/day.

2. Acute Reference Dose

The acute RfD for fenitrothion is 0.03 mg/kg bw/day. The RfD was derived from a NOEL of 0.33 mg/kg bw, based on plasma and erythrocyte ChE inhibition in an acute human dosing study. This outcome is supported by studies in rats.

3. Poisons Scheduling

No change to the current schedule of Schedule 6 of the SUSDP is proposed for fenitrothion.

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 Australian National Occupational Health and Safety Commission (formerly Worksafe Australia).

No changes to the current first aid directions (fenitrothion: a, h) and T-value (currently 20) are recommended.

5. Clearance Status

No change is recommended to the clearance status on fenitrothion.

TOXICOLOGICAL FINDINGS WITH POSSIBLE REGULATORY IMPLICATIONS

It has been claimed in some published reports examined in this review that fenitrothion has a comparable efficacy with parathion-methyl despite its lower acute mammalian toxicity. Although no efficacy data were assessed in this review, it would seem prudent to confirm this assertion, since the compound could possibly provide a safer alternative to the parathions.

Sunlight-induced degradation of a fenitrothion EUP resulted in the formation of uncharacterised breakdown products with greater toxicity than the parent compound. This observation may necessitate that some consideration being given to setting a date limit on EUPs.

On the basis of the greater acute oral toxicity of fenitrooxon (metabolite and manufacturing by product) and S-methyl fenitrothion (isomer) relative to technical fenitrothion, the current limits on the level of these components in cleared TGACs (viz. 10 g/kg for S-methyl fenitrothion and 100 mg/kg for fenitrooxon) remain appropriate.

There is evidence that fenitrothion is one of the OPs capable of inducing “Intermediate Syndrome”. This information needs to be readily available, in the event that clinicians are required to treat cases of acute poisoning.

In view of emerging concerns about the neuropathy potential of some OPs and the paucity of data that specifically address this issue for fenitrothion, some consideration to allay such concerns should be pursued. As the major source of public exposure to fenitrothion will be via ingestion of residues in food, it would seem prudent that appropriate long term dietary studies be performed, with a special emphasis on nerve histopathology and and behavioural aspects (Functional Observation Battery). Sponsors should be requested to provide such studies.

SUMMARY OF ACUTE TOXICOLOGY HAZARD

Date of Preparation:	December, 1997
Chemical name:	Fenitrothion
Worst oral LD50 in rats:	235 mg/kg bw
Worst oral LD50 in other species:	500 mg/kg bw in guinea pigs.
Worst dermal LD50:	890 mg/kg bw in rats
Worst inhalation LC50:	Not quantified but >2210 mg/m^3 in rats
Skin irritation:	Non- irritating to the skin of rabbits
Eye irritation:	Not irritating to the eyes of rabbits
Skin sensitisation:	Not a skin sensitiser to guinea pigs
T-value:	20
NOEL:	0.2 mg/kg bw/day (1-year dog study)

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Fenitrothion is an organophosphorothioate that has been available internationally since 1959. It is used in agriculture to control insect pests in a wide range of horticultural and agricultural crops. Fenitrothion has been registered in Australia since August 1968, with initial use on pastures to control a variety of pests, with use later approved in stored grain and on a variety of horticultural crops.

In Australia, public health standards for agricultural and veterinary chemicals, such as the poison schedule, first aid and safety directions and an acceptable daily intake (ADI), are set by the Department of Health and Family Services. Poisons schedules are set by the National Drugs and Poisons Schedule Committee (NDPSC) of the Australian Health Ministers' Advisory Council (formerly the Drugs and Poisons Schedule Committee (DPSC) of the National Health and Medical Research Council (NHMRC). In the case of maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Committee (PACC) of the NHMRC, however, in 1992, the Department of Health became directly responsible for establishing MRLs, a function subsequently transferred to the National Registration Authority (NRA) in June 1994.

The health regulation history of fenitrothion in Australia is tabulated in summary below.

History of public health consideration of fenitrothion in Australia

Date	Decision
April 1968	PACC: Require data to make a recommendation
August 1968	PACC: Cleared fenitrothion for use on pastures
March 1971	PACC: Agree to non-food use of fenitrothion
November 1975	PACC: Amend MRLs to 0.02 mg/kg sugar cane, 0.3 mg/kg soybean, 6 mg/kg sorghum
March 1976	PACC: Request from States to market fenitrothion for locust control
August 1977	PACC: Amend entry for raw cereals
May 1979	PACC: Increase MRL for white wheat flour to 3 mg/kg, adopt MRL 0.03 mg/kg for water
August 1979	PACC: New MRLC for gluten 5 mg/kg, amend MRL for red cabbage to cabbage
February 1981	PACC: MRLs of 0.1 mg/kg set for nuts, other fruit and vegetables

February 1982	PACC: Amend water MRL to 0.06 mg/kg
August 1982	PACC: Delete MRLs for gluten and bread (white)
November 1983	PACC: Agree fenitrothion should be included in 1984 Market Basket Survey
November 1984	PACC: Add MRL for wheatgerm 20 mg/kg
February 1985	PACC: Maintained MRL for cereal grains at 10 mg/kg
May 1985	PACC: Additional toxicity studies reviewed. ADI set at 0.003 mg/kg bw/day, applying 100-fold safety factor to NOEL of 0.3 mg/kg bw/day based on ChE inhibition.
August 1985	PACC: Residue data for wheat flour (white) requested
December 1985	PACC: Acknowledged receipt of monkey study - no change to ADI requested
May 1986	PACC: Residue data received - no change to current MRLs
September 1986	PACC: Water MRLs changed to 0.02 mg/kg. Toxicity of impurities noted
February 1987	PACC: Additional residue data and protocols requested
July 1987	DPSC: Maximum impurity level defined - S-methyl fenitrothion 1%, fenitrooxon 0.01%
November 1987	PACC: Human studies considered, however considered inadequate to change the ADI
February 1989	PACC: Recommend deletion of rice MRL, with rice covered by MRL for raw cereal grain
November 1989	PACC: Considered deletion of withholding period statement for treated grain
June 1991	PACC: Cleared TGAC for submission 329-1-185N
August 1991	DPSC: Noted revised batch specification for TGAC 329-1-185N

PACC - Pesticide and Agricultural Chemicals Committee; DPSC - Drugs and Poisons Schedule Committee

Health Standards

NOEL/ADI

The PACC established an acceptable daily intake (ADI) of 0.003 mg/kg bw/day (May, 1985). This ADI was set on a "no observable effect level" (NOEL) of 0.3 mg/kg/day based on plasma ChE inhibition in the rat.

Poison Schedule

Fenitrothion is in Schedule 6 (S6) of the Standard for Uniform Scheduling of Drugs and Poisons (SUSDP).

MRLs

Fenitrothion has MRLs set for fruits, vegetables, meats, milks and cereal grains. The current MRLs for fenitrothion are detailed in the residues section of this report or can be accessed from the National Registration Authority Website on http://www.dpie.gov.au/nra/welcome.html.

Existing Chemicals Review Program

Fenitrothion is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. Following data call-in processes, a number of additional studies on the toxicology of fenitrothion have been received from industry. These data, together with all previously submitted data have been evaluated and are detailed in the report below. The data submission details covering toxicological and public health aspects of fenitrothion are summarised in **Appendix I**.

1.2 International Toxicology Assessments

WHO/FAO

Fenitrothion was evaluated by the Joint FAO/WHO Expert Committee on Pesticide Residues (JMPR) in 1969, 1974, 1976, 1977, 1979, 1982, 1983, 1984, 1986, 1987, 1988, and 1989 (FAO/WHO, 1970, 1975, 1977, 1978, 1980, 1983, 1984, 1985, 1986, 1987, 1988, 1989). An ADI of 0.005 mg/kg bw/day was allocated by JMPR in 1974. Fenitrothion was re-evaluated in 1982 by the JMPR, and a temporary ADI of 0.001 mg/kg bw/day was set because of concerns over the reliability of the studies conducted by Industrial BIO-TEST Laboratories (IBT). In 1983, a temporary ADI of 0.003 mg/kg bw/day was established, however, there was no adequate teratogenicity study. In 1986 an acceptable rat teratology study was examined, and the ADI was again set as 0.005 mg/kg bw/day.

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

Rat: 10 mg/kg in the diet, equivalent to 0.5 mg/kg bw/day
(based on brain ChE inhibition and reproduction).

Dog: 50 mg/kg in the diet, equivalent to 1.25 mg/kg bw/day.

Man: 0.08 mg/kg bw/day (highest dose tested)*.

(***** Although this human study was cited in the 1988 JMPR toxicology report to support the ADI, no reference was given. Correspondence with Dr J Herrman at the WHO indicated that the study

was first described in the 1969 JMPR report (Nosál & Hladká, 1968) but not cited in any of the susequent reports until 1988. The reason for this omission was not known.)

US EPA

The 1995 US EPA Reregistration Eligibility Decision (R.E.D) determined that an ADI (or RfD) should be based on a NOEL of 0.125 mg/kg bw/day for systemic effects (histopathological changes in the lymph nodes) and plasma ChE inhibition observed at 0.25 mg/kg bw/day in a long-term feeding study in dogs. An uncertainty factor of 100 was used to account for the inter-species extrapolation and intra-species variability. On this basis an ADI of 0.0013 mg/kg bw/day was calculated.

2. KINETICS AND METABOLISM

A generalised mammalian metabolic pathway for fenitrothion is shown in Figure 1 (end of section 2.1). This pathway was derived using data described in many of the studies in the following section.

2.1 Absorption, distribution, metabolism and excretion

Kohli JD, Hasan MZ & Gupta BN (1974) Dermal absorption of fenitrothion in rats. Bull Environ Contam Tox 11:3 285-290

Young adult ITRC rats (source and age not specified, both sexes used, weight 160-180 g) were used. Back skin between shoulders and hind quarters was clipped and 0.1 mL (approx. 0.73 g/kg bw) fenitrothion (95% pure, density 1.32 g/mL, source not specified) was applied over an area of 2 x 2 cm. The animals were divided into 5 groups of 8 animals each. After 2, 4, 8, 24 and 48 h, blood samples were taken. A higher dose of 0.5 mL (approx. 3.69 g/kg bw) fenitrothion applied to 4 x 4 cm was also investigated, with blood samples taken after 48 h. The effect of repeated applications were also examined. Animals were treated with fenitrothion as above, with groups receiving 0, 1, 2 or 3 applications. Blood samples were taken 8 h after application. ChE activity in whole blood was estimated, as were fenitrothion levels. Unabsorbed fenitrothion was extracted from the skin with chloroform.

After a single dermal application of 0.1 mL fenitrothion, the concentration in plasma was approximately 33 µg/mL 2 h after application. The maximum concentration of 43 µg/mL was reached after 8 h. At 48 h, the levels had decreased to 27 µg/mL. The degree of whole blood ChE inhibition appeared to correspond with fenitrothion levels in plasma, with maximal inhibition at 8 h. Following extraction with chloroform, it was determined that 55% of the fenitrothion remained unabsorbed (in and on the skin) after 24 h exposure.

When 0.5 mL fenitrothion was applied, the fenitrothion level at 48 h after application was 250 µg/mL plasma. ChE inhibition was approximately 100%, and the animals were in a moribund state with fine to coarse tremors.

Multiple 0.1 mL applications resulted in increased plasma levels of fenitrothion and increased ChE inhibition. The maximum fenitrothion levels in plasma was 43, 51 and 60 µg/mL following 1, 2 and 3 applications respectively. There was 20% ChE inhibition following a single application, 42% inhibition following 2 applications and 64% inhibition following 3 applications.

Miyamoto J, Mihara K & Hosokawa S (1976) Comparative metabolism of m-methyl-^{14}C-Sumithion in several species of mammals in vivo. J Pesticide Sci 1: 9-21

^{14}C-fenitrothion, labelled at the *m*-methyl position (specific activity 3.16 mCi/mmole, radiochemically 99.8% pure, prepared by the Institute for Biological Sciences, Sumitomo Chem Co) was used. Radioactivity in faeces, blood and tissues was quantified using a liquid scintillation spectrometer after combustion.

Eight week old ICR mice and male Beagle dogs (supplied by Nihon Crea Co, Osaka), HLA-Wistar rats and native Japanese rabbits (supplied by Nihon Dobutsu Co, Osaka) were used. ^{14}C-fenitrothion was orally administered to mice, rats, rabbits, and dogs at 15 mg/kg bw. Animals were housed in metabolism cages. Urine and faeces were collected and refrigerated to prevent decomposition of metabolites. Urinary radioactive metabolites were separated using column chromatographic separation and thin layer chromatograms. Faecal metabolites were similarly separated following homogenisation with water and filtering to remove solid matter.

Expired air from the rats was collected and analysed for the presence of radioactive carbon dioxide. Blood samples were taken from all treated animals to determine levels of fenitrothion and fenitrooxon. After 0.5, 1.5, 6 and 24 h, rats and mice (unspecified number) were euthanised and whole body autoradiograms taken to examined the absorption and distribution of the radioactivity.

At 30 min after first oral administration of labelled fenitrothion to mice and rats, the majority of the radioactivity was detected in the stomach and intestines. Autoradiographic grain density suggest lesser amounts in the lung, liver and kidney while only trace amounts were present in brain, spleen, muscle, eyeball and fat. After 24 h radioactivity was barely detectable.

In all species tested, more than 80% of the radioactivity was excreted in the urine within 24 h after oral administration. Small amounts (<5%) were excreted in the faeces. No labelled carbon dioxide was detected in the expired air of rats. After 2 days, the recovered radioactivity from mice was 92%; rats 91-92%; rabbits 94%, and male dogs 88%. Faecal metabolites in rats were 3-methyl-4-nitrophenol, desmethylfenitrothion desmethylfenitrooxon and unchanged fenitrothion, with the proportion of the total radioactivity in faeces being 70.3%, 13.2%, 6.0% and 13.2% respectively. Overall, less than 1% of ingested fenitrothion was excreted from the body unchanged.

Blood samples were taken at 1, 3, 6, 9, 24, 48, 72, 96 and 120 h after dosing with 15 mg/kg bw from 10 mice, 7 rats, 5 rabbits and 3 dogs of each sex and tested for fenitrothion levels and total ^{14}C. In all species, fenitrothion levels in the blood reached a maximum between 1 and 3 h after dosing. In mice and rabbits, levels were below the limit of detection (0.001 ppm) at 48 h after dosing. In rats, fenitrothion was not detected by 96 h after dosing, while in dogs fenitrothion was

present at detectable levels (limit of detection 0.005 ppm) in the blood 120 h after dosing. Therefore, there appears to be a significant species difference in the metabolism and excretion of fenitrothion, with the persistence being (in increasing order) mice < rabbits < rats < dogs.

A major metabolite identified in the blood was fenitrooxon. The main urinary metabolites are desmethylfenitrothion, desmethylfenitrooxon and 3-methyl-4-nitrophenol (both free and conjugated with sulfate or -glucuronic acid). All species conjugate fenitrothion metabolites prior to excretion, although there was species variability in the amount of conjugation that occurred.

Ten male rats were euthanised 1 and 24 h after oral administration of 15 mg/kg bw of labelled fenitrothion. The blood, brain, lung, thymus, liver, kidney, pancreas, spleen, thyroid, adrenals, pituitary, salivary gland, testis, muscle, fat, stomach and intestine and intestinal contents were collected and analysed for total radioactivity, unchanged fenitrothion and some metabolites. At 1 h, fenitrothion was found in the stomach, intestine and gastrointestinal content at 2-3 ppm. There were a number of metabolites, including fenitrooxon, 3-methyl-4-nitrophenol, and some water soluble metabolites present in the stomach and intestine, which may indicate gastrointestinal biodegradation. Water soluble metabolites were present in the kidney at approximately 9 ppm, indicating a relatively rapid metabolism and excretion. Approximately equal amounts of radioactivity were present in the liver and the blood; mainly uncharacterised water soluble metabolites (around 2 ppm). At 24 h, the levels of fenitrothion and its metabolites present in the rat organs had greatly diminished, with levels <0.1 ppm in all organs. Radioactivity was present in approximately equal amounts in the intestines, liver and in the kidney, at around 0.07 ppm. Unchanged fenitrothion was present in the fat, while there were low levels (around 0.01 ppm) in the brain, adrenals and thyroid. No fenitrooxon was detected at 24 h in any tissue.

Fenitrothion administered to rats at 105 mg/kg bw showed a similar excretion pattern to that seen following the lower 15 mg/kg bw dose. Additionally, rats treated with fenitrothion at 15 mg/kg bw every second day for 5 days showed a similar excretion pattern to rats given a single dose.

In addition to the above experiments, groups of 5 male rabbits were fed unlabelled fenitrothion at 0, 3 or 10 mg/kg bw/day for 6 months. At the end of the feeding period, the animals were euthanised and the levels of fenitrothion and fenitrooxon in the blood, skeletal muscle and fat was determined by gas chromatography with a limit of detection for fenitrothion of 0.005 ppm and for fenitrooxon of 0.01 ppm. No detectable amounts of either compound were found in either blood or muscle, while fenitrothion (but not fenitrooxon) was detected in the fat. A level of 0.131 ppm in the fat resulted from feeding fenitrothion at 10 mg/kg bw/day, while levels of 0.045 ppm in the fat resulted from feeding fenitrothion at 3 mg/kg bw/day.

Kumar R, Roy S, Rish R & Sharma CB (1993) Metabolic fate of fenitrothion in liver, kidney and brain of rat. Biomed Chromatog 7: 301-305

Fenitrothion (Bayer (India) Ltd, Bombay India) dissolved in 0.2 mL groundnut oil was administered to male albino rats (*Rattus rattus* - from laboratory stock) by IM injection at 50 mg/kg bw. Food and water were supplied *ad libitum*. Rats were euthanised 1, 6, 12, 24 and 36 h after injection, and the liver, kidney and brain were removed and used immediately for extraction of fenitrothion and its metabolites, which were separated by High Performance Liquid Chromatography (HPLC). Metabolites were then characterised by infra-red spectroscopy. Electron microscopy studies of liver tissue was done on samples obtained following 6, 12 and 24 h of fenitrothion exposure.

The metabolites identified in tissue were O,O-dimethyl-O-3-methyl-4-aminophenyl phosphorothioate (metabolite I; aminofenitrothion), O,O-dimethyl phosphorothioate (metabolite II; not shown in Figure 1) and O,O-dimethylphosphate (metabolite III; not shown in Figure 1). At 1 h after administration, the parent compound was found in liver, kidney and brain. At 6 h after administration, metabolite I was found in liver, kidney and brain, while metabolite II was found in the brain. Twelve h after administration metabolite I and II were present in all tissues, while by 24 h metabolite III was detectable in the liver. This metabolite was detectable after 36 h in the kidney, but was not detected in the brain. The quantity of fenitrothion present in the tissues decreased over time. Metabolite I remained the most prominent metabolite over time.

The pathway proposed for the metabolism of fenitrothion involves an initial reduction to metabolite I, then hydrolytic cleavage of this metabolite to produce metabolite II. While this metabolite is produced directly from fenitrothion, particularly in plants, insects and soil, the kinetic data obtained favour a metabolic pathway via metabolite I in rats. Metabolite II undergoes oxidative desulfuration to produce metabolite III.

Electron microscopy of liver tissue indicated that 12 h after administration of fenitrothion, there was a loss of nuclear integrity shown by a distorted membrane. The smooth endoplasmic reticulum and Golgi apparatus were enlarged. After 24 h, the cells appear to be regenerating, as the nuclear membrane was intact and a well defined nucleus and nucleolus were visible. This appears to suggest that the majority of liver damage occurs soon after ingestion, and possibly could be related to fenitrothion rather than its metabolites.

Douch PGC, Hook CER & Smith JN (1968) Metabolism of Folithion (dimethyl 4-nitro-3-methylphenyl phosphor-thionate) Aust J Pharmacy, 29:584, Supp 6, S70 - S71

Fenitrothion (50 to 500 mg/kg bw) was administered PO to mice and SC to guinea pigs and rats. For all animal groups, the number, source and strain was not specified. Urine was collected for an unspecified period after administration. Following a number of steps involving hydrolysing the urine, as well as concentrating and re-extracting the solutes, the urine was found to contain 5-hydroxy-2-nitrobenzoic acid (not shown in Figure 1) and 3-methyl-4-nitrophenol.

Spectrophotometric analyses for these 2 products in the urine were made at unspecified intervals, and 90% of the dose was recovered after 3 days. In a number of experiments, recovered 5-

hydroxy-2-nitrobenzoic acid made up 13 - 25% of the administered dose in mice, 15% in rats and 18% in guinea pigs. This metabolite was also extracted from mouse urine without hydrolysis: 80% of the metabolite was present in an unconjugated form.

Mouse liver homogenate was incubated with 0.3 mg fenitrothion. After 1 h the mixture was acidified and extracted with ether. Thin layer chromatography on silica gel enabled several metabolites to be identified viz; a demethylated compound, dimethyl 4-nitro-3-carboxyphenylphosphorothionate, fenitrooxon, 3-methyl-4-nitrophenol and unchanged fenitrothion.

A quantitative analysis revealed that after 30 min incubation, 47% of the metabolites consisted of nitrophenols, 30% of desmethylated products, 15% of dimethyl aryl phosphates and 8% 5-hydroxy-2-nitrobenzoic acid. There appeared therefore to be an efficient metabolism process for fenitrothion in the mouse liver, which may contribute to the relatively low toxicity of fenitrothion in comparison to parathion-methyl.

Kawamura Y, Takeda M & Uchiyama M (1981) Biological effect of organophosphorus pesticides at low concentration. I. The detoxification of fenitrooxon at low concentration by mouse liver preparation. Eisei Kagaku 27(4): 252-256

Fenitrooxon (purity 97.5%, obtained from Sumitomo Chem Co) and *m*-methyl-^{14}C-fenitrooxon (purity 99%, specific activity 6.7 mCi/mmole, obtained from Sumitomo Chem Co) were used. Nuclear, mitochondrial, microsomal and supernatant fractions of homogenised liver were prepared from the livers of sacrificed male ddY mice.

Fenitrooxon was incubated with the tissue homogenate and the anti ChE activity was determined. The metabolite concentration was determined by chromatographic analysis. Additionally, a series of fenitrooxon solutions were incubated with a known quantity of liver microsomal fraction or soluble fraction to determine the reaction rate.

It was found that low concentrations of fenitrooxon were rapidly metabolised in the presence of mouse liver homogenate, with 80% of a 2.5 x 10^{-6} M solution being transformed in 10 min. At a concentration 100 times greater, 20% of the fenitrooxon had been transformed in 120 min. Metabolism with tissue homogenates of liver, kidney, brain, small intestine and blood revealed that liver was the most active tissue with regard to fenitrooxon transformation, with an activity around 15 times greater that kidney. There was minimal activity in brain and blood, while no transformation of fenitrooxon occurred with intestine homogenate.

In comparing the activity of each of the liver fractions, the soluble fraction has the highest activity, approximately 4 times the activity seen in the microsomal fraction, which is in turn twice that seen in the nuclear or mitochondrial fractions.

Analysis of the metabolites produced following incubation of radiolabelled fenitrooxon with liver homogenates indicated that desmethyl fenitrooxon was the only metabolite produced. No 3-methyl-4-nitrophenol was produced, and thus desmethylation was the only reaction observed in this experiment.

Kawamura Y, Takeda M & Uchiyama M (1982) Biological effect of organophosphorus pesticides at low concentration. II. The detoxification of fenitrothion at low concentration by mouse liver preparation. Eisei Kagaku 28(2): 65-70

Fenitrothion (purity 98%, obtained from Sumitomo Chem Co) and *m*-methyl-^{14}C-fenitrothion (purity 99%, specific activity 6.7 mCi/mmole, obtained from Sumitomo Chem Co) were used. Male ddY mice (source not specified) were used. These animals were sacrificed and their liver, kidney, brain, small intestine and blood was removed. Nuclear, mitochondrial, microsomal and supernatant fractions of homogenised liver were obtained. Crude tissue homogenates of the other tissues obtained were prepared. Fenitrothion was reacted with tissue homogenate for a specific length of time (not given), and then the reaction was stopped by immersion in a boiling water bath. The concentration of fenitrothion in the mixture was determined by gas chromatography. The fenitrooxon concentration was determined by measuring the ChE inhibition, determined by the change in pH of the mixture and reference to a calibrated curve. The concentration of 3-methyl-4-nitrophenol was determined, and other metabolites were identified and the quantities present determined.

Fenitrothion was most rapidly metabolised by mouse liver tissue homogenate incubation, with 90% of the added fenitrothion being metabolised in 60 min. In comparison, 7% of the added fenitrothion was metabolised by incubation with blood, 6% with brain and 2% with either kidney or intestine. Following incubation with the liver subcellular fractions, it was found that the soluble fraction was most efficient, metabolising 52% of added fenitrothion, while the nuclei metabolised 10%, the microsomes 4% and the mitochondria 1%.

Following the metabolism of fenitrothion the 2 main metabolites produced were desmethyl fenitrothion (not shown in Figure 1) and desmethyl fenitrooxon (as shown in Figure 1). Fenitrooxon and 3-methyl-4-nitrophenol were present in negligible amounts.

Mihara, K Okuno Y, Misaki Y & Miyamoto J (1978) Metabolism of fenitrothion in goats. J Pesticide Sci 3: 233-242

^{14}C-Fenitrothion (radiochemical purity 99%, obtained from Sumitomo Chem Co) was mixed with 200 g crushed hay, and administered in the morning as part of the diet to 6 female Japanese Saanen goats (obtained from Nihon Dobutsu Co, Osaka) at 0.5 mg/kg bw/day for 7 consecutive days. Goats were fed a balanced diet, maintained in metabolism cages, and milked twice daily. Evening milk samples were combined with the morning samples from the following day. The fenitrothion metabolites present were determined. Urine and faeces were collected throughout the experiment, and the fenitrothion metabolites present were extracted and purified. Blood samples for determination of ChE activity were taken at intervals throughout the study.

Two goats were euthanised each of 1, 7 and 18 days after the final administration of the radiolabelled fenitrothion. Blood and the following organs and tissues were removed for radio analysis: brain, lung, heart, thymus, liver, kidney, pancreas, mammary gland, salivary gland, omental fat, mesenteric fat, peri-renal fat, quadriceps femoris muscle, abdominal muscle, thyroid gland, adrenal, bone marrow, spleen, femur, tongue, eye, spinal cord, pituitary, uterus, ovary,

gallbladder, urinary bladder, rumen, reticulum, omasum, duodenum, jejunum, ileum, caecum, colon, rectum and skin.

Radiolabelled fenitrothion was incubated with rumen fluid under anaerobic conditions. Over 30 min, the fenitrothion was completely converted to aminofenitrothion.

At 1 day after the last administration of fenitrothion, there were relatively high levels of radioactivity present in the GI tract (between 0.1 and 0.8 ppm). These levels had decreased by day 7 to around 0.004 ppm and were negligible (0.002 ppm) by day 18 after the last administration. The fatty tissue had radioactivity equivalent to 0.01 ppm on both day 1 and day 7 after the last administration; the levels had decreased to 0.003 ppm by day 18.

Analysis revealed that unchanged fenitrothion was not found in any organ or tissue analysed. Aminofenitrothion was found in small amounts in the intestinal tract. Urinary excretion eliminated almost 50% of the labelled compound, while faecal excretion was responsible for approximately 40% of the excretion in the goat. The major metabolites in the urine included aminofenitrothion (20% of administered radioactivity), desmethylacetylaminofenitrooxon (11%), N-sulfoaminofenitrothion (7%) and N-sulfoaminofenitrooxon (5%). Other metabolites included desmethylaminofenitrothion (3%), aminofenitrooxon (3%) and 3-methyl-4-acetylaminophenyl (2%). In the faeces, 31% of administered radioactivity was present as aminofenitrothion, while all other metabolites were present at very low levels.

Milk levels were, at a maximum, 0.011 ppm, and excretion in the milk accounted for less than 1% of the total excretion. Milk levels were comparable with blood levels, and both decreased rapidly when fenitrothion was withdrawn from the diet. The metabolites detected in milk were N-sulfoaminofenitrothion, N-sulfoaminofenitrooxon acetylaminofenitrooxon and desmethylacetylaminofenitrooxon. There were also residues present which were not identifiable. The dosing level of fenitrothion administered produced little ChE inhibition.

Kovacicova J, Batora V & Truchlik S (1973) Hydrolysis rate and in vitro anticholinesterase activity of fenitrothion and S-methyl fenitrothion. J Pesticide Sci 4: 759-763

The ChE inhibitory activity of S-methyl fenitrothion, an isomer of fenitrothion which can be found as a contaminant, was tested using horse serum, human serum and fly heads as the source of ChE. S-Methyl fenitrothion was found to be 200-300 times as potent an inhibitor of ChE as the parent compound. A mixture with 1% S-methyl fenitrothion in fenitrothion is 10 times more active as a ChE inhibitor than fenitrothion alone, while the addition of 5% S-methyl fenitrothion decreases the concentration required to produce to 50% inhibition by 30 times in comparison to fenitrothion alone. It was recognised that it was difficult to predict the effects in biological systems, and *in vivo* tests were required.

Vardanis A & Crawford LG ((1964) Comparative metabolism of O,O-Dimethyl-Nitrophenol phosphorothioate (methyl parathion) and O,O-Dimethyl O-(3-Methyl-4-nitrophenol) phosphorothioate (Sumithion). J Econ Entomol 57:1 136-139

The metabolism of fenitrothion and parathion-methyl was examined in order to determine a justification for the lower mammalian toxicity of fenitrothion, given that the 2 compounds have comparable insecticidal efficacy.

The antiChE activity of parathion-methyl was increased following incubation with mouse liver slices in comparison to the activity of the compound without incubation, both with fly head ChE and bovine erythrocyte ChE. A slight decrease in ChE inhibition was seen for fenitrothion following incubation with mouse liver slices.

Incubation of both compounds with cockroach body fat demonstrated an increase in the antiChE activity in comparison to the activity of the compound without incubation. When the compounds were incubated with isolated mouse liver microsomes, there was a clear increase in the antiChE activity of both compounds, particularly when measured using fly head ChE. This was followed by testing the metabolism of fenitrothion, fenitrooxon and parathion-methyl by the supernatant fraction of mouse liver. The activation and degradation rates of fenitrothion were on the order of 3 times greater than parathion-methyl.

Therefore, it was suggested that the lower mammalian toxicity of fenitrothion in comparison to parathion-methyl is due to the increased metabolism of fenitrothion and its metabolites which occurs in the mammalian liver, in comparison to the metabolism of parathion-methyl.

Hollingworth RM, Metcalf RL & Fukuto TR (1967) The selectivity of Sumithion compared with methyl parathion. Metabolism in the white mouse. J Agr Food Chem 15(2): 242-249

The metabolism of P^{32}-labelled parathion-methyl and fenitrothion (purity and source not given) in male Swiss mice (source not given) was investigated at a number of dosage levels. Parathion-methyl was administered at 3 mg/kg bw, a dose which caused slight symptoms of intoxication and at 17 mg/kg bw, a dose which caused severe cholinergic symptoms. Fenitrothion was given at 3 and 17 mg/kg bw, to compare metabolism at these doses with that of parathion-methyl. It was also given at 200 and 850 mg/kg bw. These doses produced slight and severe cholinergic signs respectively. Mice were housed individually in metabolism cages, and urine and faeces collected for 72 h following dosing.

Fenitrothion was excreted rapidly and relatively completely. Within 24 h, 55% of the highest dose administered was collected in the urine. For the 3 lower doses, more than 75% of the administered dose had been excreted within 24 h. After 72 h, >90% of the administered dose of fenitrothion, at all levels, had been excreted in the urine or faeces. For parathion-methyl, initial excretion followed a similar pattern to fenitrothion, however excretion slowed at around 24 h, and only 85% of the administered dose had been excreted by 72 h.

The metabolite profile following administration of fenitrothion at low doses differed substantially from the profile seen at high (and toxic doses). At 3 mg/kg bw, >30% of the urinary metabolites

of fenitrothion consisted of dimethyl phosphoric acid. Desmethyl phosphate (not shown in Figure 1) made up 26% of the metabolites, while desmethyl phosphorothioate (not shown in Figure 1) made up 21% of metabolites. At 850 mg/kg bw, desmethyl phosphorothioate made up 66% of urinary metabolites, while desmethyl phosphate made up 17%. Other metabolites were present in negligible quantities at this dose. In comparison, the main urinary metabolite of parathion-methyl was dimethyl phosphoric acid, being >50% of the urinary metabolites at 3 mg/kg bw and 32% of the urinary metabolites at 17 mg/kg bw. Less than 20% of the urinary metabolites was made up of desmethyl phosphorothioate.

Thus it appears that desmethylation is an important step in the metabolism of fenitrothion, with a lesser importance for parathion-methyl. There appears, additionally to be a delay in the oxidation of the P=S bond to P=O bond in fenitrothion in comparison to parathion-methyl This is indicated by the higher proportion of P=O metabolites in the urine of parathion-methyl treated animals (71.5% at 3 mg/kg bw) in comparison to fenitrothion treated animals (64.2% at 3 mg/kg bw). This decrease or delay in activation may contribute both to the lower mammalian toxicity of fenitrothion, and also to the increase in the desmethylation metabolism.

Hladka A, Krampi V & Kovac J (1974) Effect of malathion on the content of fenitrothion and fenitrooxon in the rat. Bull Env Contam Tox 12(1): 38-45

Female Wistar rats (source and number not given) were given 200 mg/kg bw fenitrothion, 200 mg/kg bw malathion or 200 mg/kg bw malathion plus 200 mg/kg bw fenitrothion by gavage in a water emulsion with Tween 80. Animals were euthanised at unspecified time intervals between 30 min and 24 h after dosing. The level of the parent compound and the oxons was determined in the liver, muscle and blood.

The levels of fenitrothion detected in the liver were lower when malathion was administered concurrently than when fenitrothion was administered alone. In blood and muscle, the level of fenitrothion following joint administration was initially lower than when administered alone, but increased gradually with a peak at 12 h. By 24 h after administration, there were no significant differences in the levels of fenitrothion between the animals receiving fenitrothion alone, and the animals receiving both fenitrothion and malathion.

Fenitrooxon levels also peaked later when malathion was given, with levels in muscle still increasing at 24 h. With fenitrothion alone, fenitrooxon levels declined progressively from the first measurement at 30 min.

El-Sebae AG, Enan EE, Soliman SA, El-Fiki S & Khamees E (1981) Biochemical effects of some organophosphorus insecticides on new targets in white rats. J Environ Sci Health 16(4): 475-491

Adult white rats (*Rattus-rattus norvigicus*) (source - High Institute of Public Health, Alexandria University, number, sex and strain not specified) were used for a number of tests of 4 organophosphorus insecticides. The insecticides tested were RH 218 (60% purity), profenofos (98.8% purity), prothiophos (96.6% purity) and fenitrothion (96.6% purity). The source or batch number of the chemicals was not stated.

The 2 main areas investigated were *in vitro* bioassay testing of detoxification of insecticides, and *in vivo* effects of sub-lethal doses of the insecticides. For the *in vitro* tests, untreated rats were euthanised, liver and brain removed and homogenised. The homogenates were centrifuged, and the supernatant used directly for enzyme assay.

The effect of incubation period on hepatic protein interaction with brain ChE inhibition by the tested insecticides was investigated. The insecticides were incubated with liver tissue for 15, 30, 45 or 60 min, followed by boiling to stop any enzymic reactions. Following this, 0.5 mL was incubated with brain homogenate, and the ChE activity was determined.

The effect of different hepatic protein concentrations was also investigated. The insecticides were each incubated with 0, 10, 17, 31.5, 45, 60 or 71.5 mg hepatic protein for a set period of time (not given), and the effect of brain ChE tested as above. Of the insecticides tested, profenofos was a potent ChE inhibitor without incubation with liver tissue (91% inhibition). The inhibition shown by the other insecticides was: RH 218 - 61%, fenitrothion - 37% and prothiophos - 32%. Incubation with liver tissue did not alter the ChE inhibition of either profenofos or prothiophos. The inhibition of RH 218 was reduced to 34% by incubation with 71.5 mg liver tissue, and the inhibition of fenitrothion was increased to 60% by incubation with 71.5 mg liver tissue. These changes in inhibition were dose related.

The effect of different concentrations of insecticides was investigated. Liver homogenate (71.5 mg) was incubated with a range of concentrations of the insecticides, from 10^{-7} to 10^{-4} M, with the ChE activity tested as above. At very low concentrations (1×10^{-7} M), there was minimal ChE inhibition from any of the pesticides. Fenitrothion at 5×10^{-7} M produced >20% inhibition of brain ChE following incubation with the liver fraction. Profenofos showed inhibition at 1×10^{-6} M, prothiophos showed inhibition at 1×10^{-5}M and RH 218 showed inhibition at 2.5×10^{-5} M.

In vivo effects of the insecticides were tested, by administering oral doses equal to 1/10 of LD50 suspended in corn oil to 5 rats/sex. All rats including controls were examined at 1, 2, 4 and 6 h after treatment, then 1, 3, 5, 10, 14, 21 and 28 days after treatment, and blood sugar and blood urea were determined. Fenitrothion appeared to produce a transitory lowering of blood glucose and increase in BUN levels, however as the numbers of tested animals were small, and there was significant variability in the results obtained, this is of limited significance, as is the experiment in general.

Miyamoto J (1964) Studies on the mode of action of organophosphorus compounds. Part IV. Penetration of Sumithion, methyl parathion and their oxygen analogs into guinea pig brain and inhibition of cholinesterase in vivo. Agr Biol Chem 28(7):422-430

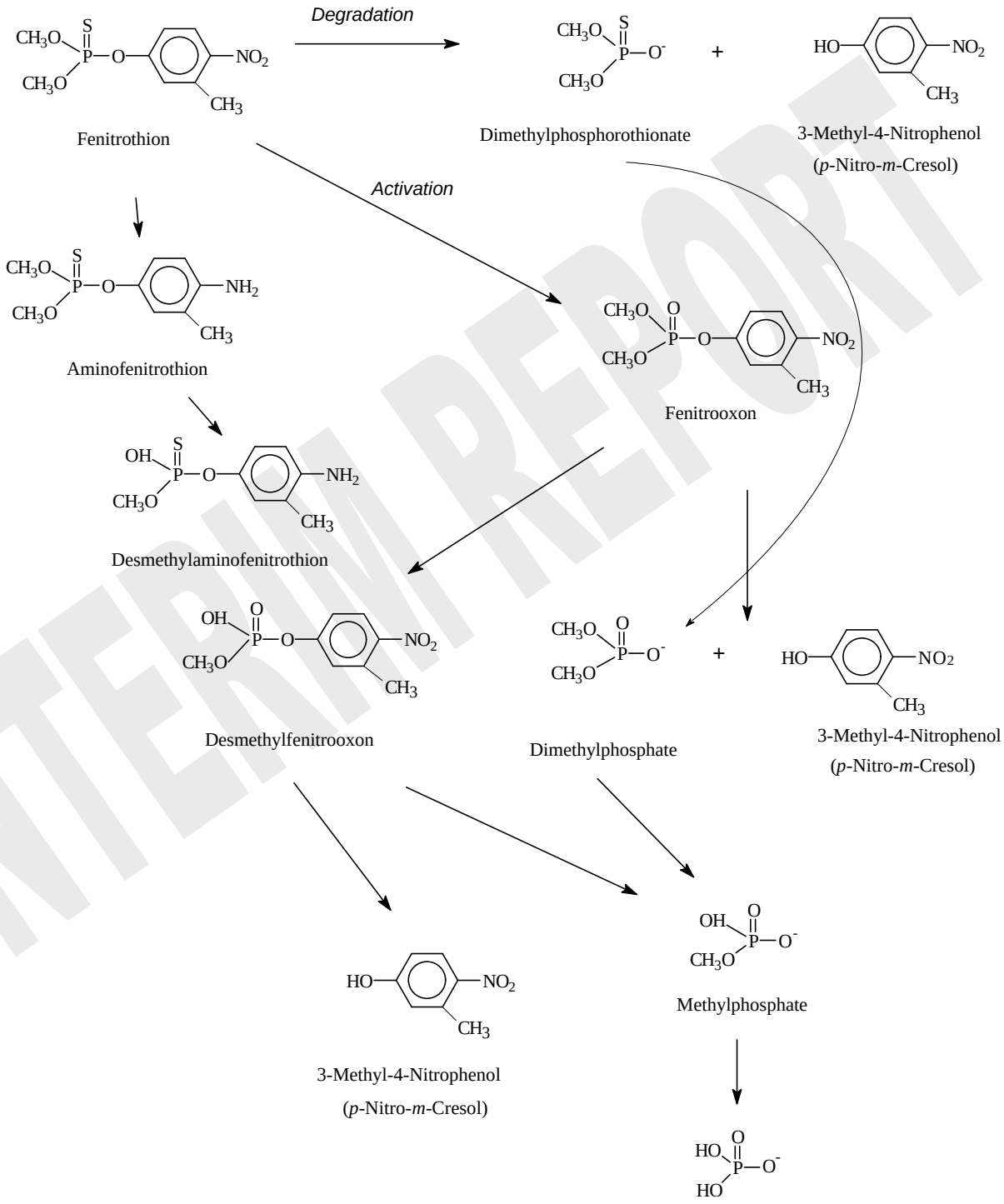
Fenitrothion, parathion-methyl, fenitrooxon and paraoxon-methyl, labelled with ^{32}P were synthesised using ^{32}P trichloride. The specific activity of the OP compounds was 10-13 mCi/g, and the purity was greater than 98%. Male guinea pigs (240 - 290 g bw, source, strain and age not specified) were given 2 mL IV of a 50% EC of OP diluted with distilled water. At intervals (not specified) after treatment animals were euthanised and the brain dissected out and homogenised.

The ChE activity of the brain tissue was determined, and the total and acid-precipitable radioactivity levels were determined.

Radioactivity in the brain peaked 5 min after administration of either parathion-methyl or fenitrothion. Most of the radioactivity was in an acid-precipitable form, and the levels of radioactivity in the brain appeared to be higher following dosage with parathion-methyl than after fenitrothion treatment (derived from graph). ChE inhibition was both more rapid and more significant with parathion-methyl treatment, with maximum inhibition of 80% (from graph) occurring 15 min after treatment with 40 mg/kg bw. ChE inhibition following fenitrothion administration reached its maximum of 50% at 30 min after dosing at the same level.

The radioactivity in the brain peaked 30 min after administration of paraoxon-methyl and the high dose of fenitrooxon, however when a low dose of fenitrooxon (5 mg/kg bw) was administered, radioactivity peaked at 5 min after administration. Levels of radioactivity in the brain were lower (interpreted from graph) following fenitrooxon treatment that following paraoxon-methyl treatment, even though the dose of fenitrooxon was higher than the dose of the paraoxon-methyl. ChE inhibition following treatment with paraoxon-methyl was higher than inhibition following fenitrooxon treatment, with approximately 5 times the dose of fenitrooxon required for 80% ChE inhibition relative to paraoxon-methyl.

Figure 1 **Major Metabolic Pathway of Fenitrothion**



2.2 Effects on hepatic enzymes

Ecobichon DJ & Zelt D (1979) The acute toxicity of fenitrothion in weanling rats and effects on tissue esterases and mono-oxygenases. Toxicol 13: 287-296

Fenitrothion (purity 97%, batch no. not given, Sumitomo Chem Co, Japan) was administered in peanut oil by gavage to young male Wistar rats (Charles River strain, number not specified) at 50 mg/kg bw/day. Control animals received peanut oil. In the initial study, animals were dosed on 5 consecutive days; dosing ceased due to signs of acute toxicity. Survivors were euthanised in groups of 4-5 animals 2, 4, 6 or 16 days after the last treatment. In a second study, fenitrothion was administered to groups of 4-5 young male rats (as above) at 50 mg/kg bw for 1, 2 or 3 consecutive days. Animals were killed 24 h after the last dose, and tissues were removed for enzyme analysis.

In a third study, groups of 4 non-fasted young male Wistar rats received a single oral dose of fenitrothion at 300 mg/kg bw. Groups were euthanised at hourly intervals up to 8 h after administration, and tissues were removed for analysis of the levels of fenitrothion.

Plasma and erythrocyte ChE levels were determined. Hepatic and renal non-specific carboxylesterase activities were also determined. The levels of 2 hepatic microsomal mono-oxygenase enzymes (*p*-nitroanisole O-demethylase and aniline hydroxylase) were determined. An *in vivo* assessment of hepatic function was done on control and treated animals by measuring the pentobarbitone sleeping time. Liver glycogen content was determined. Fenitrothion levels in blood and tissue were determined.

Moderate signs of toxicity were seen in many animals within 1 h of receiving the 3rd daily dose of 50 mg/kg bw. Severe signs of toxicity were seen in animals receiving 5 doses, including generalised tremors and clonic convulsions. By 4 days after the final dose, survivors showed no clinical signs.

Liver and kidney tissue examined 24 h after the 3rd dose of fenitrothion had levels ranging between 0.03 and 1.54 ppm and 0.28 to 0.83 ppm respectively. Plasma and erythrocyte ChE inhibition was determined following the 3rd dose of fenitrothion. There was significant inhibition of ChE in both plasma and erythrocytes, with levels less than 25% of control animals. Erythrocyte ChE levels were decreased in comparison to controls (ie more than 20% inhibition) at 6 days after the final dose, but had returned to normal at 17 days after the final dose. Plasma ChE levels had returned to normal levels 6 days after the final dose. Brain ChE levels were also significantly decreased in the post-dosing period, and had returned to normal 17 days after the final dose.

In the second study, blood tests performed 24 h after 1, 2 or 3 daily oral doses of 50 mg/kg bw fenitrothion revealed significant brain, plasma and erythrocyte ChE inhibition by a single dose. There was approximately 50% inhibition seen in all tissues. Levels were further decreased by a second dose, with approximately 75% inhibition, however this inhibition was not changed by the administration of a 3rd dose of fenitrothion.

In the third study, blood and tissue analyses performed at hourly intervals following administration of a high dose of fenitrothion (300 mg/kg bw) demonstrated that peak levels were obtained 2 h after administration in both liver and plasma. Liver levels were approximately 5 times those seen in plasma at this time, and decreased rapidly to levels comparable with plasma levels by 8 h after administration. Clinical signs of toxicity were seen within 30 min of administration, with greatest severity observed at 2 h, and signs disappearing by 3-4 h post administration.

Following 3 consecutive daily doses of 50 mg/kg bw fenitrothion, there were significantly ($p<0.05$) reduced levels of hepatic microsomal *p*-nitroanisole O-demethylase and aniline hydroxylase activity, with levels approximately 40% of those of control animals. Additionally, the pentobarbitone sleeping time was significantly ($p<0.05$) increased (by approximately 40%) in fenitrothion treated animals while liver glycogen levels were decreased approximately 35%.

Mihara K, Isobe N, Ohkawa H & Miyamoto J (1981) Effects of organophosphorus insecticides on mitochondrial and microsomal functions in the liver of rat with special emphasis on fenitrothion. J Pesticide Sci 6: 307-318

Fenitrothion (96.5% purity, Sumitomo Chem Co), diazinon (96.5% purity, Sankei Chem Co) and parathion-methyl (98.6% purity, Sumitomo Chem Co) were suspended in taurocholate (with the addition of corn oil for parathion-methyl), and administered orally to Sprague- Dawley rats (Shizuoka Agricultural Co-operative Association for Laboratory Animals) using different dosing regimens.

In the first regimen, 5 rats/sex/group received single oral doses of fenitrothion at 5, 25 or 250 mg/kg bw, or of diazinon at 300 mg/kg bw, or of parathion-methyl at 10 mg/kg bw. In the 2nd, 5 rats/sex/group received fenitrothion at 5 or 25 mg/kg bw/day on 3 consecutive days per week for 12 weeks. In the 3rd, an 11% EC formulation of fenitrothion was administered at 227 mg/kg bw/day (equal to 25 mg/kg bw/day of fenitrothion technical) on 3 consecutive days per week for 12 weeks. A vehicle control and control group was maintained.

Rats were sacrificed at various time intervals (not specified) after dosing, and liver mitochondrial and microsomal fractions prepared. The mitochondrial respiratory control ratio, the ADP/O ration, the rate of mitochondrial succinate oxidation and the mitochondrial ATPase were determined. The cytochrome P-450 content in microsomes was determined, as was the activity of aniline hydroxylase, aminopyrine N-demethylase and the protein content in mitochondria and microsomes.

Following single doses of the insecticides, the mitochondrial respiratory control rate decreased following fenitrothion at 250 mg/kg bw. This decrease was seen after 24 h in males and 72 h in females. The ADP/O ratio was not altered following insecticide administration. ATPase activity was significantly decreased ($p<0.05$) in both males and females following fenitrothion at 250 mg/kg bw, with the decrease seen at 6 h in males and 12 h in females. ATPase activity was also significantly decreased ($p<0.01$) in females 1 h after dosing with parathion-methyl. The cytochrome P-450 content was significantly ($p<0.01$) decreased in males following treatment with either fenitrothion at 250 mg/kg bw or diazinon. In females significant ($p<0.01$) decreases in

cytochrome P-450 were seen after fenitrothion at all doses and after diazinon. Parathion-methyl produced significant ($p<0.05$) decreases in cytochrome P-450 content in females after 3 h. Aniline hydroxylase activity was significantly ($p<0.01$) decreased in both males and females following treatment with all insecticides tested. Aminopyrine N-demethylase activity was significantly ($p<0.01$) decreased in males after fenitrothion at 250 mg/kg bw or diazinon treatment, while levels in females were only decreased following diazinon treatment.

After multiple dosing, aniline hydroxylase activity was significantly ($p<0.01$) increased in males following 4 weeks of treatment with fenitrothion at 25 mg/kg bw/day. Aminopyrine N-demethylase activity was significantly ($p<0.01$) increased in both males and females following 4 weeks of fenitrothion at 25 mg/kg bw, whether administered as the technical grade or as an 11% EC formulation. No other significant changes were identified following chronic fenitrothion administration.

Miyamoto J, Mihara K, Kadota T & Okuno Y (1977a) Toxicity and metabolism in vivo of fenitrothion in rats with experimental hepatic lesion J Pesticide Sci 2: 271-277

Five-week old HLA Wistar rats (Nihon Dobutsu Co, Osaka) were treated with CCl_4, DDM, or fed a low protein-high fat (LPHF) diet for 8 consecutive weeks to cause liver damage, and then used in tests of toxicity of fenitrothion and fenitrooxon. The above treatments continued during and after fenitrothion application.

Groups of 5-8 rats were treated by gavage with a single dose of 10 mL/kg bw with technical fenitrothion (Sumitomo Chem Co, Lot No 31137, purity 98.5%), suspended in 10% Tween 80. Rats were observed for 14 days, and the LD50 was calculated. The IV LD50 of fenitrooxon was determined after 1 mL/kg bw was injected IV into the femoral vein of groups of 5-7 rats.

m-Methyl-^{14}C-fenitrothion at 15, 50 or 150 mg/kg bw was administered to groups of 4 rats which had not been pretreated with liver toxicants. Rats were maintained in metabolism cages either to collect urine and faeces, or to obtain blood samples. Some rats (number unspecified) had been pretreated for 4 weeks with fenitrothion in the drinking water at 2.8 mg/kg bw/day. Plasma and erythrocyte ChE activity in these rats was decreased to 40-70% of control values at the time of administration of the test dose. Fenitrothion and fenitrooxon levels in the blood were determined. Urinary radioactive metabolites were separated and identified, mainly by 2 dimensional thin layer chromatography.

Pre-treatment with CCl_4 or DDM decreased the toxicity of fenitrothion, with the LD50 changing from 480 mg/kg bw in untreated controls to 980 mg/kg bw in treated animals. The LPHF diet marginally decreased the LD50 value to 530 mg/kg bw. A marked effect was present when the minimum toxic dose was evaluated. This was 25 mg/kg bw for untreated controls, while animals pretreated with liver damaging chemicals tolerated 500 mg/kg bw prior to toxic signs. The animals on a LPHF diet also had an increased minimum toxic dose, being 250 mg/kg bw. Fewer differences were seen between pretreated and control animals for IV injections of fenitrooxon, with only DDM-treated animals showing more tolerance for fenitrooxon.

In another series of experiments, each 6 rats from the above 4 treatment groups (control, DDM, CCl_4 & LPHF) were sacrificed 1, 3, 6, 9, 24 and 48 h after oral dosing with 15, 50 or 150 mg/kg bw fenitrothion, and plasma, erythrocyte and brain ChE levels determined. In this examination, there was a great deal of variability in the results, and they did not correlate well with the toxicity pattern described above. The results were not reported in detail, however the comment was made that ChE inhibition was greatest within 6 h of administration and was more severe at higher doses.

In the tests using radiolabelled fenitrothion there were some dose-dependent differences in metabolism pattern. At the 2 lowest doses (15 and 50 mg/kg bw), blood fenitrothion levels were maximal at 1 h post administration. The low protein-high fat diet animals had the highest blood fenitrothion levels, with CCl_4>DDM>control animals. At the highest dose (150 mg/kg bw), the low protein high fat diet animals had a delayed maximal fenitrothion concentration, with the highest levels reached 3 h post administration. This level was lower than that reached by the other pretreated animals, although otherwise the metabolism pattern was similar at this dose. Fenitrooxon was below the level of detection (0.001 ppm) in the 2 lowest doses. At the highest dose, 0.003 ppm fenitrooxon was detected in control animals immediately after administration.

The extent of fenitrothion excretion into urine and faeces was examined after acute administration of 15, 50 or 150 mg/kg bw of radiolabelled fenitrothion, and also after 15 mg/kg bw fenitrothion following a 4 week pre-treatment with 2.9 mg/kg bw fenitrothion. At the 2 lowest doses, excretion of fenitrothion was complete after 2 days, with the majority being found in the urine. The excretion profile was not changed by pre-treatment with fenitrothion. At 150 mg/kg bw, there were detectable metabolites in the urine 5 days after administration. In rats treated with hepatotoxins, the percentage of desmethylfenitrothion in the urine was increased and the percentage of desmethylfenitrooxon decreased following fenitrothion treatment at 15, 50 or 150 mg/kg bw. However, at 150 mg/kg bw the percentage of desmethylfenitroxon excreted was limited to approximately 12% in all treatment groups including control. Excretion of 3-methyl-4-nitrophenol was highest in controls, with decreased levels in the animals exposed to hepatotoxins. Pretreatment of liver damaged animals with fenitrothion shifted the excretion pattern to be more similar to that seen in control animals. The depression in the oxidative capacity of the livers of these animals, as well as the decrease in capacity for glucuronidation and sulfate conjugation can account for these differences in the metabolite profile.

Thus, in summary, rats treated with 3 liver toxicants were less susceptable to poisoning by fenitrothion as revealed by a higher LD50. The elimination profile after either a single or with repeated doses was essentially similar among all treatment groups except at the highest fenitrothion dose (150 mg/kg bw). Toxicant-treated rats produced more desmethylfenitrothion except at 150 mg/kg bw where the quantitative and qualitative differences in fenitrothion metabolites formed among control and toxicant-treated rats were minimal.

Miyamoto J, Kadota T & Mihara K (1977b) Experimental hepatic lesions and drug metabolizing enzymes in rats. J Pesticide Sci 2: 257-269

Male HLA-Wistar rats (Nihon Dobutsu Co, Osaka) were maintained in 4 groups; Group A - a control group (fed basal diet), Group B - maintained on DDM at 1000 ppm in the diet, Group C -

administered 0.75 mL CCl_4 IM twice weekly, and Group D - maintained on a low protein-high fat (LPHF) diet. The animals were maintained on these regimens for up to 12 weeks.

At certain intervals (not given) 6-12 animals per group were euthanised. The liver was dissected out, weighed, chilled and examined both histopathologically and by electron microscopy. The serum was analysed for total protein, albumin, bilirubin, AP, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, cholesterol and lactic dehydrogenase. Plasma, erythrocyte and brain ChE inhibition were measured.

Bromosulphalein (BSP) clearance time was tested. Bile flow from animals with a bile duct cannula surgically implanted was measured for 4 h. The liver water and fat content was determined. Hepatic microsomal fractions were prepared. Microsomal aminopyrine N-demethylase, aniline hydroxylase, P-450 and p-nitrophenol acetate hydroxylase activities were determined. Additionally, glutathione S-alkyltransferase activity was assayed.

The *in vitro* metabolism of fenitrothion by either the supernatant or microsomal fraction of a centrifuged liver homogenate was investigated. Four tests, to investigate different pathways were done These included; O-demethylation of fenitrothion and fenitrooxon by the supernatant, hydrolysis of fenitrothion to 3-methyl-4-nitrophenol by the supernatant, oxidation of fenitrothion to fenitrooxon, and oxidative cleavage to 3-methyl-4-nitrophenol by the microsomal fraction, and oxidation of fenitrooxon to *m*-hydroxymethyl analog by the microsomal fraction.

Treatment with DDM, CCl_4or LPHF diet caused a decrease in body weight and a variety of liver changes, including changes in weight, water percentage, fat percentage, BSP retention and bile excretion. Erythrocyte ChE was decreased with the LPHF diet. DDM did not change the level of metabolising enzymes present in the liver, while the other treatments significantly reduced the levels of these enzymes.

Metabolic conversion rates of fenitrothion and fenitrooxon to other metabolites are detailed in the Table below.

Metabolic transformation rates of fenitrothion.*

Group	to fenitrooxon	to desmethylfenitrothion	to 3-methyl-4-nitrophenol
Control	1.30	18.00	0.53
DDM treated	0.90	14.66	0.43
CCl_4 treated	0.15	10.32	0.13
LPHF diet	0.17	4.40	0.20

*µmoles/h/g liver, mean of 2 trials with 4 rats

Metabolic transformation rates of fenitrooxon.*

Group	to *m*-hydroxymethyl analog	to desmethylfenitrooxon	to 3-methyl-4-nitrophenol
Control	3.83	26.62	2.12
DDM treated	1.83	26.61	2.01
CCl_4 treated	0.67	17.35	0.81
LPHF diet	0.67	8.73	0.62

*µmoles/h/g liver, mean of 2 trials with 4 rats

From the Table, it appears that the metabolic transformation rate of fenitrothion and fenitrooxon was reduced by liver damage. An exception was the metabolism of fenitrooxon to desmethylfenitrooxon, which was not reduced by DDM. It is of significance that the activation of fenitrothion to fenitrooxon was significantly reduced by liver damage, which may account for the changes in the LD50 seen in a previous study (Miyamoto et al, 1977a).

Dubois KP & Kinoshita FK (1963) The acute toxicity of Bayer 41831 in combination with other anticholinesterase insecticides. Lab: University of Chicago. Sponsor, Bayer.

Female Sprague-Dawley rats were given fenitrothion (as Bayer 41831) in 20% ethanol and 20% propylene glycol, at half the LD50 in combination with half the LD50 of a range of other insecticides. Prior to conducting these potentiation tests, the LD50 of each sample was verified. The combination of administration of fenitrothion with 1 of: parathion-ethyl, parathion-methyl, systox, di-syston, malathion, EPN, guthion, trithion, phosdrin, dalvav, schradan, ethion, sevin, diazinon, foles, co-ral and ronnel indicated that no potentiation occurred following the concurrent administration.

2.3 Effects on hormonal balance

Gradowska-Olszewska I, Brzezinski J & Rusiecki W (1984) Excretion and peripheral metabolism of 1,2-^{3}H-testosterone and androgens in rats following intoxication with organophosphorus insecticides. J Appl Toxicol 4(5): 261-264

Methylbromphenvinphos (110 mg/kg bw) or fenitrothion (260 mg/kg bw) in corn oil was administered PO by gastric intubation to mature male Wistar rats (source not given). Controls received corn oil. All animals had *ad libitum* access to food and water. Plasma testosterone was determined 15 and 30 min and 1, 2, 5, 24, 48, 72, 96 and 120 h after the pesticide dose. During the test period, animals were housed in metabolic cages, and 24-h urine samples were collected for 5 days. Testosterone metabolites androsterone, etiocholanolone and dehydroepiandrosterone levels in urine were determined.

Additionally the peripheral metabolism of 1,2-^{3}H-testosterone was examined. Five min and 24 or 48 h after pesticide administration, the rats were given an IP dose of 50µCi 1,2-^{3}H-testosterone.

Twenty four h urine samples were collected, and labelled testosterone and its metabolites were isolated.

Fenitrothion administration produced an initial lowering of the plasma testosterone levels, with level decreased to 25% of control animals at 24 h after administration. Levels were increased to 50% of that of control animals at 48 h after dosing, and there appeared to be a rebound increase at 72 h, which then decreased to normal at 96 h after administration. The urinary excretion of testosterone metabolites was decreased after fenitrothion administration. It therefore appears that high doses of fenitrothion can produce a short term alteration in testosterone synthesis.

Clos MV, Ramoneda M & Garcia G (1994) Modification of testicular cytochrome P-450 after fenitrothion administration. Gen Pharmacol 25(3): 499-503

Fenitrothion (96.8% purity, obtained from Sumitomo Chem Co) in corn oil was administered PO to males Sprague-Dawley rats (source not stated) in 1 of 3 dosing strategies; 165 mg/kg bw in a single dose, 55 mg/kg bw/day for 3 days or 5.5 mg/kg bw/day for 30 days.

Rats were decapitated 24 h after the last administration of fenitrothion. Liver and testes were homogenised, and a microsomal fraction prepared. Tissue cytochrome P-450, NADPH cytochrome c reductase and cytochrome b_5 activities were determined. The plasma levels of testosterone were also quantified. Acute and subacute administration of fenitrothion resulted in a significant reduction of cytochrome P-450 enzyme activities in liver and testes, with levels reduced to approximately 50% that seen in controls for both these tissues. Chronic administration did not produce these changes. The acute and subacute treatments also resulted in decreases in plasma testosterone levels, with the concentration reduced to 2% of control values following the administration of fenitrothion. While chronic administration resulted in a decrease of testosterone concentration, this was on the order of 15-20% and was not considered biologically significant. The decrease in plasma testosterone is in excess of the effects that would be expected from the decrease in cytochrome P-450 levels. These inhibitory effects appear to occur only at high doses of fenitrothion, and are not produced by chronic dosing at 5.5 mg/kg bw/day.

Yamatomo T, Egashira T, Yoshida T & Kuroiwa Y (1982) Increase of adrenal weight in rats by the repeated administration of fenitrothion. Toxicol Lett 11: 187-191

This published paper investigated the effect of fenitrothion on adrenal weight and plasma corticosterone and glucose concentration during short-term repeat-dose treatment.

Technical fenitrothion (source or purity not stated) in olive oil was administered to male Wistar rats (4/group) by gavage at doses of 0 (vehicle), 7.25 or 14.5 mg/kg bw/day for 28 consecutive days. Blood was collected at weekly intervals and the plasma corticosterone and glucose concentration measured. Adrenal weights were recorded at weekly intervals..

The relative weight of the adrenals increased by 15% (not significant) and 35% ($p<0.05$) at 7.25 or 14.5 mg/kg bw/day respectively after 2 weeks of treatment, but both had returned to control weights at the end of treatment.

The concentration of plasma corticosterone increased 2-fold at 7.25 mg/kg bw/day (not significant) and 2.5 fold at 14.5 mg/kg bw/day ($p<0.05$) at week 1, but gradually returned to control levels by week 4. Glucose concentration was increased 30% at 14.5 mg/kg bw/day (not significant) at week 1, and then reduced to be below control values at week 4. No treatment-related changes on blood glucose was observed at 7.25 mg/kg bw/day.

In conclusion, the results suggest that a transient increase in adrenal weight, plasma corticosterone and glucose concentration occurs in rats after repeat administration of fenitrothion for 28 days.

3. ACUTE TOXICITY

3.1 Technical Grade Active Constituent

3.1.1 Median Lethal Dose Studies

A summary of submitted and published findings of acute median lethal dose studies with technical fenitrothion is shown in the Table below.

Median Lethal Dose Studies

Species	Sex	Route	Vehicle	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Mouse (strain ?)	M/F	PO	Glycerol:ethanol	1336 (M), 1416 (F)	OMS 43 (1964)
Mouse (dd)	M/F	PO	Tween 80	1030 (M), 1040 (F)	Kadota et al (1972)
Mouse (Wistar)	M/F	PO	Cremophor EL	775 (M), 901 (F)	Heimann (1982)
Mouse (dd)	M/F	PO	Corn oil	1400 (M), 1270 (F)	Mikami et al (1977)
Mouse (dd)	M/F	SC	Tween 80	1350 (M), 1530 (F)	Kadota et al (1972)
Mouse (dd)	M/F	Dermal	Undiluted	>2500 (M & F)	Kadota et al (1972)
Mouse (dd)	M/F	Dermal	Corn oil	>5000 (M & F)	Mikami et al (1977)
Mouse (?)	M	Dermal	Acetone	2776 (M)	Ueda & Lizuka (1961)
Mouse (dd)	M/F	IP	Tween 80	500 (M), 440 (F)	Mikami et al (1977)
Mouse (CF)	M/F	IP	Ethanol: propylene glycol	115 (M), 110 (F)	Dubois & Puchala (1960a)
Mouse (?)	M	IP	Propylene glycol	300 (M)	Valecha et al (1990)
Rat (Wistar)*	M/F	PO	?	940 (M), 600 (F)	Benes & Cerna (1970)
Rat (Sherman)*	M/F	PO	Peanut oil	740 (M), 570 (F)	Gaines (1969)
Rat (SD)*	M/F	PO	Ethanol: propylene glycol	250 (M & F)	Dubois & Puchala (1960a)
Rat (Holtzman)	F	PO	Ethanol: propylene glycol	235 (F) (mean of 4 expts)	Dubois & Kinoshita (1970)
Rat (?)	M/F	PO	Glycerol:ethanol	503 (M), 673 (F)	OMS 43 (1964)
Rat (Wistar)	M	PO	?	608 (M)	Carmargo et al (1970)

Rat (SD)	M/F	PO	Tween 80	330 (M), 800 (F)	Kadota et al (1972)
Rat (Wistar)	M	PO	Olive oil	700† (M)	Rosival et al (1976)
Rat (Wistar)	M	PO	Olive oil	490 (M)	Rosival et al (1976)
Rat (Wistar)	M/F	PO	Water	1970 (M), 3344 (F)	Hixson (1982a)
Rat (SD)	M/F	PO	Corn oil	660 (M), 1050 (F)	Mikami et al (1977)
Rat (?)	F	PO	Corn oil	1050 (F)	Mikami et al (1977)
Rat (?)	F	Dermal	Undiluted	3500 (F)	OMS 43 (1964)
Rat (Wistar)	F	Dermal	Not stated	1002 (F)	Carmargo et al (1970)
Rat (SD)	M/F	Dermal	Undiluted	890 (M), 1200 (F)	Kadota et al (1972)
Rat (SD)	M/F	Dermal	Corn oil	2700 (M), 5000 (F)	Mikami et al (1977)
Rat (SD)	M/F	SC	Tween 80	840 (M), 1300 (F)	Kadota et al (1972)
Rat (SD)	M/F	Inhal (8 h)	Kerosene:xylene	>186 (M & F)	Kohda & Kadota (1979a)
Rat (SD)	M/F	Inhal (4 h)	Corn oil	>2210 (M & F)	Kohda et al (1986)
Rat (SD)	M	Tracheal	Corn oil	950 (M)	Chevalier et al (1982)
Rat (SD)*	M/F	IP	Ethanol: propylene glycol	135 (M), 160 (F)	Dubois & Puchala (1960a)
Rat (SD)	M	IP	Corn oil	300 (M)	Chevalier et al (1982)
Guinea pig (?)*	?	PO	Sorpol 2001	1850	Miyamoto et al (1963)
Guinea pig (?)	M	PO	Glycerol:ethanol	1000 (M)	OMS 43 (1964)
Guinea pig (?)*	M	PO	Ethanol: propylene glycol	500 (M)	Dubois & Puchala (1960a)
Guinea pig (?)	M	IP	Ethanol: propylene glycol	110 (M)	Dubois & Puchala (1960a)
Rabbit (NZW)	M/F	Dermal	Water	3290 (M), >2000 (F)	Hixson (1982b)

*Studies not reviewed but cited in IPCS, EHC No. 133; † Purified fenitrothion.

Irritation studies ***Irritation***

Skin	Rabbit [NZW]	0.5mL of 24.12 % soln/ intact and abraded sites/24 & 72 h	mildly irritating	Hixson (1982c)
Skin	Rabbit [NZW]	0.5mL/ abraded sites/24 h	non-irritating	Hara & Suzuki (1981)
Skin	Rabbit [NZW]	?	non-irritating	Thyssen & Lorke (1982)
Eye	Rabbit [NZW]	0.1mL/conjunctival sac, unrinsed & rinsed	non-irritating	Hara & Suzuki (1981)
Eye	Rabbit [NZW]	?	non-irritating	Thyssen & Lorke (1982)
Eye	Rabbit [NZW]	0.1mL of 24.12% soln/ conjunctival sac, unrinsed & rinsed	non-irritating (rinsed); slight irritant (unrinsed)	Hixson (1982c)

Skin sensitisation studies

Skin sens.	Guinea pig [H]	induction (12 M)	non-sensitiser	Kohda et al (1972)

[NZW] New Zealand White; [H] Hartley;

3.1.1.1 Oral

Heimann KG (1982) S5660 -Test for acute oral toxicity on mice. Institute of Toxicology, Germany. Report date: April 27, 1982.

Technical fenitrothion (S 5660) (Batch No. 27685500; purity 97.4%) in Cremophor EL was administered PO by gavage to Wistar albino mice (10/sex/group; Winklemann, Borchen, Germany) at dose levels of 100, 500, 600, 750, 900 or 1000 mg/kg bw. Animals were observed daily for 14 days following treatment. Gross necroscopy was performed on all animals. No reference was made to the QA/GLP status of the study, or to the use of any vehicle control groups.

At the end of the 14-day observation period, deaths among treatment groups was 0/10, 0/10, 1/10 (2/10 for females), 4/10, 8/10 and 10/10, respectively (mortality rates for females the same as for males except where indicated). In all groups receiving a dose of 750 mg/kg bw or above toxicity was observed 1 h after administration and consisted of rigidity, shivering, salivation, reduced mobility and laboured respiration. Surviving animals were normal in appearance and behaviour 6 days after administration. No pathomorphological findings could be attributed to treatment.

The acute PO LD50 was 775 mg/kg bw for males and 901 mg/kg bw for females.

Kadota T, Kagoshima M, Yamazaki H & Miyamoto J (1972) Acute oral, subcutaneous and dermal toxicities of Sumithion technical in mice and rats. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: September 30, 1972.

and

Miyamoto J & Kadota T (1972) Toxicological studies with Sumithion. Research Department, Pesticides Division Sumitomo Chemical Co, Osaka, Japan. Report date: December, 1972. (Same data)

In a series of acute toxicity studies fenitrothion technical (Sumitomo Chem Co; Lot No. 417; purity 97.2%) in 10% Tween-80 was administered PO to dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) aged 6-7 weeks. Animals were observed for 7 days post treatment. No reference was made to the QA status of the study.

Fenitrothion was administered PO by gavage to mice (8/sex/group) at doses of 0, 500, 700, 980, 1370 or 1920 mg/kg bw. Deaths among treatment groups was 0/8, 1/8, 4/8 (3/8 for females), 6/8, and 8/8, respectively (mortality rates for females the same as for males except where indicated). At 1920 mg/kg bw, all animals died within 24 h, whereas no toxicity was observed at 500 mg/kg bw. In groups receiving a dose at or in excess of 700 mg/kg bw, decreased spontaneous activity, dyspnoea, ataxia, lacrimation, laboured respiration, salivation, slight tremor and clonic convulsions were observed 30 min after administration.

The acute PO LD50 was 1030 mg/kg bw for males and 1040 mg/kg bw for females.

Fenitrothion was administered PO by gavage to Sprague-Dawley rats (8/sex/group) at doses of 0, 52, 73, 102, 143, 200, 280, 392, 550 or 770 mg/kg bw; and, for females 0, 52, 73, 102, 200, 280, 392, 550, 770, 1080 and 1510 mg/kg bw for females. Mortality in the treatment groups for males was 0/8, 0/8, 0/8, 0/8, 0/8, 2/8, 2/8, 5/8, 6/8, 8/8 respectively; and for females 0/8, 0/8, 0/8, 0/8, 0/8, 0/8, 1/8, 1/8, 2/8, 5/8, 8/8 respectively.

No toxicity was observed at 52 mg/kg bw. At 770 mg/kg bw for males and 1510 mg/kg bw for females, all animals died within 24 h. In groups receiving between 73-102 mg/kg bw, muscular twitch, tremor, ataxia, dacryohaemorrhoea, salivation, exophthalmus and bladder incontinence were observed 10-30 min after administration. These clinical signs were no longer apparent after 3-4 h. Above 143 mg/kg bw, toxicity was more severe. Surviving animal recovered within 48-96 h but with tremors, ataxia, dacryohaemorrhoea, and exophthalmus still apparent.

The acute PO LD50 was 330 mg/kg bw for males and 800 mg/kg bw for females.

Rosival L, Vargova M, Szokolayova J & Cerey K (1976) Contribution to the toxic action of S-methylfenitrothion. Pesticide Biochem Physiol 6: 280-286

The acute oral toxicity of technical fenitrothion (Chemické závody J. Dimitrova, Bratislava, Czechoslovakia; batch not stated, purity 96%), purified fenitrothion (derived from the technical fenitrothion) and the S-methyl isomer (SMF; freshly prepared by demethylation) were compared.

Rosival L, Vargova M, Szokolayova J & Cerey K (1976) Contribution to the toxic action of S-methylfenitrothion. Pesticide Biochem Physiol 6: 280-286

The acute oral toxicity of technical fenitrothion (Chemické závody J. Dimitrova, Bratislava, Czechoslovakia; batch not stated, purity 96%), purified fenitrothion (derived from the technical fenitrothion) and the S-methyl isomer (SMF; freshly prepared by demethylation) were compared.

All test compounds were suspended in olive oil and administered to male Wistar rats (number not reported) by gavage. The LD50 for purified fenitrothion, technical fenitrothion and SMF was calculated to be 700 mg/kg bw, 490 mg/kg bw and 315 mg/kg bw respectively.

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

In a series of acute toxicity studies fenitrothion technical (Sumitomo Chem Co; Lot No. 60823, purity 95.9%) in corn oil was administered PO to dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) aged 6-7 weeks. Animals were observed for 14 days post treatment. Gross necroscopy was performed on animals found dead during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

Technical fenitrothion was administered PO to mice (10/sex/group) at doses of 500, 650, 845, 1000, 1300, 1700, 2200 or 2860 mg/kg bw. At the end of the 14-day observation period, mortality in the dosed males was 0/10, 0/10, 0/10, 1/10, 3/10, 8/10, 10/10, and 10/10 respectively;

and, for females 0/10, 0/10, 0/10, 4/10, 4/10, 9/10, 10/10, and 10/10 respectively. The acute PO LD50 was 1400 mg/kg bw for males and for females 1270 mg/kg bw.

Technical fenitrothion was administered PO to rats (10/sex/group) at doses of 100, 200, 346, 450, 590, 770, 1000, 1300, or 2000 mg/kg bw. At the end of the 14 day observation period, mortality in the dose groups for males was 0/10, 0/10, 2/10, 3/10, 4/10, 6/10, 7/10, 9/10, and 10/10, respectively; and, for females 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 5/10, 8/10, and 10/10, respectively. The acute PO LD50 was 660 mg/kg bw for males and for females 1050 mg/kg bw.

Clinical signs in mice and rats consisted of decreased spontaneous activity, dyspnoea, ataxia, lacrimation, laboured respiration, salivation, slight tremor and clonic convulsions (no reference was made to the dose at which clinical signs occurred). The onset of clinical signs was within 30-60 min in mice and rats and deaths were observed within 24-48 h. Surviving animals had no clinical signs 2-3 days post treatment. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

Hixson EJ (1982a) Acute oral toxicity of fenitrothion concentrate use dilution. Corporate Toxicology Department, Mobay Chemical Corporation, USA. Report date: May 3, 1982.

Technical fenitrothion (Mobay Chem Corp: Batch No.18-R-92-143; purity 75.8%) was administered PO to Wistar rats (10/sex/group; Sasco, Inc., Nebraska, USA) at dose levels of 160, 224, 313, 442, 625, 884, 1250, 1768, 2500, 3535, or 5000 mg/kg bw for males; and 160, 224, 442, 625, 884, 1250, 1768, 2500, 2973, 3535, 4204, or 5000 mg/kg bw for females. Rats were observed daily for 14 days following treatment after which gross necroscopy was performed. The study was conducted in accordance with GLP guidelines, however, no reference was made to the use of any vehicle control groups.

Deaths among treatment groups were 1/10, 0/10, 1/10, 0/10, 0/10, 0/10, 0/10, 4/10, 8/10 (4/10 for females), 10/10, and 10/10, respectively (mortality rates for females the same as for males except where indicated). Toxicity was observed at all dose levels 30 min after administration and consisted of salivation, diarrhoea, lacrimation, pilo-erection, ptosis, exophthalmos, muscle fasciculations, tremors, convulsions, ataxia, and decreased activity. No pathomorphological findings could be attributed to treatment.

The acute PO LD50 was 1979 mg/kg bw for males and 3344 mg/kg bw for females.

3.1.1.2 Subcutaneous

Kadota T, Kagoshima M, Yamazaki H & Miyamoto J (1972) Acute oral, subcutaneous and dermal toxicities of Sumithion technical in mice and rats. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: September 30, 1972.

and

Miyamoto J & Kadota T (1972) Toxicological studies with Sumithion. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: December, 1972. (Same data)

In a series of acute dermal toxicity studies fenitrothion technical (Sumitomo Chem Co; Lot No. 417; purity 97.2%) in 10% Tween-80 was administered PO to dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) aged 6-7 weeks. Animals were observed for clinical signs and mortality for 14 days post administration. No reference was made to the QA status of the study.

Technical fenitrothion was administered SC to dd mice (8/sex/group) at doses of 0, 500, 750, 1130, 1690 or 2540 mg/kg bw. At the end of the 14-day observation period, deaths among treatment groups were 0/8, 0/8, 1/8, 2/8, 5/8 (4/8 for females), and 7/8, respectively (mortality rates for females the same as for males except where indicated). At 500 mg/kg bw, a slight decrease in spontaneous activity was observed for 1 h post treatment. At doses at and above 750 mg/kg bw, clinical signs observed were dyspnoea, decrease of spontaneous activity, ataxia, lacrimation, and salivation 30-60 min after administration. These clinical signs were no longer apparent after 10 days. No notable differences in toxicity and mortality rate were observed between males and females. The acute SC LD 50 was 1350 mg/kg bw for males and 1530 mg/kg bw for females.

Technical fenitrothion was administered SC to Sprague-Dawley rats (8/sex/group) at doses of 0, 50, 100, 250, 500, 715, 1000, 1400, 1960, or 2740 mg/kg bw. At the end of the 14-day observation period, mortality in males was 0/8, 0/8, 0/8, 1/8, 4/8, 5/8, 4/8, 7/8, 7/8, and 8/8 respectively; and, for females 0/8, 0/8, 0/8, 0/8, 0/8, 2/8, 2/8, 3/8, 7/8, and 8/8 respectively. At doses at and above 100 mg/kg bw in males and 250 mg/kg bw in females, clinical signs developed 2 to 3 h after administration and included muscular twitch, tremor, ataxia, dacrohaemorrhoea, salivation, exophthalmus and incontinence of the urine. The toxic signs persisted up to 7-10 days post treatment, in particular the hyperexcitability and tremors in animals receiving doses above 500 mg/kg bw. Clinical signs in surviving animals subsided after 12-14 days.

The acute SC LD50 was 840 mg/kg bw for males and 1300 mg/kg bw for females.

3.1.1.3 Intraperitoneal

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

Technical fenitrothion (Sumitomo Chem Co; Lot No. 60823; purity 95.9%) in 10% Tween-80 was administered IP to dd mice (Nihon Dobutsu Co., Japan) aged 6-7 weeks (10/sex/group) at doses of 100, 130, 170, 220, 286, 372, 500, 650, 845 or 1000 mg/kg bw. Mice were observed for 14 days post treatment. Gross necroscopy was performed on animals found dead during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

Deaths in the dose groups were 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 5/10 (6/10 females), 8/10, 9/10, and 10/10, respectively (mortality rates for females the same as for males except where indicated). Clinical signs (observed 15-20 min post dose) consisted of decreased motor activity, irregular respiration, ataxia, salivation, and tremors and deaths were observed within 24-48 h. Surviving animals had no clinical signs 2-3 days post treatment. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

The acute LD50 was 500 mg/kg bw for males and 440 mg/kg bw for females.

3.1.1.4 Intratracheal

Chevalier G, Bastie-Sigeac I & Cote MG (1982) Morphological assessment of fenitrothion pulmonary toxicity in the rat. Tox Appl Pharmacol 63: 91-104

A morphological assessment was performed in rats to determine the pulmonary toxicity of fenitrothion after IP and intratracheal administration. The LD50 was determined for both routes of exposure. Following anaesthesia, technical fenitrothion (Sumitomo Chem Co, purity 95%) in corn oil was administered intratracheally with a microsyringe (0.25 mL/kg) to groups of 5 male Sprague-Dawley rats (Charles River Labs, Quebec) at dose levels of 2, 20 or 200 mg/kg bw. The highest dose chosen for the pulmonary morphological assessment was based on complete survival in the LD50 determination. Some rats (numbers not stated) were sacrificed at 1, 3, 7, 21, 60 and 180 days post injection and lungs sections examined by light and electron microscopy.

The LD50 determined was 300 mg/kg bw and 950 for mg/kg bw respectively for IP and intratracheal administration. Clinical signs following intratracheal administration were typical of cholinergic intoxication and at 200 mg/kg bw produced tremors, convulsions and death (the number of deaths and the associated clinical signs for IP administration were not discussed).

The microscope findings only apply to rats that received intratracheal administration of fenitrothion. From microscopy it was ascertained that acute focal pulmonary lesions occurred at all doses. Typically, during week 1, alveolar type 1 pneumocytes were damaged and replaced by type 2 pneumocytes, concurrently with white blood cell infiltration and collagen fibrillogenesis, followed by mild fibrosis at day 21. Generally, pulmonary changes at 60 and 180 days were limited, consisting of fibrotic thickening of the interstitial septa, particularly at 200 mg/kg bw, however, no histological or ultrastructural changes were detected in alveolar tissue 180 days after 20 mg/kg bw, or 60 days after 2 mg/kg bw. It was concluded from the study that fenitrothion induces limited pulmonary lesions.

Khan MF, Abidi P, Anwer J, Ray PK & Anand M (1990) Pulmonary biochemical assessment of fenitrothion toxicity in rats. Bull Envir Contam Toxicol 45: 598-603

The objective was to study whether any alterations in the biochemistry of lung fluid and lipid peroxidation in lung mitochondria occurred, as these parameters have been suggested as indicators of lung tissue damage following acute fenitrothion exposure.

Fenitrothion technical (Bayer India Ltd; purity and batch No. not stated) in ground nut oil was administered intratracheally to groups of 4-5 male Wistar rats at a dose of 0 or 30 mg/kg bw. Control rats received an equivalent volume of vehicle. Groups were sacrificed at 1, 4, 7, 14, 21, and 30 days post injection and the lungs lavaged. Following centrifugal separation, a cell free aliquot (volume not stated) was assayed for levels of lactate dehydrogenase (LDH), ascorbic acid, sialic acid, protein and phospholipids. Additionally, lung mitochondria were prepared and lipid peroxidation (nmoles malonaldehyde formed/h/mg of protein) measured.

LDH activity was significantly ($p<0.05$) increased at day 1, 4 and 7 post treatment (3.2, 6.4 and 4 fold respectively relative to controls). Thereafter, the activity declined but at 30 days was significantly ($p<0.05$) higher than controls (1.2 fold). Protein and sialic acid levels were significantly ($p<0.05$) increased at 1, 4, 7, 14 and 21 days post treatment (sialic acid levels not-significantly increased at day 21) with protein showing a maximum increase at day 1 (1.4 fold) whereas, sialic acid had a maximum increase at day 4 (3.3 fold). At 30 days post treatment, protein and sialic acid levels had returned to control values.

Ascorbic acid levels decreased (non-significantly) days 1-7 days post treatment, then a significant ($p<0.05$) increase at day 14 (38%) and day 21 (33%) was observed. At 30 days post treatment, levels had returned to control values. Similarly, phospholipid levels decreased initially (days 1-7, significant at day 1 only) post treatment, then significantly ($p<0.05$) increased at day 14 (1.2 fold), before declining to control`values at day 30 post treatment. Lung mitochondrial lipid peroxidation was significantly ($p<0.05$) increased from days 1-7 post treatment (maximum increase 7.8 fold at day 7), before, decreasing to control values at day 30.

These results indicate that significant biochemical changes following lung tissue damage take place following acute fenitrothion exposure. However, it is unclear whether these alterations are due to parent compound or a metabolite.

3.1.1.5 Dermal

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

Technical fenitrothion (Sumithion, Lot No. 60823, purity 95.9%) in corn oil was applied dermally to groups of dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) aged 6-7 weeks (10/sex/group). The animals backs were clipped free of hair and the test compounds applied. Tape was applied to prevent ingestion then removed 24 h later and the site cleaned with cotton soaked with ether. Gross necroscopy was performed on animals dying during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

No clinical signs or deaths were recorded in male and female mice administered fenitrothion at doses of 1000, 2500 or 5000 mg/kg bw. The acute LD50 was >5,000 mg/kg bw.

Technical fenitrothion was administered to male and female rats at doses of 250, 500, 750, 1000, 2500, or 5000 mg/kg bw. At the end of the 14-day observation period, mortality in males was

0/10, 0/10, 0/10, 3/10, 4/10 and 7/10, respectively; and for females, 0/10, 0/10, 0/10, 1/10, 3/10 and 5/10, respectively.

Clinical signs (observed 16 h post dose) consisted of decreased motor activity, irregular respiration, ataxia, salivation, and tremors and deaths were observed 2-7 days post treatment. Surviving animals had no clinical signs 7-13 days post treatment. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

The acute dermal LD50 was 2700 mg/kg bw for males and 5000 mg/kg bw for females.

Kadota T, Kagoshima M, Yamazaki H & Miyamoto J (1972) Acute oral, subcutaneous and dermal toxicities of Sumithion technical in mice and rats. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: September 30, 1972.

and

Miyamoto J & Kadota T (1972) Toxicological studies with Sumithion. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: December, 1972. (Same data)

Technical fenitrothion (Sumitomo Chem Co; Lot No. 417; purity 97.2%) was applied to the skin of dd mice (8/sex/group; Nihon Dobutsu Co., Japan) at doses of 1250 or 2500 mg/kg bw. There were no deaths or toxicity observed during the 21-day observation period. Consequently, the LD50 for dermal application in male and female mice was >2500 mg/kg bw.

Technical fenitrothion was applied to the skin of Sprague-Dawley rats (8/sex/group; Nihon Dobutsu Co., Japan) at doses of 310, 625, 1250, 2500 or 5000 mg/kg bw. At the end of the 21-day observation period, deaths among treatment groups was 0/8, 1/8, 7/8 (5/8 for females), 8/8 (7/8 for females), and 8/8 respectively (mortality rates for females the same as for males except where indicated). Slight hyperexcitability was observed 2 to 4 days post administration at 310 mg/kg bw. At the next higher dose, bladder incontinence, muscular twitch, rapid respiration, hyperexcitability and exophthalmus were observed 24 h after treatment and persisted for 5 days. These clinical signs were more severe as the dose was increased. Symptoms in surviving rats subsided 2-3 weeks post treatment.

The acute dermal LD 50 was 890 mg/kg bw for males and 1200 mg/kg bw for females.

Hixson EJ (1982b) Acute dermal toxicity of fenitrothion concentrate use dilution in rabbits. Toxicology Department, Mobay Chemical Corporation, USA. Report date: April 19, 1982.

In an acute dermal test, technical fenitrothion (Mobay Chem Corp, Batch No. 18-R-92-143; purity 75.8%) diluted with water to give a 24.12% (w/v) solution was applied to the shaved intact dorsal skin of 4 to 5 (male and female) New Zealand White rabbits at doses of 0, 1000, 2000, 3000 or 4000 mg/kg bw in males; and 0, 1000 or 2000 mg/kg bw in females for a period of 24 h. The test site was covered with gauze and adhesive tape and following exposure, the site was cleaned, and the animals observed for 14 days. Animals that died or were euthanised at the end of

the study underwent gross necroscopy immediately after death. No reference was made to the GLP status of this study, however, it was stated that it was reviewed by a QA unit.

Mortality in groups were 0/4, 0/4, 0/4, 2/4, and 3/4 for the males; no deaths were recorded in females. Clinical signs consisted of salivation, muscle fasciculations, tremors, ataxia, agitation, decreased activity, miosis and an unkept appearance. No pathomorphological signs could be attributed to treatment.

The LD50 was calculated to be 3290 mg/kg bw for males and >2000 mg/kg bw for females.

3.1.2 Skin Irritation and Sensitisation Studies

Hixson EJ (1982c) Eye and dermal irritation of fenitrothion concentrate use dilution. Toxicology Department, Mobay Chemical Corporation, USA. Report date, March 10, 1982.

In an acute irritation study, technical fenitrothion (Mobay Chem Corp, Batch No. 18-R-92-143; purity 75.8%) was diluted with water to give a 24.12 % (w/v) dilution and applied to the shaved skin (under a 2.5 cm gauze patch) in aliquots of 0.5 mL to New Zealand White rabbits (6/sex). Four test sites on each animal (2 with an abraded stratum corneum and the other 2 left intact) were used. The animals trunks were wrapped in a plastic sheet and secured with tape. After 24 h, the sites were cleaned with tissue paper soaked in water, and skin reaction graded and scored according to the system of Draize at 24 and 72 h. The study was conducted in accordance to GLP and all phases of the study was reviewed by a QA unit.

All rabbits had slight erythema 24 h after treatment on the abraded sites; 3/6 had erythema on the 2 intact sites, 2/6 on 1 intact site**,** and 1/6 did not exhibit erythema on any intact sites. At 72 h, 5/6 had no erythema but the remaining rabbit had erythema that cleared after an additional 120 h. Oedema was not observed at any stage. A primary irritation score of 0.46 was calculated from the erythema scores of intact and abraded skin. Under the conditions of this study, diluted fenitrothion technical (24.12%) was considered to be a mild skin irritant.

Hara S & Suzuki T (1981) Primary eye and skin irritation tests of Sumithion technical in rabbits. Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Japan. Report Date: March 31, 1981.

Technical fenitrothion (0.5 mL, Sumitomo Chem Co, Lot No. 90305; purity 96.5%) was applied to the abraded shaved skin on the backs of 6 New Zealand White rabbits (Inoue Laboratory Animal Centre Co Ltd, Japan), then covered with gauze and occlusive dressings for 24 h post application, at which time the test sites were wiped to remove the test material. Observations for skin reactions were made 24, 48, 72 h and 1 week after application, and graded and scored according to the system of Draize. No indication was given as to the GLP status of the study.

No evidence of erythema or oedema was observed. Under the conditions of this study, technical fenitrothion was not considered to be a skin irritant to rabbits.

Kohda H, Kagoshima M, Kadota T & Miyamoto J (1972) Skin sensitisation test of Sumithion technical in guinea pigs. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report Date: October 20, 1972.

and

Miyamoto J & Kadota T (1972) Toxicological studies with Sumithion. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: December, 1972.

Technical fenitrothion (Sumitomo Chem Co, Lot No. 417, purity 97.2%) was administered intradermally to the shaved abdomens of 2 groups of 6 male Hartley Guinea pigs (Nihon Dobutsu Co., Japan) at a dose of 1 and 5% fenitrothion in corn oil (volume 0.05 mL/animal for the first application, 0.1mL/animal from second application) on alternate days for 14 days. A vehicle control group of 9 males received corn oil, whilst another 5 males received 0.05% 2,4-dinitrochlorobenzene (DNCB) (volume 0.1 mL/animal) as a positive control for the same period as the treated animals. The injection sites were changed so as not to overlap the site of previous applications.

Following the last application, animals remained untreated for 14 days when they were challenged with fenitrothion at a different site from the original injection site. Fenitrothion treated animals received 1 or 5% fenitrothion intradermally (0.05 mL/animal) or by dermal application (0.03 mL/animal), DNCB controls 0.1% intradermally and dermally (0.03 mL/animal for both methods) and animals that received corn oil as induction treatment received dermally 5% corn oil (0.03 mL/animal). Skin reactions were evaluated at 24 h post challenge.

Following the initial sensitising injection with 1 and 5% fenitrothion, DNCB, and corn oil slight swelling was observed, as was found with 1 and 5% fenitrothion injected for challenge. Intradermal injection of 5% fenitrothion for challenge caused swelling and hyperaemia with the same severity as that observed at sensitisation; whereas no abnormality was found in animals challenged by dermal application of 5% fenitrothion. The positive control group produced the expected severe haemorrhage and swelling 3-4 h after challenge.

In conclusion, fenitrothion did not induce skin sensitisation in the guinea-pigs under the conditions of this assay.

Thyssen J & Lorke D (1982) S 5660 (fenitrothion, Folithion active ingredient). Test for irritant effect on skin or mucous membrane. Institute of Toxicology, Bayer AG, Germany. Report date: March 8, 1982.

Technical fenitrothion (Source not stated, Batch No. 2606; purity 97.4%) was applied to skin on the backs of 6 male and female New Zealand White rabbits (Degenfeld, Germany) for 24 h, according to the 1973 guidelines of the US Department of Agriculture. The exact amount applied and details of the methodology was not stated. Observations for skin reactions were made at 24 and 72 h.

In the skin irritation studies, no evidence of erythema or oedema was observed. Under the conditions of this study, technical fenitrothion was not considered to be a skin irritant to rabbits.

3.1.3 Inhalational Studies

Miyamoto J & Kadota T (1972) Toxicological studies with Sumithion. Research Department, Pesticides Division Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: December, 1972.

and

Kohda H & Kadota T (1979a) (Amended report) Acute inhalational toxicity study of Sumithion in rats. Institute for Biological Science, Japan. Report date: June 7, 1979.

The acute inhalational toxicity of fenitrothion (Sumitomo Chem Co, Lot No. 417, 97.2% purity) dissolved in a mixture of deodorised kerosene and xylene was assessed in a dynamic aerosol exposure apparatus (whole body), with Sprague-Dawley rats (Clea Japan Inc.; 8/sex/group) exposed for 2, 4 or 8 h at doses of 10, 70 or 186 mg/m^3. The inhalational particle size was assessed as being in the range 1-3 µm, however, the mass mean diameter was not stated. The generation of the desired fine particles at concentrations above 186 mg/m^3 was considered to be technically too difficult so this concentration was the highest tested. Development of any clinical signs and alterations in body weight were recorded during exposure and for 7 days post exposure. A group administered solvent only [kerosene:xylene (85:15)] served as the vehicle control.

After exposure to solvent only for 2 or 4 h, the control group showed no abnormal behaviour, reduction in body weight or other clinical signs. At 10 mg/m^3, rats displayed similar behavioural characteristics to controls when exposed for 2 or 4 h. At the end of the 8 h exposure, piloerection and incontinence of urine was noted, with recovery occurring within 2 h. The mean body weight of male rats exposed for 8 h was slightly but not significantly decreased 1 day after exposure, but returned to control values after 2 days.

At 70 mg/m^3, urinary incontinence was observed in 1 male and 1 female rat exposed for 2 h, 4 males and all the females exposed for 4 h, and in all animals exposed for 8 h. Rapid respiration and dyspnoea developed after 4 and 8 h. Slight piloerection, ataxia and salivation were observed after an 8 h exposure group, but these signs disappeared within 24 h. A non-significant transient decrease in body weight was observed in animals exposed for 8 h, with a return to control values within 2 days.

At 186 mg/m^3, similar clinical signs to the 70 mg/m^3 group were observed except for muscular fibrillation and tremors at 4 and 8 h. Additionally exophthalmus developed after 8 h. These clinical signs at 8 h were more severe in males and culminated in 2 deaths 3 days after exposure.

The LC50 established in this study was estimated to be greater than 186 mg/m^3.

Kohda et al (1986) Acute inhalational toxicity of Sumithion in rats. Laboratory of biochemistry and toxicology, Takarezuka Research Centre, Sumitomo Chemical Co., Ltd. Japan. Report date: March 26, 1986.

The acute inhalational toxicity of fenitrothion (Sumitomo Chem Co, Lot No. 40805, purity 96.6%) dissolved in corn oil was assessed in a dynamic aerosol exposure apparatus (whole body), with 5-week old Sprague-Dawley rats (Shizuoka Laboratory Animal Centre; 10/sex/group) exposed for 4 h at mean concentrations of 0 (vehicle control), 0 (air, negative control), 3.91, 8.90, 38.2, 1010 or 2210 mg/m^3. The inhalational particle size was assessed as being in the range 0.59-0.82 µm. Development of any clinical signs and alterations in body weight were recorded during exposure and for 14 days post exposure. Plasma, erythrocyte and brain ChE levels were determined. For this part of the study additional groups of rats (5/sex/group) were used to determine ChE activities at various times and doses post exposure (ranging from day 1-56).

Gross necroscopy was performed on rats that died during the study and on survivors killed at the end of the exposure period. The study was performed according to GLP.

One male rat dosed at 2210 mg/m^3 died on day 6. No clinical signs were observed at 38.2 mg/m^3 or below but at 1010 mg/m^3 irregular respiration, nasal discharge, decreased spontaneous activity, lacrimation, salivation, and urinary incontinence (muscular fibrillation was additionally observed only in male rats) were observed. At 2210 mg/m^3 additional clinical signs like hyperpnoea and intermittent tremors were observed; tonic convulsions, ataxia and soft faeces were only observed in male rats. Clinical signs commenced at 30 min post exposure and disappeared within 9 days after termination of exposure in the surviving rats.

A significant decrease in body weight gain was observed in male ($p<0.01$) and female ($p<0.05$) rats dosed at 2210 mg/m^3 (58% for males and 24% for females; compared with vehicle control values) at the end of the observation period.

Plasma ChE levels (when compared with vehicle controls) were reduced 17% (non-significant), 34%, 60%, 87% and 93% ($p<0.01$) at doses of 3.91, 8.90, 38.2, 1010 and 2210 mg/m^3 respectively, in male rats at day 1 post exposure. At day 14 post exposure in males, plasma ChE activity was reduced by 10%, 4%, 12%, 3% and 12% for the respective doses (non-significant at all doses). For females, plasma ChE levels were 5% (non-significant), 50%, 72%, 95% and 94% ($p<0.01$) at doses of 3.91, 8.90, 38.2, 1010 and 2210 mg/m^3 respectively, at day 1 post exposure; with levels of 12%, 0%, 14%, 0% (all non-significant) and 24% ($p<0.05$) respectively, at day 14. From day 21 (measured only for animals dosed at 1010 and 2210 mg/m^3), none of the animals (males or females) showed ChE levels reduced below control values.

Erythrocyte ChE activities were unchanged at 3.91 and 8.9 mg/m^3 but reduced by 10% ($p<0.05$), 74% and 84% (both $p<0.01$) at doses of 38.2, 1010 and 2210 mg/m^3 respectively, in male rats at day 1 post exposure. At day 14 post exposure, levels were the same except that the reduction at the two highest doses was 48% and 64% respectively (both $p<0.01$). Significant ($p<0.01$) reductions in activities were observed at 1010 mg/m^3 at day 21 and 28 (30% and 22%) respectively, however, at day 35 and 42 (final measurement) activities were both only reduced by 9% (non-significant). Significant ($p<0.01$) reductions in activities were also observed at the dose

of 2210 mg/m^3 at day 21 and 28 post dose (42% and 30%) respectively, however, at day 35 and 56 (final measurement) activity was reduced by 4% and 5% respectively (both non-significant).

For females, erythrocyte ChE activity was reduced by 12% (non-significant), 14% ($p<0.05$), 32%, 79% and 85% ($p<0.01$) at doses of 3.91, 8.90, 38.2, 1010 and 2210 mg/m^3 respectively, at day 1 post exposure. By day 14 the reduction in activity was 7%, 7%, 15% ($p<0.05$), 53% and 60% (both significant; $p<0.01$) respectively. Significant ($p<0.01$) reductions in activities were observed at the dose of 1010 mg/m^3 at day 21 and 28 post dose (40% and 22%) respectively, however, at day 35 and 42 (final measurement) levels of activities were 10% and 8% respectively (both non-significant). Significant ($p<0.01$) reductions in activities were also observed at the dose of 2210 mg/m^3 at day 21, 28 and 35 post dose (47%, 25%, 15%) respectively, however, at day 42, 48 and 56 (final measurement) levels of activities were 7%, 5% and 7% respectively (all non-significant).

Brain ChE activity was unchanged except at the two highest doses (1010 and 2210 mg/m^3) where the reduction in activity was 65% and 85% respectively (both significant; $p<0.01$) in male rats at day 7. At day 14, activity was unchanged except at the upper three doses (14%, 40% and 58%; $p<0.01$ for upper 2 doses). At day 42 (dose 1010 mg/m^3) and day 56 (dose 2210 mg/m^3) levels were reduced by 6% (non-significant) and 19% ($p<0.01$) respectively.

For females, brain ChE activity was unchanged at 3.91 and 8.90 mg/m^3 but was reduced by 12% (non-significant), 61% and 88% ($p<0.01$) at 38.2, 1010 and 2210 mg/m^3 respectively, at day 7 and 13% ($p<0.05$), 45% and 58% (both significant; $p<0.01$) respectively, at day 14. At day 42 (dose 1010 mg/m^3) and day 56 (dose 2210 mg/m^3) activity levels were both reduced by 11% ($p<0.05$).

In summary, inhibition of ChE activity was observed 1-7 days after initiation of inhalation exposure. Specifically, reduced plasma ChE activity in males and females at or above 8.90 mg/m^3; erythrocyte ChE in males exposed at or above 38.2 mg/m^3 and in females dosed at 8.9 mg/m^3; and brain ChE activities in males and females dosed at 1010 mg/m^3. These activities recovered between 3-56 days post exposure, with the brain ChE activities remaining reduced at day 56 at the highest dose used (2210 mg/m^3).

No pathomorphological findings could be attributed to treatment. The LC50 was estimated to be in excess of 2210 mg/m^3 for both male and female rats.

3.1.4 Eye Irritation Studies

Hixson EJ (1982c) Eye and dermal irritation of fenitrothion concentrate use dilution. Toxicology Department, Mobay Chemical Corporation, USA. Report date: March 10, 1982.

Fenitrothion (Mobay Chem Corp, batch No. 18-R-92-143, purity 75.8%) diluted to give a 24.12% (w/v) solution was instilled into the conjunctival sac of the left eye of 9 New Zealand White rabbits (Small Stock, Inc., Arkansas, USA) (7 females and 2 males) in aliquots of 0.1 mL to determine the eye irritation potential of the test material. The right eye served as a control. Three of the rabbits (2 males and 1 female) had their treated eyes flushed with water 45 sec post treatment, the remainder left unflushed. The treated eyes were examined for signs of irritation at 1, 2, 3, 4, and 7 days after treatment. Rabbits whose eyes exhibited responses on day 7 were further examined until day 14. Irritation was graded and scored according to the method of Draize and the criteria for eye irritants of the Federal Hazardous Substances Act, USA, 1972. Studies were conducted in accordance with GLP guidelines.

Rabbits (6/6) that did not receive flushing of the eyes post treatment exhibited mild to moderate erythema (score 1-2; range 0-4) 1 day after treatment, however, erythema was not observed after day 3 (2/6 animals), day 4 (2/6 animals), or day 7 (2/6 animals). No corneal opacity, or iritis was observed following treatment. Slight chemosis (score 1; range 0-3) was observed on day 1 only in 1 female rabbit. A moderate to severe discharge (score 1-3; range 0-3) was observed in all animals, however, this cleared by day 8 post treatment.

In rabbits whose eyes were flushed following treatment exhibited no corneal opacity or iritis. Mild to moderate erythema (score 1-2; range 0-4) was observed in all 3 animals clearing by day 4 (2/3 animals), or day 7 (1/6 animal). Slight chemosis (score 1; range 0-3) was observed in 2 rabbits (1 female; 1 male). Under the conditions of this study, diluted technical fenitrothion (24.12%) was considered to be an eye irritant, although it was reversible by day 8 post exposure.

Hara S & Suzuki T (1981) Primary eye and skin irritation tests of Sumithion technical in rabbits. Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Japan. Report Date: March 31, 1981.

Technical fenitrothion (Sumitomo Chem Co, Lot No. 90305, purity 96.5%) was instilled into the conjunctival sac of 1 eye (right or left not stated) of 9 male New Zealand White rabbits (Inoue Laboratory Animals, Japan) in aliquots of 0.1 mL to determine the eye irritation potential of the test material. In 3 animals the treated eyes were flushed, 30 sec after treatment, with water for 1 min. The other eye served as a control. The treated eyes of each rabbit were examined for signs of irritation at 1, 24, 48, 72, 96 h and 1 week after treatment. Irritation was graded and scored according to the Draize system. No indication was given as to the GLP status of the study.

No evidence of eye irritation other than slight conjunctival redness in the unwashed eyes of rabbits was observed 1 h after application. However, this redness disappeared after 48 h. Under the conditions of this study, technical fenitrothion was not considered to be an eye irritant.

Thyssen J & Lorke D (1982) S 5660 (fenitrothion, Folithion active ingredient). Test for irritant effect on skin or mucous membrane. Institute of Toxicology, Bayer AG, Germany. Report date: March 8, 1982.

Technical fenitrothion (Source not stated, Batch No. 2606a, purity 97.4%) was instilled into the conjunctival sac of 1 eye (right or left not stated) of 6 New Zealand White rabbits (Degenfeld, Germany). The exact amount instilled and details of methodology were not provided, however, the applicant stated that the tests were performed in accordance with the 1981 Interagency Regulatory Liaison Group, USA. The treated eyes of each rabbit were examined for signs of irritation at 1, 24, 48, 72 h, then at 1 week, 2 week and 3 weeks thereafter. Irritation was graded and scored according to a scale outlined in the 1972 US Federal Register.

No evidence of corneal damage or eye irritation was reported in this study other than slight redness of the conjunctiva after 1 h but not after 24 h. Under the conditions of this study, technical fenitrothion was not considered to be an eye irritant.

3.2 Isomers, Metabolites and Impurities

3.2.1 Median Lethal Dose Studies

A summary of submitted and published findings of acute median lethal dose studies for the metabolites and impurities of technical fenitrothion is shown in the Table below.

Median Lethal Dose Studies

Species	Sex	Route	Vehicle	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Fenitrooxon (active mammalian metabolite)					
Mouse (?)*	?	PO	?	90	Miyamoto et al (1963) Miyamoto (1969)
Mouse (Swiss)*	?	PO	?	120	Hollingworth et al (1967)
Mouse (CD1)	M	IP	Propylene glycol	44.5 (M)	Myatt & Ecobichon (1975)
Rat (?)*	?	PO	?	24	Miyamoto et al (1963) Miyamoto (1969)
Rat (?)*	?	IV	?	3.3	Miyamoto et al (1963) Miyamoto (1969)
Guinea-pig (?)*	?	PO	?	221	Miyamoto et al (1963) Miyamoto (1969)
Guinea pig (?)*	?	IV	?	32	Miyamoto et al (1963) Miyamoto (1969
3-methyl-4-nitrophenol (mammalian metabolite)					
Mouse (dd)	M	PO	Arabian gum	250 (M)	Kadota & Miyamoto (1979)
Mouse (dd)	M	Dermal	Ethanol	>5000 (M)	Kadota & Miyamoto (1979)
Mouse (dd)	M	IP	Arabian gum	136 (M)	Kadota & Miyamoto (1979)

Rat (Wistar)	M/F	PO	Arabian gum	2300 (M), 1200 (F)	Sumitomo Chem Co. (1971)
Rat (Wistar)	M/F	PO	Arabian gum	2300 (M), 1200 (F)	Kadota & Miyamoto (1979)
S-methylfenitrothion (isomer)					
Mouse (dd)	M/F	PO	Corn oil	550 (M), 420 (F)	Mikami et al (1977)
Mouse (dd)	M/F	Dermal	Corn oil	1750 (M), 1900 (F)	Mikami et al (1977)
Mouse (dd)	M/F	IP	Tween 80	54 (M), 65 (F)	Mikami et al (1977)
Mouse (CD1)	M	IP	Propylene glycol	112 (M)	Myatt & Ecobichon (1975)
Rat (SD)	M/F	PO	Corn oil	460 (M), 540 (F)	Mikami et al (1977)
Rat (Wistar)	M	PO	Olive oil	315 (M)	Rosival et al (1976)
Rat (SD)	M/F	Dermal	Corn oil	1450 (M), 1750 (F)	Mikami et al (1977)
Bis-fenitrothion (impurity)					
Mouse (ICR)	M/F	PO	Corn oil	612 (M), 500 (F)	Suzuki (1982)

* Studies not reviewed but cited in IPCS, EHC No. 133.

3.2.1.1 Oral

Rosival L, Vargova M, Szokolayova J & Cerey K (1976) Contribution to the toxic action of S-methylfenitrothion. Pesticide Biochem Physiol 6: 280-286

The acute oral toxicity of technical fenitrothion (Chemické závody J. Dimitrova, Bratislava, Czechoslovakia; batch not stated, purity 96%), purified fenitrothion (derived from the technical fenitrothion) and the S-methyl isomer (SMF; freshly prepared by demethylation) were compared.

All test compounds were suspended in olive oil and administered to male Wistar rats (number not reported) by gavage. The LD50 for purified fenitrothion, technical fenitrothion and SMF was calculated to be 700 mg/kg bw, 490 mg/kg bw and 315 mg/kg bw respectively.

Suzuki T (1982) Acute oral toxicity of Bis-Sumithion, O-methyl O,O-di (3-methyl-4 nitrophenyl) phosphorothionate, in mice. Research Department, Pesticide Division, Sumitomo Chemical Co., Ltd., Japan. Report date: January 6, 1982.

In an acute toxicity study, O-methyl O,O-di (3-methyl-4 nitrophenyl) phosphorothionate (Sumitomo Chem Co, Lot No. N 10506, purity 98.8%) in corn oil was administered PO by gavage to ICR mice (10/sex/group) at doses of 0, 125, 250, 500, 750 or 1000 mg/kg bw. The animals were observed and body weights recorded at 0, 7 and 14 days post treatment. Surviving animals were subjected to gross necroscopy. No reference was made to the GLP status of the study, however, the data was reviewed by a QA unit.

At the end of the 14-day observation period, mortality in the dose groups were 0/10, 0/10, 0/10, 2/10 (0/10 for females), 8/10 (10/10 for females), and 10/10, respectively (mortality rates for females the same as for males except where indicated). In groups receiving a dose at and above 500 mg/kg bw toxicity was observed 4 h after administration and consisted of muscular fibrillation, hyperpnoea followed by dyspnoea, ataxia, limb paralysis, and excretion of an oily substance into the faeces. Gross necroscopy revealed no remarkable macroscopic changes other than blood in the stomach in all dead animals.

The acute PO LD50 was 612 mg/kg bw for males and 500 mg/kg bw for females.

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

S-methylfenitrothion (Source and Lot No. not stated, purity 99%) in corn oil was administered PO to dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) aged 6-7 weeks. Animals were observed for 14 days post treatment. Gross necroscopy was performed on animals that died during the study and on the survivors killed at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

S-methylfenitrothion was administered PO to mice (10/sex/group) in a corn oil vehicle at doses of 100, 130, 170, 220, 286, 372, 500, 650, 845 or 1000 mg/kg bw. At the end of the 14-day observation period, deaths among male treatment groups were 0/10, 0/10, 0/10, 0/10, 0/10, 1/10, 4/10, 7/10, 10/10 and 10/10, respectively; and in females, 0/10, 0/10, 0/10, 0/10, 1/10, 3/10, 8/10, 10/10, 10/10 and 10/10 respectively. The acute PO LD50 was 550 mg/kg bw for males and for females, 420 mg/kg bw.

S-methylfenitrothion was administered PO to rats (10/sex/group) at doses of 100, 130, 170, 220, 250, 284, 385, 500, 650, 845 or 1000 mg/kg bw. At the end of the 14 day observation period, deaths among male treatment groups were 0/10, 0/10, 0/10, 0/10, 2/10, 2/10, 4/10, 6/10, 7/10, 9/10 and 9/10, respectively; and in females 0/10, 0/10, 0/10, 0/10, 1/10, 1/10, 3/10, 6/10, 6/10, 8/10, and 9/10 respectively. The acute PO LD50 was 460 mg/kg bw for males and for females, 540 mg/kg bw.

Clinical signs in mice and rats consisted of decreased spontaneous activity, dyspnoea, ataxia, lacrimation, laboured respiration, salivation, slight tremor and clonic convulsions. The onset of clinical signs was within 20-30 min post treatment in mice and rats and deaths were observed within 24-48 h (no reference was made to the dose at which clinical signs occurred). Surviving animals behaved normally 2-3 days post treatment. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

Kadota T & Miyamoto J (1979) Acute oral, dermal and intraperitoneal toxicities of p-nitro-m-cresol in rats and mice. Research Department, Pesticide Division, Sumitomo Chemical Co., Ltd., Japan. Report date: January 22, 1979.

In a series of acute toxicity studies 3-methyl-4-nitrophenol (Sumitomo Chem Co, Lot No. not stated, purity 99.7%) in 10% Arabic gum was administered PO by gavage to 6-7 week old dd mice and Sprague-Dawley rats (Nihon Dobutso Co, Japan). Animals were observed for 14 days post treatment. Gross necroscopy was performed on animals that died during the study and on survivors subsequently killed at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

3-Methyl-4-nitrophenol was administered PO to male dd mice (8/group) at doses of 100, 140, 200, 280, 390 or 550 mg/kg bw. Death occurred within 1 h and survivors recovered within 2-3 h and exhibited no clinical signs after 24 h. At the end of the 14-day observation period, deaths

among treatment groups were 0/8, 1/8, 3/8, 5/8, 6/8 and 8/8, respectively. At and above 140 mg/kg bw, clinical signs of slow respiration, ataxia and slight tremors were found (10 min post dosing). The acute oral LD50 was 250 mg/kg bw. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

3-Methyl-4-nitrophenol was administered PO to Wistar rats (5/sex/group) at doses of 500, 1000, 1500, 2000, or 2500 mg/kg bw. Deaths occurred within 2-4 h in males and 2-3 days in females. Survivors recovered within 3 days. At the end of the 14-day observation period, deaths among male treatment groups were 0/5, 1/5, 1/5, 2/5, and 5/5, respectively; and for females, 0/5, 2/5, 3/5, 4/5 and 5/5, respectively. At and above a dose of 500 mg/kg bw rats exhibited a decrease in spontaneous activity, slow and irregular respiration (followed by accelerated respiration at 1000 mg/kg bw) and ataxia. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

The acute PO LD50 for males was 2300 mg/kg bw and 1200 mg/kg bw for females.

3.2.1.2 Intraperitoneal

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

S-methylfenitrothion (Source and Lot No. not stated, purity 99%) in 10% Tween-80 was administered IP to dd mice (10/sex/group; Nihon Dobutsu Co, Japan) at doses of 10, 20, 28, 37, 50, 65, 85 or 100 mg/kg bw. Animals were observed for 14 days post treatment. Gross necroscopy was performed on animals dying during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

At the end of the 14-day observation period, deaths among male treatment groups were 0/10, 0/10, 0/10, 0/10, 0/10, 7/10, 9/10, and 10/10 respectively; and in females, 0/10, 0/10, 0/10, 0/10, 1/10, 5/10, 10/10 and 10/10 respectively. Clinical signs consisted of decreased spontaneous activity, dyspnoea, ataxia, lacrimation, laboured respiration, salivation, slight tremor and clonic convulsions (no reference was made to the dose at which clinical signs occurred). The onset of clinical signs was within 10-20 min and deaths were observed within 24-48 h. Surviving animals had no clinical signs 2-3 days post treatment. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

The acute IP LD50 was 54 mg/kg bw for males and for females, 65 mg/kg bw.

Kadota T & Miyamoto J (1979) Acute oral, dermal and intraperitoneal toxicities of p-nitro-m-cresol in rats and mice. Research Department, Pesticide Division, Sumitomo chemical Co., Ltd., Japan. Report date: January 22, 1979.

3-Methyl-4-nitrophenol (Source and Lot No. not stated, purity 99.7%) in 10% Arabic gum was administered IP to 10 male dd mice (Nihon Dobutso Co, Japan) aged 6-7 weeks at doses of 67, 100, 150 or 225 mg/kg bw. Animals were observed for 14 days post treatment. Gross necroscopy

was performed on animals dying during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups.

At the end of the 14-day observation period, deaths among treatment groups was 0/10, 1/10, 7/10 and 10/10 respectively. At a dose of 100 mg/kg bw and above clinical signs of rapid respiration and marked tremors were found. The surviving animals recovered after 3 h. The acute LD50 was calculated to be 136 mg/kg bw.

3.2.1.3 Dermal

Mikami N, Kohda H & Suzuki H (1977) Toxicity study of S-methylfenitrothion. Institute for Biological Sciences, Japan. Report date: April 24, 1977.

S-methylfenitrothion (fenitrothion isomer) (Source and Lot No. not stated, purity 99%) in corn oil was administered dermally to groups of dd mice and Sprague-Dawley rats (Nihon Dobutsu Co., Japan) (10/sex/group). The animals backs were clipped free of hair and the test compounds applied. Tape was applied to prevent ingestion then removed 24 h later and the site cleaned with cotton soaked with ether. Gross necroscopy was performed on animals dying during the study and on the surviving animals at the end of the observation period. No reference was made to the QA status of the study or to the use of any vehicle control groups in this study.

Mice were administered S-methylfenitrothion dermally at doses of 500, 750, 1000, 1750, 2500 or 5000 mg/kg bw. At the end of the 14-day observation period, deaths among male treatment groups were 0/10, 0/10, 0/10, 5/10, 8/10 and 10/10, respectively; and in females, 0/10, 0/10, 0/10, 4/10, 8/10 and 10/10 respectively. The acute LD50 was 1750 mg/kg bw for males and for females 1900 mg/kg bw.

Rats were dermally dosed at 100, 250, 500, 750, 1000 or 2500 mg/kg bw with S-methylfenitrothion. At the end of the 14-day observation period, deaths among treatment groups were 0/10, 0/10, 0/10, 0/10, 2/10 and 9/10 (7/10 for females), respectively (mortality rates for females the same as for males except where indicated**)**. The acute LD50 was 1450 mg/kg bw for males and for females 1750 mg/kg bw.

Clinical signs in mice and rats were the same as for PO and IP routes and consisted of decreased spontaneous activity, dyspnoea, ataxia, lacrimation, laboured respiration, salivation, slight tremor and clonic convulsions (no reference was made to the dose at which clinical signs occurred). The exception was that death was observed during 2-7 days post treatment (compared with 24-48 h via PO and IP routes) and the clinical signs in surviving animals returned to normal 5-7 days post treatment compared with 2-3 days via the PO and IP route. No significant pathomorphological findings were observed in tissues or organs at necroscopy.

Kadota T & Miyamoto J (1979) Acute oral, dermal and intraperitoneal toxicities of p-nitro-m-cresol in rats and mice. Research Department, Pesticide Division, Sumitomo chemical Co., Ltd., Japan. Report date: January 22, 1979.

3-Methyl-4-nitrophenol (source and Lot No. not stated, purity 99.7%) in ethanol was applied to the skin of groups of 10 male mice (Nihon Dobutso Co., Japan) 6-7 weeks old, at doses of 2500 or 5000 mg/kg bw. Backs were clipped free of hair and compound applied to the sites (exposure duration or occlusion details not reported). Animals were observed for 14 days post treatment. Gross necroscopy was performed on dead animals and on the surviving animals at the end of the observation period. No reference was made to any QA measures employed in the study.
There were no deaths or toxicity observed during the 14-day observation period. Consequently, the LD50 for male and female mice was >5000 mg/kg bw.

3.2.2 Eye Irritation Studies

Kadota T & Miyamoto J (1979) Primary eye irritation test of p-nitro-m-cresol technical in rabbits. Research Department, Pesticide Division, Sumitomo Chemical Co., Ltd., Japan. Report date: January 18, 1979.

3-Methyl-4-nitrophenol (Lot No. not stated, purity 94.9%) was instilled into the conjunctival sac of 1 eye of 6 male albino rabbits (Japanese strain; Nihon Dobutsu Co) to determine the eye irritation potential of the test material (50 mg of compound used). The other eye served as a control. The treated eyes were flushed with saline for 2 min and 24 h later reflushed. The treated eyes of each rabbit were examined for signs of irritation at 1, 24, 48, and 72 h after treatment. Irritation was graded and scored according to the methods listed in the 1972 US Federal Register. No reference was made to any QA measures.

No evidence of corneal damage or eye irritation was reported in this study other than slight redness of the conjunctiva 1 h after application in all animals. However, this disappeared by 48 h post application. Under the conditions of this study, 3-methyl-4-nitrophenol was not considered to be an eye irritant.

3.3 End Use Products

3.3.1 Median Lethal Dose Studies

A summary of findings of acute dose studies on the End Use Products of fenitrothion is shown in the Table below. For all studies doses refer to active constituent.

Median Lethal Dose Studies

EUP	Species	Sex	Route	LD50(mg/kg bw) or LC50 (mg/m^3)	Reference
Folithion 50 EC	Mouse (NMRI)	M/F	PO	2166 (M), 3524 (F)	Flucke & Thyssen (1980)
MEP	Mouse (ICR)	M/F	PO	410 (M & F)	Hiraoka et al (1989)

E 5660 50 EC 182	Rat (Wistar)	M/F	PO	817 (M), 782 (F)	Flucke (1984)
Folithion 50 EC	Rat (Wistar)	M/F	PO	602 (M), 925 (F)	Heimann & Lorke (1981)
Folithion 50 EC	Rat (Wistar)	M/F	PO	517 (M), 508 (F)	Thyssen & Kimmerle (1975)
Folithion 50 EC	Rat (Wistar)	M/F	Dermal	>500 (M & F)	Thyssen & Kimmerle (1975)
E 5660 50 EC 182	Rat (Wistar)	M/F	Dermal	>2500 (M & F)	Flucke (1984)
Folithion 50 EC	Rat (Wistar)	M/F	Dermal	>2500 (M & F)	Heimann & Lorke (1981)
Folithion 50 EC	Rat (Wistar)	M/F	Inhal (1 h)	>1103 (M & F)	Flucke & Thyssen (1980)
Folithion 50 EC	Rat (Wistar)	M/F	Inhal (4 h)	1261 (M), >1261 (F)	Flucke & Thyssen (1980)
19.24% EUP	Rat (SD)	M/F	Inhal (4 h)	>1089 (M & F)	Sangha (1982)
Folithion 50 EC	Rabbits (NZW)	M/F	Dermal	555 (M & F)	Rao (1982)

Irritation studies ***Irritation***

Skin	Rabbit (NZW)	0.5mL/intact /24 h (50% formulation)	strong irritant	Flucke & Thyssen (1980)
Eye	Rabbit (NZW)	0.1mL/conjunctival sac, unrinsed(50% formulation)	moderate irritant	Flucke & Thyssen (1980)

(NZW) New Zealand White

Details of these studies are provided below.

3.3.1.1 Oral

Flucke W (1984) E 5660 50 EC 182 c.n. fenitrothion (ISO). Study for acute oral and dermal toxicity. Institute of Toxicology, Bayer AG, Germany. Report date: June 28, 1984.
An end use product containing fenitrothion (E 5660; 50% EC fenitrothion) (Lot No. 513/182, source not stated) was administered PO by gavage to groups of 5-10 male and 5-10 female fasted (overnight) Wistar rats (Winklemann, Borchen, Germany) at dose levels of 1, 5, 100, 500, 600, 710, 850, 1000 or 1400 mg/kg bw for males; and, 5, 10, 250, 400, 710, 850, 1000 or 1120 for mg/kg bw for females. Animals were observed daily for 14 days following treatment when gross necroscopy was performed. The study was performed according to OECD guidelines, but no reference was made to the use of any vehicle control groups.

At the end of the 14-day observation period, male deaths observed among treatment groups were 0/5, 0/5, 0/5, 1/5, 1/5, 1/5, 2/5, 4/5, and 5/5 respectively; and for females, 0/5, 0/5, 0/5, 1/5, 1/5, 1/5, 2/5, 7/10, and 5/5, respectively. In all groups receiving 5 mg/kg bw (10 mg/kg bw in females) or above, clinical signs commenced 30 min following treatment and consisted of tremor, convulsions, twitching, spasms, salivation, spastic gait, dyspnoea, apathy and reduced mobility. A range of macroscopic effects were observed in animals that died during the study, including distended lungs, dark and patchy livers, pale and patchy spleens, reddened stomach and patchy kidneys. No treatment-related pathomorphological findings were found in surviving animals.

The acute PO LD50 was calculated to be 817 mg/kg bw for males and 782 mg/kg bw for females (calculated relative to active constituent).

Heimann KG & Lorke D (1981) Folithion 50 EC. Test for acute oral and dermal toxicity. Institute of Toxicology, Bayer AG Germany. Report date: September 30, 1981.

An end use product containing fenitrothion (Folithion EC; containing 50% technical fenitrothion E 5660, Batch number 125655, source not stated) was administered PO by gavage to groups of 10 male and 10 female Wistar rats (Winklemann, Borchen, Germany; fasted for 16 h) at dose levels of 5, 10, 100, 250, 300, 350, 400, 500, 600, 1000, 1500 or 2500 mg/kg bw for males; and 10, 25, 100, 500, 750, 1000, 1250, 1500 or 2000 mg/kg bw for females. Rats were observed daily for 14 days following treatment when gross necroscopy was performed.

At the end of the 14-day observation period, deaths among treatment groups were 0/10, 0/10, 0/10, 1/10, 2/10, 3/10, 4/10, 5/10, 6/10, 6/10, 9/10 and 10/10 respectively for males; and 0/10, 0/10, 0/10, 2/10, 3/10, 6/10, 6/10, 9/10 and 10/10 respectively, for females. In animals receiving 10 mg/kg bw or above, clinical signs were observed 10 min following treatment and consisted of dyspnoea, reduced mobility, convulsions, salivation, partial prostration and dacryohaemorrhoea, apathy and bristling coat. A range of macroscopic effects were observed in animals that died during the study, including distended lungs, dark and patchy livers, pale and patchy spleens, reddened stomach and patchy kidneys.

The acute oral LD50 was calculated to be 602 mg/kg bw for males and 925 mg/kg bw for females (calculated relative to active constituent).

Thyssen F & Kimmerle D (1975) Folithion 50 EC/acute oral and dermal toxicity. Institute of Toxicology, Bayer AG Germany. Report date: June 4, 1975.

An end use product containing fenitrothion (Folithion 50% EC; Lot No. and source not stated) was administered to groups of 10-20 male and 10-20 female Wistar rats (Winklemann, Borchen, Germany) at dose levels of 250, 350, 500, 750 or 1000 mg/kg bw for males; and 350, 500 or 750 mg/kg bw females. Animals were observed daily for 14 days following treatment.

At the end of the 14-day observation period, deaths among treatment groups was 0/10, 2/10, 4/10, 17/20, and 10/10 respectively for males; and 1/10, 5/10, and 9/10 respectively for females. Clinical signs were observed within minutes following treatment (exact time not stated) and consisted of muscular tremors, cramps, and increased salivation.

The acute PO LD50 was calculated to be 517 mg/kg bw for males and 508 mg/kg bw for females (calculated relative to active constituent).

Flucke W & Thyssen J (1980) Folithion (S 5660=E 5660) 50 EC/India. Acute toxicity studies. Institute of Toxicology, Bayer AG Germany. Report date: June 18, 1980.

An end use product containing fenitrothion (Folithion 50% EC India; containing 50% technical fenitrothion E 5660, batch number not stated; to be used at a concentration of 0.2% aqueous) was

administered PO in a Cremophor EL distilled water vehicle to NMRI mice (Winklemann, Borchen, Germany; 10/sex/group; fasted overnight) at dose levels of 50, 100, 500, 1000, 1500, 2000, 2500, 4000 or 5000 mg/kg bw for males; and 50, 100, 500, 1000, 2500, 3500 or 5000 mg/kg bw females. Mice were observed daily for 14 days following treatment when gross necroscopy was performed.

At the end of the 14-day observation period, deaths among treatment groups were 0/10, 0/10, 0/10, 0/10, 2/10, 4/10, 7/10, 9/10 and 10/10 respectively for males; and 0/10, 0/10, 0/10, 0/10, 2/10, 5/10, or 8/10 respectively for females. Clinical signs of apathy, dyspnoea, occasional prostration and lateral recumbency were observed within 5 to 20 min. Post necroscopy findings consisted of emphysematous lung alterations and pale discolouration of the livers, spleens and kidneys.

The acute PO LD50 was calculated to be 2166 mg/kg bw for males and 3524 mg/kg bw for females (calculated relative to active constituent).

3.3.1.2 Intraperitoneal

Hiraoka Y, Imada A, Tanaka J & Okuda H (1989) The effects of fenitrothion emulsion (organic phosphorous pesticide) and its degraded solution on mice. Hiroshima J Med Sci 38: 209-212

Studies were performed to assess changes in the lethality of a fenitrothion emulsion following sunlight-induced degradation. A fenitrothion emulsion (consisting of 50% fenitrothion technical and 50% organic solvent (not named), emulsifier and other unnamed products) referred to in this study as MEP was adjusted to a pH level of 8, 10 and 14 by addition of a buffer solution and then exposed to natural sunlight at 30^0C for 8 h/day so as to degrade the formulation. Exposure was maintained until 90% of the fenitrothion had been degraded as determined by gas chromatography with a flame photometric detector.

Untreated MEP emulsion and the 3 pH-degraded solutions were separately administered IP to ICR mice (10/sex/group). As there were several uncharacterised degradation products at unknown concentrations in the degraded solutions the administered dose was calculated relative to undegraded fenitrothion.

The LD50 of sunlight-degraded fenitrothion ranged from 60-120 mg/kg bw depending on pH. The greatest increase in toxicity was associated with the emulsion degraded at pH 14. However, all degraded products were substantially more toxic than the parent compound (LD50, 410 mg/kg bw).

3.3.1.3 Dermal

Flucke W (1984) E 5660 50 EC 182 c.n. fenitrothion (ISO). Study for acute oral and dermal toxicity. Institute of Toxicology, Bayer AG Germany. Report date: June 28, 1984.

In an acute dermal lethality test, the animals backs were clipped free of hair and a fenitrothion 50 EC formulation (Lot No. 513/182, source not reported) applied to intact skin. Prior to application to groups of Wistar rats (5/sex/group; Winklemann, Borchen, Germany) at doses of 250, 500, 1250, 1775 or 2500 mg/kg bw in males and 50, 125, 250, 500, 1250 or 2500 mg/kg bw in females, the formulation was thickened by the addition of cellulose powder. The application site was then covered with aluminium foil and an occlusive dressing and left for 24 h. Following exposure, the site was cleaned with soap and water. The study was performed according to the OECD guidelines but no reference was made to the use of any vehicle control groups in this study.

Male deaths among treatment groups were 0/5, 0/5, 0/5, 2/5, and 2/5 respectively; and for females 0/5, 0/5, 1/5, 1/5, 1/5 and 1/5 respectively. In excess of 125 mg/kg bw for females and 1775 mg/kg bw for males clinical signs were observed 10 min following treatment and consisted of dyspnoea, reduced mobility, convulsions, salivation, partial prostration and dacryohaemorrhoea, apathy and bristling coat. Macroscopic effects observed in animals that died during the study were distended lungs, dark and patchy livers, pale and patchy spleens, reddened stomach and patchy kidneys. Consequently, the dermal LD50 of this 50% formulation in male and female rats was in excess of 2500 mg/kg bw.

Thyssen F & Kimmerle D (1975) Folithion 50 EC/acute oral and dermal toxicity. Institute of Toxicology, Bayer AG Germany. Report date: June 4, 1975.

In an acute dermal lethality test, male and female Wistar rats (Winklemann, Borchen, Germany; numbers used not reported) were clipped free of dorsal hair and 1 mL/kg of a fenitrothion formulation (Folithion 50% EC; batch No. not reported) applied undiluted (500 mg/kg bw) for 24 h. Animals were observed daily for 7 days.

No deaths were observed following treatment. Clinical signs were observed following treatment (onset time not reported) and consisted of muscular tremors, cramps, and increased salivation. Consequently, the dermal LD50 for male and female rats was in excess of 500 mg/kg bw.

Heimann KG & Lorke D (1981) Folithion 50 EC. Test for acute oral and dermal toxicity. Institute of Toxicology, Bayer AG Germany. Report date: September 30, 1981.

In an acute dermal lethality test, Wistar rats (Winklemann, Borchen, Germany; 5/sex/group except for females receiving in excess of 500 mg/kg bw where 10/group were used) were shaved and a formulation containing 51% fenitrothion (Folithion 50 EC; batch No. 125655, source not stated) applied to intact dorsal skin. Prior to application the formulation was thickened by addition of cellulose powder. Males were dosed at 125, 250, 500, 1250 or 2500 mg/kg bw (250, 500, 1000, 2500 or 5000 µL/kg bw) whereas for females it was at 125, 250, 500, 1250, 1750 or 2500 mg/kg bw (250, 500, 1000, 2500, 3500 or 5000 µL/kg bw). The application site was covered with an occlusive dressing and left for 24 h. Following exposure, the site was cleaned with soap and water and the animals observed for 14 days.

No deaths were observed in male rats but the mortality rate was 0/5, 0/5, 0/5, 0/10, 2/10, and 4/10 respectively in females. Clinical signs were observed within 24 h following treatment and consisted of dyspnoea, reduced mobility, and convulsions. A range of macroscopic effects were observed in females that died during the study, including distended lungs, dark and patchy livers, pale and patchy spleens, reddened stomach and patchy kidneys.

Consequently, the dermal LD50 for males and females was in excess of 2500 mg/kg bw (5000 µL/kg bw).

Rao RV (1982) Acute dermal LD50 toxicity study of Folithion 50 EC in rabbits. Haffkine Institute, India. Report date: March 22, 1982.

In an acute dermal lethality test, the backs of rabbits were clipped free of hair and formulation (Folithion 50 EC; batch No. FTL ½, Bayer India Ltd, India; sp. gravity 1.15 g/mL) applied to the intact dorsal skin. The formulation was applied undiluted to New Zealand White rabbits (2/sex/group) at doses of 0, 287, 431, 471, 511 or 575 mg/kg bw (0.5, 0.75, 0.82, .0.88 or 1 mL/kg bw) for a period of 24 h. Following exposure, the site was cleaned, and the animals observed for 30 days. Gross necroscopy was performed on animals that died during treatment and on surviving animals at the end of treatment. No reference was made to the QA/GLP status of the study.

Deaths observed among treatment groups was 0/4, 0/4, 1/4, 1/4, 3/4 and 4/4. Clinical signs consisted of excitation, followed by depression, loss of appetite, diarrhoea, and respiratory disturbances. No pathomorphological findings could be attributed to treatment.

The dermal LD50 for male and female rabbits was calculated to be 555 mg/kg bw (0.97 mL/kg bw).

3.3.2 Skin Irritation Studies

Flucke W &Thyssen J (1980) Folithion (S 5660=E 5660) 50 EC/India. Acute toxicity studies. Institute of Toxicology, Bayer AG Germany. Report date: June 18, 1980.

Folithion 50 EC (India; containing 50% technical fenitrothion E 5660, batch number not stated) was applied undiluted or as a 0.2% (v/v) dilution in water (application volume - 0.5 mL) to the hairless skin of an ear auricle of New Zealand White rabbits (Hacking & Churchill, England; gender used in study not reported). The site was then covered with adhesive strips for a total contact time of 1, 2, 4, 8 or 24 h (1-2 rabbits per contact duration). The animals were observed for 7 days post treatment. No indication was given as to the GLP status, or the test guidelines used and there was no grading scale employed in assessing the degree of skin irritation.

Although there was no observed effect in the 2 rabbits treated with undiluted formulation after 1 h, there was mild to moderate erythema in the 1 rabbit tested at 2 h. In each of the rabbits at 4 and 8 h the erythema was classified as very severe. The erythema persisted for 3 days after the 2 hour exposure but was in excess of 7 days after longer contact duration. Oedema was only observed after a 4 h contact duration. In contrast, the 0.2% solution did not cause any skin irritation irrespective of contact duration.

Thus, the undiluted end use fenitrothion formulation (50% emulsifiable concentrate) was judged to be a strong dermal irritant whereas a 0.2% (v/v) dilution was considered to be a non-irritant.

3.3.3 Inhalational Studies

Sangha GK (1982) Acute inhalational toxicity study with fenitrothion concentrate end use dilution (19.24% AI) in rats. Corporate Toxicology Department, Mobay Chemical Corporation, USA. Report date: July 15, 1982.

The acute inhalational toxicity of a 19.24% (active ingedient) fenitrothion EUP (Batch number 81-R-92-143), prepared by mixing 24.36 g of fenitrothion concentrate (Mobay Chem Corp, purity 96%) suspended in 75.78 g of water with 10.5% (w/w) Dowanol TPM and 10.5% (w/w) Atlox 340F was assessed in a dynamic aerosol exposure apparatus using Sprague-Dawley rats (Sasco, Inc., Nebraska, USA). Rats (10/sex/group) were exposed for 4 h (nose-only) at 1089 mg/m^3 calculated as a EUP concentration. A control group was exposed to air only. The inhalational particle size was assessed and showed that 50% of the particle mass sampled had a mass mean diameter below 2µm, and 98% of particle mass below 10µm. Development of any clinical signs and body weights was recorded during exposure and for 14 days post exposure. After 14 days the rats were euthanised and a gross necroscopy performed. No reference was made to the GLP status of this study, however, it was reviewed by a QA unit.

No deaths were recorded. Clinical signs consisted of salivation, lacrimation, muscle fasciculations, tremors, decreased activity, convulsions, and runny noses. A decrease in mean body weight was observed during the observation period but significant only in males. Macroscopic effects observed were lung atelectasis, interstitial pneumonitis, hepatocellular vacuolation, pericholangitis, focal hepatitis, nephritis, pyelitis of the kidneys.

From this study, the inhalational lethality (LC50) of this 19.24% (active ingedient) fenitrothion EUP in Sprague-Dawley rats was greater than 1089 mg/m^3.

Flucke W & Thyssen J (1980) Folithion (S 5660=E 5660) 50 EC/India. Acute toxicity studies. Institute of Toxicology, Bayer AG Germany. Report date: June 18, 1980.

The acute inhalational toxicity of an end use product containing fenitrothion (Folithion 50 EC India; containing 50% technical fenitrothion E 5660, batch number not stated, to be used at a concentration of 0.2% aqueous) was assessed in a dynamic aerosol exposure apparatus, with Wistar rats (10/sex/group; Winklemann, Borchen, Germany) exposed for 1 h (nose only) at doses of 149, 497 or 1103 mg/m^3. Additionally, groups of 10/sex were exposed for 4 h at doses of 8, 52, 192, 467 or 1261 mg/m^3. No details of the particle size or mass mean diameter were provided. Clinical signs and body weights changes were recorded during exposure and for 14 days there. Gross necroscopy was performed at the conclusion of the observation period.

In the 1-h exposure groups clinical signs consisted of mild behavioural changes up to 3 days post treatment (exact dose that clinical signs commenced were not stated). No changes in body weight were observed. No pathomorphological signs were attributed to treatment. The LC50 established in this study was estimated to be greater than 1103 mg/m^3.

In the 4-h exposure groups, clinical signs during the first 24 h post exposure period consisted of muscle twitching, salivation, drowsiness and lateral recumbency (exact dose that clinical signs commenced were not stated). No changes in body weight were observed. At the end of the 14-day observation period there were 5/10 deaths for males and 1/10 for females at 1261 mg/m^3. Gross necroscopy on animals that died revealed dilated lungs, whereas in survivors there were no changes that could be attributed to treatment. The LC50 in male rats was calculated to be 1261 mg/m^3, and greater than 1261 mg/m^3 for female rats.

Chevalier G, Henin JP, Vannier H, Canevet C, Cote MG & Le Bouffant L (1984) Pulmonary toxicity of aerosolised oil-formulated fenitrothion treated rats. Toxicol App Pharmacol 76: 349-355

Groups of 9 male Sprague-Dawley rats (Iffa-Credo, France) were exposed to aerosols of a fenitrothion formulation at 0 (air only to simulate field use conditions), 2 or 500 mg/m^3 for 1 h in a nose-only exposure chamber. The formulation contained 15% (v/v) fenitrothion technical (Sumitomo Chemical Company; Lot No. not stated; purity 95%), 35% (v/v) petroleum solvent Cytosol 63 (Shell Chemical Canada) and 50% (v/v) diluent oil 585 (Texaco Canada). The inhalational particle size was assessed as being in the range 0.72 to 1.6 µm. Rats were observed daily for clinical signs and weighed weekly. Animals (6/9) were sacrificed at 3, 7, 21, and 60 days after treatment, and in addition 5 control rats were sacrificed at day 3. The remaining rats were killed at day 60 and their lungs examined by light and electron microscopy.

No deaths were observed during the treatment period. Control rats showed no clinical signs other than transient eye irritation. Clinical signs in fenitrothion treated animals at both doses consisted of exophthalmos, piloerection, irritability and chromodacryorrhoea. No reduction in body weights occurred during the treatment period.

No changes in the lungs of control animals was observed. In animals dosed at 500 mg/m^3 discrete foci of inflammation in the lungs including interstitial oedema, cellular infiltration, and increased

numbers of alveolar macrophages were detected at day 3. These changes decreased at day 7 and by day 21 or 60 the tissues were normal. More limited changes in the lungs at day 3 were observed at the lower dose, with no modifications seen at day 7, 21 or 60.

It appears that fenitrothion at a concentration of 500 mg/m^3 presents no serious long-term pulmonary hazard.

Coulombe PA, Lortie S & Cote MG (1986) Pulmonary toxicity of the insecticide fenitrothion in the rat following a single field exposure. J Appl Toxicol 6: 317-323

The pulmonary toxicity of fenitrothion in rats was evaluated following a single exposure to an aerially sprayed formulation in the field.

Four groups (including a control group) of 40 male Sprague-Dawley rats (Charles River, Quebec), confined to an open top wooden box, were placed directly under the aerial lines of spraying, at intervals of 1 km. A control group was located 8.2 km from the site of spraying. The sprayed formulation contained technical fenitrothion (Sumitomo Chem Co, Canada, at 11.3% v/v), 28% (v/v) petroleum solvent Cyclosol 63 (Shell Chem Canada), 59.5% (v/v) oil Diluent 585 (Texaco Canada), and 1% (v/v) Automate Red B dye. The aircraft flew at an altitude less than 100 m, and the animals were exposed for 65-100 min to the aerial spray. The degree of exposure was monitored at ground level by air sampling and visual evidence of droplet activity deposition. The recorded concentrations are shown in the Table below.

Field Exposure to Fenitrothion

Group	Exposure Duration (h)	Concentration (µg/m^3)	
		After 30 min	After 60 min
1	1.1	0	0
2	1.7	6.19	4.32
3	1.5	33.20	2.80
4 (control)	2	0	0

After exposure, the animals were observed for 7 days for clinical signs. Plasma ChE activity was measured from blood samples collected from the jugular vein within 12 h following exposure, and the pulmonary alveoli ultrastructure examined by light and electron microscopy at 3, 7, 21, 60 and 180 days after exposure (4 rats sacrificed/group for groups 1, 2 and 4; 8 rats for group 3).

No deaths or adverse clinical signs were observed. Plasma ChE was not significantly different for any exposed groups relative to controls. Lung pathology was limited to small and discrete foci showing inflammation and slight interstitial oedema, which was reversible within 2 months. Thus, field exposure of aerially applied fenitrothion does not result in irreversible damage of lung alveoli.

3.3.4 Eye Irritation Studies

Flucke W & Thyssen J (1980) Folithion (S 5660=E 5660) 50 EC/India. Acute toxicity studies. Institute of Toxicology, Bayer AG Germany. Report date: June 18, 1980.

An end use product of fenitrothion (Folithion 50 EC India; containing 50% technical fenitrothion E 5660, batch number not stated) was instilled into the conjunctival sac of 1 eye of 2 New Zealand White rabbits (sex not stated). In this eye irritancy test, 0.1mL of undiluted or of a 0.2% (v/v) dilution of the formulation was used and reactions monitored daily for 1 week. No reference was made to the scale used to grade the eye irritations.

The 2 rabbits treated with 50% formulation showed mild to moderate redness of the conjunctivita and chemosis, a reddened and swollen iris, and a opacified cornea up to 2 days post treatment. However, this disappeared after 2 days post application. No evidence of eye irritation was observed following treatment with 0.2% aqueous solution. Under the conditions of the study the undiluted formulation was classified as a moderate eye irritant whereas a 0.2% formulation solution was classified as a non-irritant.

3.4 Antidote Studies

A number of studies have been conducted to assess the efficacy of various therapeutic treatments for fenitrothion poisoning in animals. The results are summarised in the Table below.

Antidote Studies

Putative Antidote (mg/kg bw)	Fenitrothion (mg/kg bw)	Species	Outcome	Reference
Atropine (5) IP; or Clonidine (0.3 or 1) IP; or Methyldopa (50 or 200) IP; or Propanolol (5 or 10) IP	(300) IP	Mice	No change in clinical signs with any treatment.	Valecha et al (1990)
Atropine (25 or 50) IP; or 2-PAM (50) IP; or Atropine (25) & 2-PAM (50) IP	(200 or 800) PO	Mice	Atropine alone or in combination with 2-PAM increased survival rate. 2-PAM alone increased survival time but no change in LD50.	Kadota et al (1974)
Atropine (10, 25 or 50) SC; or 2-PAM (50 or 100) IP; or Atropine (25) & 2-PAM (50) IP	(200 or 800) PO	Rats	Atropine (all doses tested) ameliorated clinical signs and increased survival. 2-PAM alone had no effect and in combination with atropine gave no additional improvernent.	Kadota et al (1974)
2-PAM (100) IP; or TMB-4 (75) IP	(160 or 320) IP	Rats	Survival increased with 2-PAM after fenitrothion (160)	Dubois (1960)
Atropine (50) IP*; or 2-PAM (5 or 10) IP*; or Atropine (50) & 2-PAM (10) IP*	(500 or 750) PO	Rats	Clinical signs ameliorated with atropine or atropine & 2-PAM; no effect with 2-PAM.	Kimmerle (1962)

2-PAM (50) IP; or Diphenhydramine (10) IP; or Atropine (20) IP; or TPMM (100) IP; or Scopolamine (10) IP; or Biperiden (10) IP; or Phenobarbitone (50) IP; or Glutathione (100) IP; or Diazepam (30) IP	(500) PO	Rats	Survival was enhanced with all compounds except phenobarbitone, glutathione or diazepam. Some clinical signs were ameliorated with all compounds except for diphenhydramine, phenobarbitone, glutathione and diazepam.	Matsubara & Horikoshi (1983)
Atropine (20) IP; or Scopolamine (10) IP; or Biperiden (10) IP; or 2-PAM (50) IP; or Diphenhydramine (10) IP; or Atropine (10) & 2-PAM (25) IP; or Biperiden (10) & 2-PAM (50) IP; or Scopolamine (10) & 2-PAM (50) IP	(800) PO		Antidote efficacy was ranked; atropine alone or in combination with 2-PAM was the most effective in enhancing survival and reducing toxic signs.	
Atropine (10) IP; or 2-PAM (25) IP; or Atropine (10) & 2-PAM (25) IP	(800) PO		Survival was enhanced by atropine alone or in combination with 2-PAM.	
2-PAM (50) IV 2-PAM (10) intramedullary	(75) IV	Rats	Ameliorated clinical signs and enhanced survival.	Uehara et al (1993)
2-PAM (50) IP; or 2-PAM (50) IP 2-PAM (100) IV (rabbits)	(20 or 500) PO (31) IV (rabbits)	Rats Rabbits	2-PAM reduced the extent of plasma, erythrocyte and brain ChE inhibition.	Matsubara & Horikoshi (1984)

TMB-4, 1,1-tri-methylene-bis-(4-formyl-pyridinium) bromide; TPMM, (1-(methyl morphorinium)-3-(4-hydroxyiminomethylpyridinium) propane dibromide; *delayed 15 min; repeatedly administered IP individually or in combination at intervals of 9 h (atropine) and 3 h (2-PAM) for 48 h; repeatedly administered IP at intervals of 2 h for 48 h.

Valecha N, Prabhu S & Mehta L (1990) Influence of clonidine, methyldopa and propanolol on acute toxicity of fenitrothion in mice. Ind J Physiol Pharmacol 34: 39-41

The effect of pretreatment with clonidine, methyldopa, atropine and propanolol on the acute toxicity of fenitrothion (source, purity and batch number not stated) in mice was studied.

The LD50 of fenitrothion was determined in male mice (strain not stated) in a dose-ranging experiment whereby IP administration at 150, 300, 500 or 750 mg/kg bw produced a mortality rate of 17, 54, 70 and 100%, respectively after 24 h.

Atropine (5 mg/kg bw), clonidine (0.3 or 1.0 mg/kg bw), methyldopa (50 or 200 mg/kg bw) or propanolol (5 or 10 mg/kg bw) in distilled water were administered IP prior to administration of 300 mg/kg bw fenitrothion (near the LD50) in propylene glycol IP to groups of 10 male mice. The concurrent control group received 300 mg/kg bw fenitrothion.

Clinical signs of straub tail, muscle fasciculation, and excessive salivation were observed in controls whereas a slight non-significant decrease in the incidence of muscle fasciculations and straub tail in atropine-treated rats was observed. No other changes were observed in antidote-treated mice.

The 24-h lethality was significantly ($p<0.01$) decreased from 80% in controls to 28% after atropine, whereas with propanolol a slight decrease from 80% to 50% was observed. Clonidine and methyldopa slightly increased the lethality at all the doses tested.

Kadota T, Kohda H & Miyamoto J (1974) Effect of atropine and 2-PAM as antidotes on acute intoxication of Sumithion in mice and rats. Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Japan. Report Date: July, 1974.

To test the therapeutic effect of atropine and 2-PAM after acute fenitrothion poisoning, technical fenitrothion (97.2% purity, Sumitomo Chem Co) in 10% Tween-80 was administered PO (exact dose not stated) to groups of ten male dd mice, then IP injections of atropine (25 or 50 mg/kg bw) or 2-PAM (50 mg/kg bw) or in combination (25 mg/kg bw atropine & 50 mg/kg bw 2-PAM) were administered immediately. For the control, saline was administered in place of the putative antidotes. Animals were observed for 72 h.

Fenitrothion treated mice without antidote died within 1-4 h. Following atropine treatment, survival time increased from 4 to 48 h, and from 3 to 24 h following 2-PAM treatment. Atropine at 50 mg/kg bw significantly ($p<0.01$) reduced the LD50 from 970 mg/kg bw to 1440 mg/kg bw. Treatment with 2-PAM had no effect, whereas, the atropine and 2-PAM combination raised the LD50 to a similar extent as for atropine alone.

Additionally, fenitrothion (200 or 800 mg/kg bw) was administered PO to groups of 10 male Sprague-Dawley rats. SC injections of atropine (10, 25 or 50 mg/kg bw) or IP injections of 2-PAM (50 or 100 mg/kg bw) were administered immediately after fenitrothion. As for the mouse studies, 25 mg/kg bw atropine and 50 mg/kg bw 2-PAM were tested in combination.

Clinical signs of intoxication were tremors, salivation, ataxia, lacrimation, exophthalmus, piloerection and dyspnoea. Only tremors were seen in rats given fenitrothion (200 mg/kg bw), and atropine (10, 25 or 50 mg/kg bw). At 800 mg/kg bw fenitrothion, atropine treatment at 10, 25 or 50 mg/kg bw was effective in reducing toxic symptoms and enhancing survival rate (10% survival rate in animals not receiving atropine treatment compared with 20-30% in atropine-treated animals). 2-PAM did not affect the survival or decrease toxic signs. Survival time and clinical symptoms of rats after fenitrothion administration was not further improved by co-administration of atropine and 2-PAM.

In conclusion, atropine was effective in reducing clinical symptoms and in enhancing survival rates in mice and rats following acute fenitrothion poisoning. 2-PAM or atropine & 2-PAM combination did not appear to exhibit an antidote effect.

Dubois KP (1960) The effects of pyridine-2-aldoxime methiodide (2-PAM) and TMB-4 on the acute toxicity of Bayer 41831, S 5660. Department of Pharmacology, University of Chicago, USA. Report date: November 28, 1960.

Either 2-PAM (100 mg/kg bw) or 1,1-tri-methylene-bis-(4-formyl-pyridinium) bromide (TMB-4 at 75 mg/kg bw) was administered IP to 5 female Sprague-Dawley rats, before an IP administration of fenitrothion technical (Bayer 41831, S 5660; purity not stated) at a dose of 160 or 320 mg/kg bw in 20% ethanol and 80% propylene glycol. A previous study had determined the LD50 of fenitrothion technical (Bayer 41831) to be 160 mg/kg bw.

A mortality rate of 20% was observed with 160 mg/kg bw fenitrothion and TMB-4 or 2-PAM. However, with 320 mg/kg bw fenitrothion, the mortality rate was unchanged with 2-PAM, whereas the rate was increased to 80% after TMB-4 treatment. The results suggest that 2-PAM is an effective antidote as indicated by a 2-fold increase in the LD50 from 160 mg/kg bw to 320 mg/kg bw.

Kimmerle D (1962) Effectiveness of PAM as antidote for S 5660 (Folithion). Institute of Toxicology, Bayer AG, Germany. Report date: January 10, 1962.

To test the efficacy of atropine sulphate and 2-PAM to ameliorate acute fenitrothion poisoning symptoms, technical fenitrothion (S 5660, source and purity not stated) was administered PO to male Sprague-Dawley rats (exact numbers used not stated) at a dose of 500 or 750 mg/kg bw followed by IP injections of atropine sulphate (50 mg/kg bw) or 2-PAM (5 or 10 mg/kg bw) separately or in combination, immediately after signs of toxicity (15 min post dose). Atropine reduced clinical signs of acute toxicity, whereas 5 mg/kg bw 2-PAM produced no reversal, and at 10 mg/kg bw only a slight reduction. 2-PAM and atropine sulphate in combination produced similar effects on clinical signs to the administration of atropine only. In conclusion, results from this study suggested that atropine or atropine and 2-PAM reduced the clinical signs of fenitrothion poisoning, whereas 2-PAM alone had minimal effects on clinical signs.

Matsubara T & Horikokoshi I (1983) Possible antidotes in severe intoxication with fenitrothion in rats. J Pharm Dyn 6: 699-707

The potential antidote effects of IP administration of atropine sulphate (20 mg/kg bw), scopolamine hydrobromide (10 mg/kg bw), reduced glutathione (100 mg/kg bw), biperiden lactate (10 mg/kg bw), sodium phenobarbitone (50 mg/kg bw), diphenhydramine hydrochloride (10 mg/kg bw), 2-PAM (50 mg/kg bw), diazepam (30 mg/kg bw), and (1-(methyl morphorinium)-3-(4-hydroxyiminomethylpyridinium) propane dibromide (TPMM) (100 mg/kg bw) after acute fenitrothion poisoning was studied.

In the first part of this study, technical fenitrothion (Sumitomo Chem Co; purity or batch No. not stated) in 10% Tween-80 was administered PO to groups of 10 male Sprague-Dawley rats (Shizuoka Agricultural Co-operative, Japan) at a dose of 500 mg/kg bw. A single IP administration of putative antidote was given immediately following fenitrothion, and the survival ratio, time of death (monitored until day 7 post treatment), and clinical signs observed (for 72 h post treatment).

Four rats without antidote died within 10 h, and another 2 between 24-48 h (ie survival was 40%). 2-PAM or diphenhydramine significantly ($p<0.01$) increased the survival to 100%, and atropine or TPMM significantly ($p<0.05$) increased survival to 90%. Treatment with scopolamine, phenobarbitone or biperiden did not affect survival. In contrast, glutathione or diazepam decreased (not significant) survival to 20% and 30% respectively.

Clinical signs of fenitrothion intoxication (onset after 10 min) were: tremors, salivation, ataxia, lacrimation, convulsions, urinary incontinence, miosis, fasciculation, and diarrhoea. These signs disappeared within 10 h, but reappeared 24 h later and then were not observed at 48 h post

treatment. The antidote efficacy was quantified using a four step severity scale (ranging from no signs to severe) and incidence for some clinical signs, namely salivation, miosis, fasciculation and ataxia. Salivation was significantly ($p< 0.01$) reduced from a severe grade (7/10 control animals) to no signs of slight, moderate or severe salivation following treatment with atropine, scopolamine, or biperiden (in all 10 fenitrothion treated animals) up to 10 h post treatment (at 24 h moderate salivation was observed); and by 2-PAM only up to 1 h (only effective for severe salivation as moderate salivation was observed in 8 animals and slight salivation in 2 animals). In contrast, treatment with the other drugs (TPMM, diphenhydramine, phenobarbitone, diazepam and glutathione) did not significantly reduce salivation.

Miosis was altered to mydriasis by atropine, scopolamine or biperiden up to 3 h post treatment (in all 10 fenitrothion-treated animals), while the other drugs (2-PAM, TPMM, diphenhydramine, phenobarbitone, diazepam and glutathione) had no effect. Severe fasciculation was significantly ($p<0.01$) reduced by 2-PAM and TPMM at 1 h (in 10 animals, however, slight to moderate fasciculation was still observed); the other drugs (atropine, scopolamine, biperiden, TPMM, diphenhydramine, phenobarbitone, diazepam and glutathione) had no effect. Atropine, scopolamine, biperiden and 2-PAM significantly ($p<0.01$) reduced severe ataxia in all 10 rats at 1 h (however, slight to moderate ataxia was still observed in the animals); the other drugs (TPMM, diphenhydramine, phenobarbitone, diazepam and glutathione) had no effect on the severity of ataxia.

In the second part of the study, those compounds that had demonstrated some antidote efficacy and a selected number of compound combinations were ranked. All control rats given fenitrothion at 800 mg/kg bw PO died. Treatment with atropine, scopolamine, biperiden and 2-PAM increased survival to 40%, 20%, 30% and 20%, respectively ($p<0.05$ for atropine only). Treatment with atropine & 2-PAM, scopolamine & 2-PAM, and biperiden & 2-PAM increased survival to 50%, 40% ($p<0.05$ for both) and 20% respectively (not-significant). Severe salivation and fasciculation were significantly alleviated ($p<0.01$) by each antidote treatment except for diphenhydramine at 1, 3 and 6 h.

In the third part of the study, IP injections of atropine (10 mg/kg bw), 2-PAM (25 mg/kg bw) or as a combination, were repeatedly administered over 48 h (atropine at intervals of 9 h, 2-PAM at intervals of 3 h) following PO administration of fenitrothion (800 mg/kg bw), and the survival ratio noted. Gross necroscopy was performed on rats that died during treatment and on survivors at the terminal kill.

Animals treated with fenitrothion died within 3 h. Repeat treatment with atropine significantly ($p<0.01$) increased survival rates to 80%; in contrast animals treated with 2-PAM exhibited a survival rate of 20%. Combined treatment with atropine & 2-PAM significantly ($p<0.01$) increased survival ratios to 90% in rats receiving 800 mg/kg bw fenitrothion.

In conclusion, this study found that the best therapeutic treatment against acute fenitrothion poisoning was a combination of atropine and 2-PAM.

Uehara S, Hiromori T, Isobe N, Suzuki T, Kato T & Miyamoto J (1993) Studies on the therapeutic effect of 2-pyridine aldoxime methiodide (2-PAM) in mammals following

organophosphorous compound-poisoning (report 3): distribution and antidotal effect of 2-PAM in rats. J Toxicol Sci 18: 265-275

Fenitrothion (purity 97%) at 75 mg/kg bw and malathion (purity 96%) at 105 mg/kg bw (prepared by Sumitomo Chem Co, Japan) in 10% Tween 80 was administered IV to groups of 5 male Sprague-Dawley rats (Charles River; Japan). 2-PAM was administered IV at 50 mg/kg bw or by intramedullary injection (into the subarachnoid space between L3 and L4) at a dose of 10 mg/kg bw immediately following treatment with fenitrothion or malathion (1-2 min post dose). Animals were observed for clinical signs post treatment. Blood was obtained from the orbital`plexus and plasma, erythrocyte and brain ChE measured (time of sampling not stated). Control groups were IV fenitrothion (75 mg/kg bw), IV malathion (105 mg/kg bw) and saline administered intramedullary (400 µL/kg bw), or IV Tween-80 & 2-PAM (intramedullary 10 mg/kg bw).

All rats (5/5) died within 12 min after fenitrothion administration. Clinical signs preceding death were muscular fibrillation, salivation, miosis, clonic convulsions, ataxia, decreased respiration, and dyspnoea. Co-administration of 2-PAM reduced the severity of muscular fibrillation, salivation and miosis only (the other signs persisted), prior to death (5/5). Intramedullary 2-PAM prevented death (5/5) and reduced salivation and dyspnoea (other signs persisting). Muscular fibrillation and miosis were diminished following IV and intramedullary administration of 2-PAM 10 min after fenitrothion treatment; all rats survived. Intramedullary injection of 2-PAM and vehicle following IV fenitrothion induced clonic convulsions, ataxia and hyperexcitability.

Intramedullary 2-PAM following IV fenitrothion led to a non-significant reactivation in brain ChE activity from 11% of the control value to 23% at 15 min and 28% at 60 min (means of five trials). No increase in ChE activities was observed in erythrocyte or plasma levels at comparative time points and protocols. IV 2-PAM following fenitrothion treatment resulted in no increase in brain ChE activity.

Clinical signs following malathion treatment were similar to fenitrothion, with the exception of a discharge from the nostrils and all rats (5/5) died within 6 min (cf 12 min). Administration of 2-PAM IV had no effect on clinical signs, however, an intramedullary injection of 2-PAM diminished the dyspnoea, nostril discharge and muscular fibrillation.

Intramedullary administration of 2-PAM following malathion treatment resulted in a non-significant increase in brain ChE activity at 15 min (79%) and 60 min (67%) relative to the control group (28%). IV administration of 2-PAM following malathion treatment had no effect on brain ChE activity. Plasma and erythrocyte activities were not recorded.

In conclusion, the results suggest that intramedullary administration of 2-PAM to rats following acute fenitrothion and malathion poisoning can increase survival rats, and decrease some of the accompanying clinical signs.

Matsubara T & Horikoshi I (1984) Chemical reactivations of inactivated acetylcholinesterase after 2-PAM therapy in fenitrothion-poisoned rat and rabbit. J Pharm Dyn 7: 131-137

This published study investigated the reactivation of inactivated ChE with 2-PAM in rats and rabbits after acute fenitrothion poisoning.

Fenitrothion technical (Sumitomo Chem Co; purity or batch No. not stated) in 5 or 10% Tween-80 was administered PO to groups of 4-10 male Sprague-Dawley rats (Shizuoka Agricultural Co-Operative, Japan) at 20 or 500 mg/kg bw. 2-PAM (50 mg/kg bw) was administered IP immediately following fenitrothion treatment.

In fenitrothion (500 mg/kg bw) and 2-PAM treated rats there was a significant ($p<0.05$) increase in plasma ChE activity at 30 min (23% relative to fenitrothion treatment only) and 2 h (21%). Similarly, there was a significant ($p<0.01$) increase in erythrocyte ChE (30% at 30 min; 18% at 2 h) post treatment; but no increases were observed at 6 h. Brain ChE activity at all sampling times was not altered following 2-PAM treatment.

For rats treated with 20 mg/kg bw fenitrothion and 2-PAM there was a significant increase in plasma ChE activity (34%; $p<0.01$), erythrocyte ChE (34%; $p<0.05$) and brain ChE (34%; $p<0.05$) at 2 h post treatment, but no increases were noted at 6 h. No results were recorded at 30 min.

To determine the effect of repeat 2-PAM administration on ChE reactivation, rats (6/group) were treated with 20 mg/kg bw fenitrothion PO, followed by repeat dose IP administration of 2-PAM (50 mg/kg bw) at intervals of 2 h for 48 h. Plasma, erythrocyte and brain ChE activities were measured at 2, 6, 12, 24, 48, 96 h, 1 and 2 weeks. The reactivation of ChE after repeat 2-PAM treatment was significant ($p<0.05$) in plasma, erythrocytes and the brain at 2 or 6 h. Activities of all 3 enzymes gradually returned to control or pre-treatment levels within 2 weeks.

For the rabbit studies, fenitrothion (31 mg/kg bw) in 10% Tween-80 was administered IV to groups of 5 male Japanese albino rabbits (Kitayama Labes Co, Japan) immediately followed by 2-PAM (100 mg/kg bw) IV. Erythrocyte, brain and plasma ChE was measured at 30 min and 2 and 6 h following fenitrothion treatment. Vehicle or vehicle & 2-PAM (50 mg/kg bw) was administered to the concurrent control groups.

Fenitrothion administered IV to rabbits at 31 mg/kg bw followed by IV treatment with 2-PAM (100 mg/kg bw) led to significant increases ($p<0.01$) after 30 min in plasma ChE (46% relative to fenitrothion only), erythrocyte (37%) and brain ChE (22%).

In conclusion, results from this study suggest that restoration of inactivated plasma, erythrocyte and brain ChE's could be achieved by 2-PAM treatment in rats and rabbits. Furthermore, 2-PAM would appear to be more effective as an antidote when administered to animals below the LD50 of fenitrothion (previously established as 500 mg/kg bw by these investigators) or that repeat administration is necessary to achieve antidote actions.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Oral Administration in Rats for One Month

Trottier B, Fraser AR, Planet G & Ecobichon DJ (1980) Subacute toxicity of technical fenitrothion in male rats. Toxicol 17: 29-38

Technical fenitrothion (Sumitomo Chem Co, Japan; purity 96.7%) in sodium taurocholate was administered to male CD rats (Canadian Breeding Farms and Labs, Quebec; 36/group) by gavage at doses of 0, 2.5, 5, 10 or 20 mg/kg bw/day for 30 consecutive days. Animals were observed daily for signs of toxicity and morbidity. Four rats/group were euthanised at 8, 15, 22 and 30 days after commencement of treatment; and, at 8, 15, 29, 57 and 85 days after termination of treatment. Clinical chemistry parameters determined at the various time points were: plasma, erythrocyte and brain ChE, hepatic and renal carboxylesterases, glucose, BUN, creatinine, protein, amylase, AST, and AP.

Eight of 36 rats dosed at 20 mg/kg bw/day died in the first week of treatment. Clinical signs consisted of salivation, piloerection, diarrhoea, chromodacryorrhoea, excitability, ataxia, muscle fasciculations, generalised tremors and convulsions. No significant changes were observed in organ weights or body weights during the treatment period in any of the dose groups apart from rats dosed at 20 mg/kg bw/day. At this dose a significant ($p<0.05$) reduction in mean body weights was observed at day 30 (15%), 60 (22%) and day 87 (15%). These values returned to control levels at day 115 (85 days post termination of treatment).

A significant ($p<0.05$) dose-related decrease in plasma ChE activity (ranging from 30-65%) from day 8-30 of treatment was observed in rats treated at doses ranging from 5-20 mg/kg bw/day. No significant decreases were observed in rats treated at 2.5 mg/kg bw/day. Plasma ChE activity was restored rapidly to control values by day 38 (8 days post termination of treatment). Similarly, for erythrocyte ChE a significant ($p<0.05$) dose-related decrease in activity (range 30-60%) was observed from day 8-30 of treatment (dose range 5-20 mg/kg bw/day). However, activities did not return to control levels until day 45 (15 days post termination of treatment). A significant ($p<0.05$) dose-related decrease in brain ChE activity was observed from day 8-30 of treatment at a dose of 10 or 20 mg/kg bw/day (range 60-70%). In the other two dose groups statistically significant ($p<0.05$) reductions were observed at day 30 (30%) at a dose of 2.5 mg/kg bw/day; and at day 22 (45%) at a dose of 5 mg/kg bw/day. Activities returned to control values by day 60 (30 days post termination of treatment).

Liver carboxylesterases were significantly ($p<0.05$) decreased (range 50-80%) from day 8-30 (5-20 mg/kg bw/day). Activities returned to control levels by day 45 (15 days post termination of treatment) for all doses except at 20 mg/kg bw/day where a decrease of 25% was still observed. At this dose, levels had returned to normal by day 87 (57 days post termination of treatment). A significant decrease in renal carboxylesterase (range 20-70%) was observed from day 8-30 (dose range 5-20 mg/kg bw/day). Recovery of activity was rapid and comparable to control values by day 38 (8 days post termination of treatment).
No significant treatment-related changes were observed in other clinical biochemistry parameters.

A NOEL for the study can be set at 2.5 mg/kg bw/day based on reductions in plasma and erythrocyte ChE in male rats at the next higher dose.

Durham HD, Comeau AM, Cameron PH & Ecobichon DJ (1982) Subacute toxicity in rats orally administered fenitrothion alone and in selected formulation. Toxicol App Pharmacol 62: 455-464

Fenitrothion (Sumitomo Chem Co, Japan; purity 96.7%) in ethanol:propylene glycol (25:75) in the absence or presence of 1.5% (v/v) of Atlox 3409F (Atlas Powder Coy.) and Dowanol TPM (tripropylene glycol methyl-ether) (Dow Chem Co, Canada) was administered to non-fasted Sprague-Dawley rats (45/group with males and females randomly distributed among groups; Canadian Breeding Farms and Laboratories, Quebec) by gavage at doses of 0, 0 (vehicle plus Atlox 3409F and Dowanol TPM), 1, 5, 10 or 20 mg/kg bw/day for 30 consecutive days. Rats were observed for signs of toxicity and morbidity and body weights recorded 3 times a week during the 30-day treatment period and at weekly intervals thereafter. Five rats/group were randomly selected and euthanised at 7, 14, 21 and 30 days after commencement of treatment; and, at 8, 30, 60 and 90 days after termination of treatment. Haematological and blood chemistry parameters determined consisted of haematology (MCHC, MCH, MCV, Hct, Hb, erythrocyte and total and differential WBC counts)**;** and AST, ALT, AP, liver and kidney carboxylesterases, and plasma, brain and erythrocyte ChE activity.

No deaths were observed in any of the treatment groups. No clinical signs, changes in body weight or food consumption were observed in groups other than at 20 mg/kg bw/day (technical and formulation). In males, clinical signs consisted of piloerection, salivation, hyperexcitability, and chromodacryorrhoea. Additionally, slight non-significant reductions in food consumption and body weight in males were observed (from the graphs supplied) during the treatment period but recovery to control levels had occurred 8 weeks after treatment. In females, clinical signs were the same except that muscle fasciculations were also observed. Slight non-significant reductions in food consumption and body weight in females were observed (from the graphs supplied) during the treatment period. Recovery to control levels had occurred by week 8, in the rats treated with fenitrothion technical, however, the body weight of rats in the formulation control group remained lower. No significant treatment related changes were observed in haematological or routine biochemical parameters (AST, ALT or AP).

The results for treatment with either fenitrothion technical or formulation on plasma, erythrocyte or brain ChE activities and liver and renal carboxylesterases at various doses were compared at day 30. In male rats treated with fenitrothion technical, significant ($p<0.05$) reductions in enzymes were observed at 1 (liver carboxylesterase 25%), 5 (erythrocyte ChE 40%; brain ChE 30%), or 10 mg/kg bw/day (erythrocyte ChE 50%). In comparison, in males treated with formulation, reductions ($p<0.05$) observed at each dose was at: 1 (liver carboxylesterase 50%), 5 (erythrocyte ChE 50%; brain ChE 50%), or 10 mg/kg bw/day (erythrocyte ChE 60%). No significant reductions were noted at 20 mg/kg bw/day in males treated with fenitrothion technical or formulation.

In females rats treated with fenitrothion technical, significant ($p<0.05$) reductions in enzymes were observed at 5 (erythrocyte ChE 50%; hepatic carboxylesterase 45%), 10 (hepatic carboxylesterase 60%; and renal carboxylesterase 40%), or 20 mg/kg bw/day (hepatic carboxylesterase 70%; renal carboxylesterase 40%). In comparison, formulation treated rats had reductions ($p<0.05$) observed at each respective dose: 5 (erythrocyte ChE 60%; hepatic carboxylesterase 65%), 10 (hepatic carboxylesterase 70%; and renal carboxylesterase 60%), or 20 mg/kg bw/day (hepatic carboxylesterase 80%; renal carboxylesterase 60%). No significant reductions were noted at 1 mg/kg bw/day in females treated with fenitrothion technical or formulation.

In conclusion, the results suggest that there is little difference in toxicity between the technical and formulated product. Thus, a NOEL for the study can be set at 1 mg/kg bw/day based on reductions in erythrocyte ChE in male and female rats at the next higher dose tested.

Yamatomo T, Egashira T, Yoshida T & Kuroiwa Y (1983) Production of tolerance to fenitrothion in male rats. Jap J Pharmacol 33: 691-693

This short communication investigated the tolerance potential of rats to repeat administration of sublethal doses of fenitrothion. The study was prompted by previously published reports, which indicated that rats fed fenitrothion in the diet gradually reduced the degree of ChE inhibition in various organs in proportion to the exposure duration. To confirm these findings, technical fenitrothion (Sumitomo Chem Co; purity not stated) in olive oil was administered to male Wistar rats (4 or 5/group; source not stated) PO at doses of 0 (vehicle), 7.25, 14.5 or 29 mg/kg bw/day for 28 consecutive days. These doses were selected because they represent 1/40, 1/20 and 1/10 respectively of a predetermined LD50 and were known to cause body weight loss. Thus, one end point used to identify tolerance was reduced body weight loss as a function of time. The other end point used in this study, namely lethal dose susceptibility was tested by pretreating groups of rats with fenitrothion (0 (vehicle), 7.25, 14.5 or 29 mg/kg bw/day; PO) for 5 days before high-dose fenitrothion administration (58 mg/kg bw/day PO; ie 1/5 LD50) for a further 10 days. Clinical signs and body weights were monitored for both aspects of the study.

Signs of toxicity were observed at 14.5 and 29 mg/kg bw/day; both groups experienced tremors and ataxia but those in the high dose group also had lacrimation, urination and salivation. From a supplied graph it appears as though the reduced mean body weight observed at 14.5 and 29 mg/kg bw/day was significant (ie maximum, 25% at day 10 and 30% at day 6 at 14.5 and 29 mg/kg bw/day respectively). It was reported, though no actual numbers provided, that ChE activity in the liver and plasma was reduced initially (within 1-4 days) in proportion to dose and that it showed a tendency to recover from day 6 onwards. This reduced ChE inhibition appeared to correlate with the gradual reduction in the severity of clinical signs.

Rats pretreated with fenitrothion for 5 days had significant body weight loss ($p<0.001$ at 14.5 and 29 mg/kg bw/day at day 3) though not with a clear dose relationship (ie loss at 14.5 mg/kg bw/day being greater than at 29 mg/kg bw/day). During pretreatment, rats had the expected cholinergic signs which together with body weight loss (both maximal at day 3) declined with exposure duration. Mortality rates 2-3 days after administration of 58 mg/kg bw/day were 20% for each the 14.5 and 29 mg/kg bw/day pretreated groups but very

surprisingly (given this dose was 1/5 LD50) mortality at 7.25 mg/kg bw/day and untreated controls was 60% and 100% respectively. This unexpected mortality rate for the untreated and low dose group may of course be the result of a distortion due to small group numbers.

The notion that tolerance is induced by repeat administration of fenitrothion appears to be supported by this preliminary study despite its obvious limitations due to small animal numbers in each treatment group. As indicated by the investigators further studies are warranted to elucidate the mechanism of tolerance development.

4.2 Inhalational Studies in Mice and Rats for up to One Month

Kohda H & Kadota T (1979b) Subacute inhalation toxicity study of Sumithion in rats and mice (amended report). Institute for Biological Sciences, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: June 7, 1979. (Pre-GLP)

Sprague-Dawley rats and ICR mice (Clea Japan Inc., Japan) were exposed in nose-only exposure chambers to aerosols of technical fenitrothion (Sumitomo Chem Co; Lot No. 417; purity 97.2%). Fenitrothion was dissolved with deodorised kerosene (Deo-base, Sonneborn Sons Inc., USA) and xylene (85:15, v/v). The study consisted of 3 separate protocols with differing dose and exposure duration. In protocol 1, 0 (air control), 0 (vehicle control), 15 or 62 mg/m^3/day fenitrothion was administered to 16 rats/sex/group and 15 mice/sex/group, whereby, animals were exposed for 2 h/day, 6 days/week for 4 weeks. In protocol 2, 0 (vehicle control), 7, 15 or 62 mg/m^3/day was administered to 6 rats/sex/group for 2 h/day for 5 days. In protocol 3, 0 (vehicle control), 2 and 7 mg/m^3/day was administered to 94 mice/sex/group and 24 rats/sex/group for 2 h/day, 5 days/week for 4 weeks. The inhalational particle diameter was assessed as being in the range 0.56-2.03 µm with approximately 50% being <1 µm. The rationale for experiments 2 and 3 was to measure ChE inhibition and determine a NOEL.

Animals were observed daily for general health and behavioural changes, and body weights recorded weekly (daily in protocol 2). Haematological and blood chemistry parameters were determined at the end of the exposure period in protocol 1. The clinical parameters determined on 10 rats/sex/group and 12 mice/sex/group consisted of haematology (Hct, Hb, erythrocyte and WBC counts, thrombocyte count and erythrocyte sedimentation)**;** and blood glucose, BUN, albumin, albumin/globulin (A/G) ratio, AP, total protein, AST, ALT, sodium, potassium and ChE activity. Erythrocyte sedimentation, sodium, potassium, glucose, BUN, AP, and AST levels were not examined in mice. In protocols 2 and 3 only ChE activities were determined.

After blood sampling the following organs were dissected from sacrificed animals and macroscopically examined: brain, eye, spinal cord, trachea, lung, liver, spleen, bone marrow, pancreas, kidney, testis, ovary, pituitary, thyroid, adrenal and any abnormal tissues. The absolute organ weights of the testes and ovaries, liver, lung, spleen, adrenals, kidneys, and brain were determined (mouse ovaries and adrenals were not weighed). Histopathology examination was carried out on these organs.

In protocol 1, 6 rats/sex/group were used for plasma and erythrocyte ChE activity determination (except negative control group) after 7, 14 and 28 days exposure, and also at 7 and 14 days after final exposure. These groups of rats were sacrificed 14 days after final exposure and brain ChE assayed. Additionally, plasma, erythrocyte and brain ChE levels were determined in 6/10 rats/sex in each group sacrificed 1 day after exposure. In protocol 2, plasma and erythrocyte ChE activities were determined after 1, 3 and 5 days exposure, and at termination (day 5 after exposure) for brain ChE. In protocol 3, plasma and erythrocyte ChE activities were determined in 6 rats/sex/group at 1, 3, 14 and 28 days exposure, and at 14 and 28 days after the final exposure. At 28 days after the final exposure brain ChE was assayed. Additionally, 6 rats/sex/group were sacrificed after 5 days of exposure, and at 1 and 7 days post exposure and the activities of all 3 ChE's was determined.

For the mice in protocol 1, plasma and brain ChE activities were determined at day 1 (5/sex/group), and 14 days after the final exposure (using pooled plasma and pooled brain from 3/sex/group). In protocol 3, at 1, 3, 5, 14, and 28 days during the exposure, 9-10/sex/group were sacrificed and the plasma and brain of 3-4 mice/group pooled and used for each ChE determination.

There were no deaths among rats for any treatment regimen. For rats at 62 mg/m^3/day, slight salivation and urinary incontinence was observed 3-6 days following treatment. All mice including vehicle controls developed depilation of the whole body 2 weeks after exposure, with recovery 2 weeks after termination of treatment (no reasons given). There was no significant reduction in mean body weight of mice for any treatment.

Mean leucocyte counts in mice appeared to increase dose proportionately (at 15 & 62 mg/m^3/day) relative to solvent controls (M, 14% & 22%; F, 9% & 22%) with significance ($p<0.05$) being achieved in both genders at 62 mg/m^3/day. Inexplicably the air-only control values were significantly ($p<0.05$) reduced relative to the solvent controls in male mice but not for females. Another aberration occurred with the thrombocyte count where a significant ($p<0.05$) reduction (40%) for the air-only control relative to vehicle control for males was observed but was increased for females (33%; not significant). Hence, although a significant ($p<0.01$) increase in thrombocyte count at 62 mg/m^3/day was observed in females relative to solvent control, its biological significance is uncertain. The small numbers of animals per group may be a contributing factor for the aberration.

In rats, haematological parameters were comparable to solvent control values except for a significant ($p<0.05$) reduction in Hb and Hct for females at 15 mg/m^3/day (but not at 62 mg/m^3/day). This change is unlikely to have any biological significance as there was no apparent dose relationship.

Clinical chemistry showed a significant ($p<0.05$) dose related reduction in A/G ratio (34% at 15 mg/m^3/day; 38% at 62 mg/m^3/day) relative to solvent control values (no significant differences were found between air-only and solvent controls); and, a significant dose-related increase ($p<0.01$) in ALT (34% at 62 mg/m^3/day) in male mice relative to solvent controls (however, there was a significant increase in air-only control values of 42% relative to solvent control values). No significant differences were noted in females. In male rats, a significant ($p<0.01$) dose-related

decrease in AST (20%) and, a dose-related decrease in ALT (22%) relative to solvent controls at 62 mg/m^3/day was observed (however, the ALT air-only control value was significantly increased 23% relative to the solvent control values). In female rats a significant ($p<0.05$) dose-related decrease in ALT (11%) at 62 mg/m^3/day relative to solvent controls was observed (no significant differences were found between air-only and solvent controls).

For rats in protocol 1, plasma and erythrocyte ChE activities were reduced by more than 50% (although significance was not calculated) at 62 mg/m^3/day (during the 4-week exposure), with recovery by day 14 after the final exposure (<20%) for plasma ChE, but erythrocyte ChE in males remaining reduced (20%). Brain ChE levels in male and female rats were reduced (45% and 65%) respectively at day 1 after the final exposure at the same dose, but remained reduced at day 14 after the final exposure (30% males; 40% females). At 15 mg/m^3 approximately 25% reduction of male and female plasma ChE and erythrocyte ChE in females was observed, with activities comparable to control by day 7 after the final exposure. Brain ChE levels were reduced 25% only in females day 1 after the final exposure, with a return to control values at day 14 after the final exposure.

For protocol 2, plasma, erythrocyte and brain ChE activities were significantly reduced in male and female rats by more than 50% (when measured at day 5 exposure) at a dose of 62 mg/m^3/day. At 15 mg/m^3, significant reductions were only observed in the plasma ChE at day-3 exposure (males 25%; females 45%) and day 5 (males 30%; females 50%). At 7 mg/m^3/day no significant reductions were noted. In protocol 3 at a dose of 2 mg/m^3/day no significant changes were observed in plasma, erythrocyte or brain ChE for either sex. At 7 mg/m^3/day significant reductions were only observed in plasma ChE in females at day 21 and 28 (40% and 30% respectively) of the exposure period.

In mice used in protocol 1, plasma and brain ChE activities were reduced (although significance was not calculated) in males (87% and 56% respectively) and females (60% and 32% respectively) at a dose of 62 mg/m^3/day (day 1 after the final exposure). Plasma ChE activity returned to normal control levels by day 14 after the final exposure, but brain ChE remained reduced (M & F, 25%). At 15 mg/m^3/day, significant reductions in male plasma (30%) and brain ChE activities in females (20%) were observed on day 1 post exposure, with activities comparable with control values for male plasma ChE and slight reductions in brain ChE persisting in females (13%) by day 14 after the final exposure. For protocol 3, mice at 2 and 7 mg/m^3/day had no significant changes in plasma, erythrocyte or brain ChE. There were no organ weight, gross pathology or histopathological changes that could be ascribed to treatment in either rats or mice.

The NOEL for the study was 2 mg/m^3/day, based on reductions in plasma ChE in female rats at the next higher dose.

Miyamoto J & Kadota T (undated) Subacute inhalation toxicity studies of Sumithion on rats and mice. Lab: Research Dept, Pesticides Division, Sumitomo Chemical Co Ltd. (Pre-GLP)

Two studies were done to investigate the inhalation toxicity of fenitrothion. In the first, fenitrothion (Lot No. 417, purity 97.2%, Sumitomo Chem Co, Japan) suspended in a mixture of

85% kerosene and 15% xylene at 0.07, 0.2 and 1% (not stated, but most likely v/v), was administered to Sprague-Dawley rats (source not given) for 2 h/day for 5 days, with a delivered concentration of 7, 15 or 62 mg/m^3/day and 6/sex/group (whole body exposure). The plasma and erythrocyte ChE activity of the rats were determined at days 1, 3 and 5 of treatment, and the brain ChE activity was determined at day 5. Additionally rats and mice (source and strain not given) were exposed to a fenitrothion aerosol (with the majority (?) of particles being between 1 and 3 µm in diameter) at 15 or 62 mg/m^3/day for 2 h/day, 6 days/week for 4 weeks. At the end of this period half the animals were euthanised, and haematology, clinical chemistry and histopathology examinations were done. The remainder of the animals were maintained for 14 days to determine ChE recovery.

In the rats exposed for 5 days, plasma ChE was decreased (>20%, estimated from supplied graph) at 15 mg/m^3/day from day 3. In males, plasma ChE was decreased at 62 mg/m^3/day from day 1, while in females the decrease was seen from day 3. Erythrocyte ChE was only decreased in high-dose animals, with decreases seen on day 3 and day 5. Brain ChE was also decreased in this group.

In the 4-week study, rats showed decreased plasma ChE (>20%, estimated from supplied graph) at the lowest dose tested in the first two weeks of exposure. Erythrocyte ChE was decreased in females by the lower dose, but in males only by the higher dose of 62 mg/m^3/day. Brain ChE was decreased in females by 15 mg/m^3/day and in males by 62 mg/m^3/day. Male mice had reduced plasma ChE levels and 15 mg/m^3/day while females showed reduced levels only at 62 mg/m^3/day. Brain ChE activity was decreased in both sexes only at the higher dose.

The body weights of rats treated with fenitrothion for 4 weeks was not changed in relation to controls. In contrast, all treated male mice showed a decrease in body weight (estimated from supplied graph) of around 10%. No haematological changes were seen in rats, while mice on 62 mg/m^3/day showed an increased WBC. Rats on 62 mg/m^3/day had a significant decrease in AST and ALT levels (males $p<0.01$, females $p<0.05$). This was associated in males with a significant ($p<0.05$) increase in liver weight, and histopathological examination showed fatty change in parenchymal cells.

Based on the effects on plasma ChE seen at 15 mg/m^3/day, the NOEL for the study is 7 mg/m^3/day, for a 5-day administration period. Based on the ChE effects seen at the lowest dose, no NOEL could be established in either mice or rats in the 4-week study.

Breckenridge C, Pesant M, Durham HD & Ecobichon DJ (1982) A 30-day toxicity study of inhaled fenitrothion in the albino rat. Toxicol App Pharmacol 62*: *32-43

Sprague-Dawley rats (Camm Research Laboratory Animals, New Jersey, USA) were exposed in nose-only exposure chambers to aerosols of a fenitrothion-emulsifier mixture. The formulation contained 11% fenitrothion technical (Sumitomo Chem Co; Lot No. not stated; purity 96.7%), 1.5% (v/v) Atlox 3409F (Atlas Powder Coy), and 1.5% (v/v) Dowanol TPM (tripropylene glycol methyl-ether; Dow Chem Co, Canada). Rats (16/sex/group except for a cage control group that had 20/sex; not placed in exposure chamber) were exposed for 2 h/day for 30 days at target concentrations of 0 (air control), 0 (vehicle control), 6.7, 20 and 60 µg/L air (equivalent to 0, 0,

0.0067, 0.02 and 0.06 mg/L air respectively). The actual mean concentration of fenitrothion that animals were exposed to was 5.2, 16.5 and 57 µg/L/day. The inhalational mean particle diameter was in the range 1.4 to 2.4 µm.

Animals were observed daily for general health and behavioural changes, and body weights recorded weekly. Haematology and blood chemistry were performed at day 7, 15 and 30 (terminal kill) on 10/sex/group. The remaining rats in each group were tested after a 30-day recovery period. Haematology parameters determined were MCHC, MCV, Hct, Hb, erythrocyte and total and differential WBC counts, prothrombin time, activated partial thromboplastin time, and reticulocyte count. Clinical chemistry included blood glucose, BUN, AST, ALT, AP, albumin, total protein, ornithine carbamoyl transferase, total bilirubin, total cholesterol and triglyceride, together with an assessment of plasma, brain and erythrocyte ChE activity. Urinalysis was also performed.

Macroscopic examination was performed on all rats at their scheduled death with the following organs being dissected, weighed and examined: brain, pituitary, trachea, lungs, liver, spleen, bone marrow, pancreas, kidneys, testis, ovaries, thyroid, and adrenal glands. Liver, kidney and brain were processed for histopathological examination together with samples from the nasal septum, paranasal sinuses, pharynx and buccal mucosa from rats killed at day 60. These respiratory tract tissues were examined to identify any local irritant effects.

Two deaths (1/sex) unrelated to treatment occurred. A female at 57 µg/L/day asphyxiated during exposure on day 2, while a moribund male at 5.2 µg/L/day with a fungal infection of the respiratory tract was sacrificed on day 17. Females at 57 µg/L/day showed pronounced muscular fasciculations and intermittent convulsions for 2-3 h post treatment beginning during the second week of treatment and persisting until day 30. Many (exact numbers not stated) also had a hunched posture, extension of limbs and uncoordinated movements. Males (3/16) and females (5/16) in the high-dose group produced a clear secretion from the eyes post treatment (exact time not stated). Apparently there were no treatment-related changes in body weight, haematology, biochemistry or urinalysis that occurred during the treatment period.

No individual or summarised animal results were presented, however, it was stated that there was no treatment related change in body weight. Food consumption was reduced in the first week of treatment in all groups placed in the restraining device; this was apparently not related to fenitrothion administration but this cannot be corroborated in the absence of individual animal data. No results for the haematology, clinical chemistry or urinalysis tests were presented, though it was stated that there were no significant differences among groups. Similarly, the absence of any comment suggests that no abnormalities were observed after necroscopy or the histopathological examinations.

Erythrocyte ChE was decreased by approximately 16% in the air control animals in comparison to the cage control animals; given this, the air control results were used as the standard values. It was postulated that this depression was related to the stress of restraint and handling.

In males, significant ($p<0.05$) reductions in plasma ChE were observed at 57 µg/L/day, on day 7, 15 and 30 of treatment (50%, 70% and 45% respectively). At 5.2 or 16.5 µg/L/day significant

reductions were observed at day 15 (25% and 30% respectively) and day 30 (25%; significant at 16.5 µg/L/day only). In females, significant ($p<0.05$) reductions were observed at 57 µg/L/day at day 7, 15 or 30 of treatment (85%, 90% and 85% respectively). No significant change occurred in females at 5.2 µg/L/day on day 7, whereas on day 15 and 30, significant reductions were observed (55% for both days). At 16.5 µg/L/day, significant reductions were observed on day 7, 15 and 30 (65%, 65% and 60% respectively). Recovery of activity was rapid and comparable to air controls by day 60.

The erythrocyte ChE activities for male and female rats was not significantly reduced at doses of 5.2 or 16.5 µg/L/day during treatment except in females receiving a dose of 16.5 µg/L/day at day 15 and 30 (20% on both days; $p<0.05$). At 57 µg/L/day, significant reductions in erythrocyte ChE activities in males at day 7, 15 and 30 were observed (50%, 50%, and 15% respectively); and in females (65%, 50% and 35% respectively). Recovery of activity was rapid and comparable to air control levels by day 60.

For males, significant ($p<0.05$) reductions in brain ChE activity at day 30 was observed at 16.5 and 57 µg/L/day (30% and 50% respectively). No significant reduction was observed at 5.2 µg/L/day (8%). For females, significant ($p<0.05$) reductions at day 30 was observed at all doses, ie 5.2, 16.5 or 57 µg/L/day (20%, 45% and 65% respectively). Recovery of brain ChE activity was complete by day 60 in all groups except for females at 57 µg/L/day (28%; $p<0.05$).

A NOEL for this inhalation study could not be established as significant inhibition of plasma or brain ChE activity was observed at 5.2 µg/L/day or 5.2 mg/m^3/day (measured as fenitrothion concentration).

4.3 Dermal Administration in Rabbits for Three Weeks

Suetake K, Kakino K, Kamimura H & Ichiki T (1991) 21-day dermal toxicity study in rabbits with Sumithion T.G. Safety Assessment Laboratory, Panapharm Laboratories, Japan. Report date: August 7, 1991. (GLP - US EPA)

Technical fenitrothion (Sumithion technical grade; Lot No. 90617; purity 73.7%; Sumitomo Chem Co, Japan) was applied to a shaved area between the shoulder and rump of New Zealand White rabbits (5/sex/group; Kitayama Labes Co, Japan) at 0 (distilled water), 3, 10, 50 or 250 mg/kg bw/day for a period of 21 days. At each treatment the test material was applied for 6 h under an occlusive bandage, after which the test site was washed with non-irritant soap and water.

Behaviour and general health were observed twice daily, and the test sites were examined for changes before each application, and dermal reactions were scored according to the method of Draize. Body weight and food consumption were measured weekly with haematology and blood chemistry being assessed at the end of the study. The following haematology parameters were determined: Hb, erythrocyte count, WBC, Hct, reticulocytes, MCV, MCH, MCHC, differential blood count, thrombocytes, coagulation time, and platelets. Clinical chemistry parameters determined were: sodium, potassium, inorganic phosphorus, calcium, chloride, total bilirubin,

total protein, BUN, creatinine, serum glucose, AST, ALT, AP, total cholesterol, albumin, globulin and A/G ratio together with erythrocyte, serum and brain ChE activity.

Necroscopy was performed on treated animals that either died or were sacrificed during the study and on survivors at the terminal kill. Absolute and relative organ weights of the following were determined: heart, liver with drained gall bladder, kidneys, testes/ovaries, epididymides and adrenals. A range of organs and tissues were preserved, and examined histopathologically.

At the high dose (250 mg/kg bw/day), all males and 2/5 females either died or were sacrificed moribund during treatment. Prior to death, characteristic OP poisoning signs were evident, namely hypoactivity, muscular hypotonia, tremors, bradypnea, hypothermia, salivation, clonic convulsions, loose or mucous stool, diarrhoea and a soiled periproctal region. At 250 mg/kg bw/day a reduction in body weight was observed in males at day 7 (11%), day 14 (14%) and day 20 (18%; $p<0.05$). Females showed slight reductions at day 14 (5%) and day 20 (7%) at the same dose but these were not significant. Rabbits in the treated groups showed similar food intakes to controls.

At 3, 10 and 50 mg/kg bw/day, very slight erythema (score 1; range 0-4) was observed in most males (13/15) from day 5-7 onwards and in females (10/15) from day 5-9 onwards. Very slight oedema (score 1; range 0-4) was observed in 1/15 males (from day 8-18) and 8/15 females (day 13 onwards). The mean dermal irritation scores ranged from 0.2-1.4 in males and 0.2-1.8 in females. Desquamation was observed in 6/15 males and 13/15 females (time of onset not stated). At 250 mg/kg bw/day, very slight erythema (score 1) was observed in the surviving 4 males (day 4-6) and 3 females (day 4-6); and, very slight to well defined erythema (score 1-2) in 1 male (day 7-10) and 2 females (day 7-14). Very slight oedema (score 1) was observed in 1 male (day 7 & 8) and 1 female (day 5-7). Very slight to moderate oedema (score 1-3) was noted in 1 male (day 8). The mean dermal irritation scores ranged from 0.2-2.66 in males and 0.2-2.4 in females. Desquamation was observed in 3 males and 3 females (time of onset not stated).

Although a significant ($p<0.05$) increase in mean prothrombin time (12%) and ALT (30%) in males was observed at 50 mg/kg bw/day, there was no evidence for any treatment-related haematological or biochemical changes (not including ChE levels) at 3, 10, 50 or 250 mg/kg bw/day.

Significant ($p<0.01$) decreases in mean erythrocyte ChE activity were observed in males at 10 and 50 mg/kg bw/day (46% and 49% respectively), and in females at 10, 50 and 250 mg/kg bw/day (51%, 70% and 83% respectively) 2 h after final dosing. After 24 h, significant ($p<0.01$) decreases were observed in males at 50 mg/kg bw/day (58%) and in females at 50 and 250 mg/kg bw/day (65% & 79% respectively). Similarly, significant ($p<0.01$) reductions in mean plasma ChE activity in females were observed at 10 and 50 mg/kg bw/day (73% and 89% respectively) 2 h after final dosing. After 24 h, significant decreases were observed in males at 50 mg/kg bw/day (40%; significance $p<0.05$) and in females at 10, 50 and 250 mg/kg bw/day (41%, 60% and 94% respectively; all significant at $p<0.01$). Mean brain ChE activity was also significantly reduced in males at 50 mg/kg bw/day (36%; $p<0.01$); and in females at 10 (21%; $p<0.05$), 50 (30%; significance $p<0.01$) and 250 mg/kg bw/day (79%;

p<0.01) was observed at termination. No ChE activities were measured in rats at 250 mg/kg bw/day that died or were sacrificed in a moribund condition.

An increase (25%; p<0.05) in absolute and relative (36%; not significant) weight of the adrenals was observed in females at 250 mg/kg bw/day. No other organs showed any significant differences in absolute or relative weights between treated and control groups. Histopathological examination of the skin revealed thickening of the epidermis, hyperkeratosis and inflammatory infiltrates in the corium in males and females at all doses; and, haemorrhage in the corium at 10, 50 and 250 mg/kg bw/day. At 250 mg/kg bw/day, 1 male and 1 female had slight necrosis in the liver and some animals showed changes in the digestive tract and kidney that were considered to be the cause of deterioration in general condition.

The NOEL for this study is 3 mg/kg bw/day based on the inhibition of serum and brain ChE activity at doses in excess of 10 mg/kg bw/day.

4.4 Other Studies

Nag A & Ghosh JJ (1985) Comparative toxic effect of Sumithion on rats and pigeons at the level of myelin. Neurosci Lett 56: 167-173

This published paper compared the changes in rat and pigeon myelin composition following fenitrothion treatment.

Technical fenitrothion (Rallis India Ltd; purity 97%) in ground nut oil was administered to rats and pigeons (8/group; strain and source of animals not stated) PO at 0 (vehicle), 5 (in pigeons) or 50 mg/kg bw/day (in rats) for 1, 3 or 5 days. Animals were killed 24 h after the last treatment. Brain and spinal cord were collected within 30 min of sacrifice and the myelin isolated by density gradient centrifugation. Levels of total protein, total lipids, cholesterol, cerebroside, sulphatide, phospholipid, and total ganglioside were determined.

In spinal cord myelin from pigeons, a significant decrease in total protein (41%; p<0.005), cerebroside (31%; p<0.001) and sulphatide (16%; p<0.005) and increases in cholesterol (21%; p<0.005) was observed at day 5 at a dose of 5 mg/kg bw/day. No significant changes in any of the parameters measured from the spinal cord myelin of rats dosed at 50 mg/kg bw/day was observed. The investigators state that brain proteins were apparently unaffected by treatment in rats and pigeons however, the results were not reported.

Densitometric tracings of myelin proteins from rat and pigeon spinal cords showed decreases in 2 high-molecular weight myelin proteins, Wolfgram and proteolipid protein, and an increase in basic protein at day 3, with a subsequent return to normal control levels by day 5 (ie before the end of dosing) in the rat, but not in the pigeon. The electrophoretic mobility of proteolipid and basic protein of both species increased towards the anode, indicating an increase in negative charge because of phosphorylation or acetylation at day 3, whereas at day 5 this trend occurs only in pigeons.

In conclusion, changes in the constituents of the myelin membrane in pigeons occur with repeat administration of fenitrothion whereas rats appear relatively resistant. It was suggested that phosphorylation of basic protein components by a cAMP-dependent protein kinase increases cAMP levels and could disrupt membranes in pigeons. The rat may have a detoxifying mechanism allowing recovery of myelin proteins.

5. SUBCHRONIC TOXICITY

5.1 Rat

Dubois KP & Puchala E (1960b) The subacute toxicity of Bayer 41831 to rats. Lab: Pharmacology Dept. University of Chicago. Sponsor Bayer. (pre-GLP)

Fenitrothion (Bayer 41831; batch or purity not reported) was suspended in 20% ethanol and 80% propylene glycol and administered IP to female Sprague-Dawley rats (5/group; source not reported) at 10, 25, 50 or 100 mg/kg bw/day by daily injection for 60 days. Except for rats that died within 10 days of the first dose, body weight was measured at 5-day intervals throughout the study. ChE activity in plasma, brain and submaxillary glands was determined on day 1, 3, 7, 11, 18, 25 and 60 in rats (3/time interval) at 10 mg/kg bw/day.

As shown in the Table below there appeared to be a clear relationship between dose and onset of death.

Dose (mg/kg bw/day)	**Mortality** (days after first dose)			
	(0-5)	**(5-10)**	**(10-30)**	**(30-60)**
0	0	0	0	0
10	0	0	0	0
25	1	2	2	-
50	3	2	-	-
100	4	1	-	-

After 60 days of treatment at 10 mg/kg bw/day, rats were about 10% lighter than controls as could be judged from a supplied graph. At 25 mg/kg bw/day the body weight was reduced by about 10% under their starting weight after 15 days (death of last rat in this group) and about 20% less than control (measured on day 15). There was no description of clinical signs prior to death in any treatment group. However, for rats at 10 mg/kg bw/day it was stated that cholinergic symptoms were observed for about 3 h after each daily treatment.

From a supplied graph it appears that whereas serum ChE activity was reduced by about 75% within 1 day, brain and submaxillary ChE achieved a similar degree of inhibition after approximately 10 days of treatment at 10 mg/kg bw/day. At the end of treatment the mean ChE

activity was reported to be inhibited by 80%, 69% and 83% for brain, submaxillary glands and plasma respectively. Thus, based on observed ChE inhibition, no NOEL could be set for this study.

5.1.1 Dietary Studies

Misu Y, Segawa T, Kuruma T, Kojima M & Takagi H (1966) Subacute toxicity of O,O-dimethyl-O-(3-methyl-4-nitrophenol) phosphorothioate (Sumithion) in the rat. Tox Appl Pharm 9:17-26

Technical fenitrothion (source, purity not given) was administered in the diet to male Wistar rats at 0, 32, 63, 125, 250 or 500 ppm for 90 days, with either 16 or 17 rats/group. Rats were observed daily for behavioural changes, body weight changes, food intake and the presence of protein or glucose in the urine. After 30, 60 and 90 days, 4 rats/group were euthanised and the following tissues taken for examination; brain stem, brain cortex, cerebellum, thyroid, heart, lung, liver, kidney, spleen, adrenal, testes and prostate. The ChE activity in plasma, erythrocytes, brain, liver and kidney was determined. The noradrenaline and 3-hydroxytyramine levels in the brain stem, liver and spleen were examined (though the rationale for this was not described). In the rats killed after 90 days, a histopathological examination of the above tissues was carried out. Tissues from rats dying during the study were also examined.

In addition, 2 groups of rats were maintained on 0 or 500 ppm fenitrothion in the diet. These rats were euthanised after 11 days of treatment, when the cholinergic signs in the treated group were maximal.

During the study, 1 high dose rat died. Clinical signs at 500 ppm included muscle fasciculations, ataxia, piloerection, and lacrimation. Other ophthalmic signs included corneal opacity and corneal and conjunctival bleeding, which recovered by day 30. At 250 ppm a number of rats showed muscle fasciculation and lacrimation from day 15-18. The NOEL based on clinical signs seen at 250 ppm is therefore 125 ppm.

There appeared to be a decrease in body weight in the high dose animals (estimated from a graph), which was most marked in the first 7 days of treatment. Food intake also appeared reduced over this period. There were no body weight changes observed in any other treatment groups. The NOEL for body weight changes therefore appears to be 250 ppm in the diet.

There were no changes in body relative organ weight seen at 250 ppm but at 500 ppm the weight of testes and brain appeared to be increased. In view of the body weight loss, this result would be expected. Absolute organ weights were not reported. There were no significant histopathological changes observed. Urinary tests did not show any treatment related effects on protein or glucose levels.

ChE inhibition is detailed below.

ChE Inhibition (mean percentage)

Dose (ppm)	Plasma ChE	Erythrocyte ChE	Brain ChE
32	0	20	3
63	0	26	2
125	37	62	3
250	51	70	9
500	50	82	43

From the above, it can be seen that significant plasma ChE inhibition occurs at 125 ppm. Erythrocyte ChE inhibition of >20% is seen at 63 ppm, while brain ChE inhibition is seen at 500 ppm. Therefore, the NOEL for erythrocyte ChE activity is 32 ppm based on reduced activity at 63 ppm. The NOEL for brain ChE inhibition is 250 ppm, while the NOEL for plasma ChE inhibition is 63 ppm. The noradrenaline and 3-hydroxytyramine content of brain stem, spleen and liver showed no treatment-related changes.

Overall the NOEL for the study was 32 ppm in the diet (equivalent to 3.2 mg/kg bw/day), based on the effects on erythrocyte ChE seen at 63 ppm.

Hosokawa S & Miyamoto J (1975) Effect of subchronic feeding of Sumithion, Sumioxon and p-nitrocresol on rat hepatic oxidative phosphorylation and mixed function oxidases. Botyu-Kagaku 40:33-38

Fenitrothion (purity 97.2%), fenitrooxon (purity 99%) and 3-methyl-4-nitrophenol (purity 99.5%) (all obtained from Sumitomo Chem Co) was administered in the diet to Wistar rats (4/sex/group; source not specified) for 90 days in the following dosing regimen; fenitrothion at 150 ppm, fenitrooxon at 50 ppm and 3-methyl-4-nitrophenol at 1500 ppm. A control group was also maintained. Food and water were available *ad libitum*. Body weight was determined during the study (unspecified time intervals). At the end of the feeding period, all animals were fed a control diet for 3 days, then fasted for 12 h prior to euthanasia.

Rats were then killed by decapitation, the liver removed and mitochondrial and microsomal fractions prepared. The oxygen utilisation, ADP/O ratios, respiratory control indices and mitochondrial ATPase activities were measured. The microsomal enzyme activity was determined by measuring the demethylation of aminopyrine and the aromatic hydroxylation of aniline. The cytochrome P-450 and $NADPH_2$ -cytochrome c reductase activities were determined.

Fenitrothion at 150 ppm produced a slight increase in the body weight of males and a decrease in the body weight of females at the end of the study. Fenitrooxon at 50 ppm produced a slight increase in body weight of females, and no change in the body weight of males. Treatment with 3-methyl-4-nitrophenol at 1500 ppm produced an increase of 33% in the body weight of males. There were no significant changes in the liver weights of treated animals but the total

mitochondrial protein concentration (mg/g liver) in males treated with 1500 ppm 3-methyl-4-nitrophenol was reduced by approximately 40% relative to controls.

ATPase activity was not altered by treatment with fenitrothion or fenitrooxon, however it was slightly decreased following treatment with 3-methyl-4-nitrophenol. There were no treatment related changes in mitochondrial respiration, including ADP/O ratio. It therefore appeared that long term feeding of fenitrothion did not produce any significant adverse effects on liver tissue, whilst there were some effects from the long term feeding of 3-methyl-4-nitrophenol.

Beyrouty P, Benjamin W, Robinson K & Noveroske J (1993) A 3-month dietary study of the potential effects of fenitrothion on behaviour, neurochemistry, neuromorphology in rats. Project No. 97145. Lab: Bio-Research Laboratories Ltd., Quebec, Canada. Sponsor: Sumitomo Chemical Co Ltd. Report date: June 16, 1993 with amendments on November 22, 1993. (GLP - US EPA)

Young (50-53 day old) Sprague-Dawley Crl:CD®(SD)BR rats (12/sex/group) were fed fenitrothion in the feed at concentrations of 0, 6, 20, 60 or 200 ppm (equal to 0.40, 1.32, 3.99 or 13.80 mg/kg bw/day in males and 0.46, 1.56, 4.85 or 17.60 mg/kg bw/day in females) for 13 weeks. Technical fenitrothion (Lot No. 90617; purity 94.3%) suspended in corn oil and then mixed with rodent chow was found to be suitable with respect to homogeneity, stability and concentration for the week between fresh batches. Food consumption and body weight were measured weekly and clinical monitoring was performed twice daily. A quantitative and qualitative functional observation battery (FOB) test was performed before treatment, then during week 3, 7 and 12 of treatment. Brain, erythrocyte and plasma ChE activities were measured in satellite groups of 5 rats/sex after 4, 8 and 13 weeks of treatment. At the conclusion of the study, 6 rats/sex/group were randomly selected for whole-body perfusion and subsequent processing to enable an extensive neuropathology assessment. A necropsy was performed on the remaining 6 rats/sex but the brains from control and high dose (200 ppm) rats were removed and dissected into 6 regions (cerebellum, cerebral cortex, hippocampus, striatum, thalamus/hypothalamus and the rest), then homogenised to quantify glial fibrillary acidic protein (GFAP) by dot-immunobinding assay. The assay was repeated 6 times.

The neuropathology assessment with light microscopy examined six levels of the brain, namely forebrain (through septum), cerebrum (through hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and 3 levels of the spinal cord (cervical, thoracic and lumbar). Brain weight prior to trimming was also recorded. Examination of peripheral nerves was performed under electron microscopy with samples of sciatic nerve (at mid-thigh region; cross-section, and sciatic notch; longitudinal and cross-section), sural nerve (at knee; cross-section) and tibial nerve (at knee; longitudinal and cross section). Additionally, various levels of the spinal cord (left Gasserian ganglion; cross-section, lumbar dorsal root ganglion (L4); cross-section, lumbar dorsal root (L4); cross-section, lumbar ventral root (L4); longitudinal and cross-section, cervical dorsal root ganglion (C5); cross-section, cervical dorsal root (C5); cross-section, cervical ventral root (C5); cross-section) were prepared for examination after epoxy resin embedding.

Qualitative FOB observations were performed with rats in the home cage, during handling and in a test arena. Home cage observations were body position, tremors, twitches, convulsions and

bizarre behaviour (static). For handling observations, the ease of removal from the home cage, vocalisation, lacrimation, pupil size, salivation, urinary staining, diarrhoea, body and abdominal tones, extensor thrust, corneal reflex, pinna reflex, toe and tail pinch and visual placing were assessed. Arena observations involved rearing, ataxic, hypotonic or impaired gait, overall gait incapacity, bizarre behaviour (movement) palpebral closure, tremors, twitches, convulsions, piloerection, respiratory rate/pattern, locomotor activity level, arousal, grooming, defecation, urination, auricular startle, olfactory response, air righting reflex and positional passivity when placed on top of a box. The quantitative aspects entailed grip strength (fore- and hindlimb), hindlimb splay, body temperature and motor activity (interrupting a light beam in a chamber).

No rats died during the study period. In females at 200 ppm; 4/27 had tremors during the second week of treatment; some (5/27) had a red/brown muzzle and; several (15/27) had a brown stained tail. Group mean food consumption was significantly ($p<0.01$) lower in both males (7%) and females (14%) during the first week of treatment at 200 ppm but was then significantly increased during weeks 5, 6, 7, 8 and 13 (average 12%) in females only. Bodyweights of males (4% average) and females (13% average) were significantly reduced ($p<0.01$-$p<0.05$) for the first 3 weeks of treatment at 200 ppm and this continued in females until week 8. At 60 ppm, only females had significantly reduced ($p<0.05$) bodyweight during the first week of treatment.

Although statistical significance ($p<0.05$) was achieved for reduced locomotor activity in the 20 ppm group at week 3 and in the 60 ppm group at week 7, a lack of any dose relationship suggests no treatment related effects. Similarly, a significant ($p<0.05$) change in positional passivity in males at week 12 was attributable to an unusual response in the control group (ie. controls were more passive than treated rats). Quantitative assessments did not reveal any unequivocal treatment-related effects although sporadic incidences of statistically significant reductions in the grip strength of the fore- (week 3) and hindlimbs (week 7) of females were observed at 200 ppm. Similarly at 60 ppm, a significant decrease ($p<0.05$) in the hindlimb splay of females at week 3 was not accompanied by any significant change at 200 ppm. No significant changes in body temperature or motor activity were observed.

Macroscopic tissue examination at necropsy revealed that 1/6 females at 200 ppm had a darkened area on the meninges in the cervical region of the spinal cord. Other findings were cysts on the edge of the spleen in 1/6 males at 60 ppm and 2/6 at 200 ppm. The pathologist's report indicated that cysts were common among laboratory rats and therefore these incidences were likely to be unrelated to treatment. Neuropathological examination revealed few abnormalities and of those found their incidence was equal to, or less than that observed in controls. These included dilated ventricles in the brain, vacuolation of the ganglion cell body in both cervical and lumbar dorsal root ganglia and in the gasserian ganglion; myelin bubbling/splitting in the lumbar dorsal and ventral roots and in nerves of the cervical and dorsal root ganglia; and axon degeneration in the lumbar ventral root and lumbar dorsal root ganglion. The mean percentage GFAP in males at 200 ppm ranged between 78.8% and 103.4% over all brain regions whereas for females it ranged from 85.9% to 97.6%. As US EPA guidelines suggest that GFAP percentages in excess of 115% are significant, it seems reasonable to conclude that minimal injury to astrocytes occurred at 200 ppm. No significant changes in brain weight were evident.

The observed ChE inhibition is shown in the Table below.

ChE Inhibition at Week 13 (mean percentage)

Dose (ppm)	Plasma ChE males		females§		Erythrocyte ChE males		females		Brain ChE males		females	
6	17	[13]	52	[28]	10	[6]	-14	[-2]	3	[4]	-2	[2]
20	13	[17]	66	[59]	10	[9]	-1	[11]	0	[0]	5	[11]
60	36*	[40]	77†	[74]	17	[23]	26	[30]	14**	[16]	58**	[57]
200	40**	[49]	87‡	[84]	41**	[44]	38	[46]	58**	[60]	77**	[78]

Values in square brackets represent the average inhibition measured after 4, 8 and 13 weeks of treatment; Negative values are where test levels exceed controls; * $p<0.05$, ** $p<0.01$ (Dunnett's test); § Dunnett's test of significance was used at week 8 but Dunn's test was used at weeks 4 and 13; † $p<0.01$ and ‡ $p<0.001$ (Dunn's test).

From the Table it is apparent that fenitrothion at the lowest concentration tested, namely 6 ppm produced appreciable and biologically significant plasma ChE inhibition in females. The investigators use of another statistical test (ie. Dunn's) to assess the significance of plasma ChE inhibition in females at weeks 4 and 13 but not at week 8 or elsewhere in the study (ie. Dunnett's test) appears to be an attempt to mask significance. This cannot be justified. The good agreement of the inhibition observed at week 13 relative to the average inhibition (n=3) measured at weeks 4, 8 and 13 indicate a modest degree of variability and hence support the biological significance of the observation. In addition, the clear dose-response relationship supports the assertion. Significant brain and erythrocyte ChE inhibition was observed at 60 and 200 ppm.

Thus, no NOEL can be established for fenitrothion in this study given the substantial plasma ChE inhibition observed in females at the lowest dose tested (6 ppm). The NOEL for brain and erythrocyte ChE inhibition was 20 ppm in the diet (equal to 1.32-1.56 mg/kg bw/day).

Kadota T, Kohda H & Miyamoto J (1972) Six month feeding study of Sumithion, Sumioxon and p-nitrocresol in rats. Lab: Pesticides Division, Sumitomo Chemical Co Ltd. (pre-GLP)

and

Kadota T, Kohda H & Miyamoto J (1975) Subchronic toxicity studies of Sumithion, Sumioxon and p-nitrocresol in rats and 92 week feeding study of Sumithion with special reference to change of cholinesterase activity. Botyu-Kagaku 40: 38-47

Fenitrothion (purity 97.2%, Lot No. 417), fenitrooxon (purity 99%) and 3-methyl-4-nitrophenol (purity 99.5%) (all obtained from Sumitomo Chem Co) were administered to Wistar rats (15/sex/group; source - Nihon Dobutsu Co) in the diet for 6 months, in the following dosing regimens: control, fenitrothion at 10, 30 or 150 ppm (equal to 0.6, 1.8 or 9.2 mg/kg bw/day for males and 0.6, 2.0 or 11.3 mg/kg bw/day for females); fenitrooxon at 5, 15, or 50 ppm (equal to 0.3, 0.9 or 3.0 mg/kg bw/day for male and 0.3, 1.0 or 3.7 mg/kg bw/day for females); or 3-methyl-4-nitrophenol at 150, 500 or 1500 ppm (equal to 9.2, 31 or 95 mg/kg bw/day for males and 10, 33 or 101 mg/kg bw/day for females).

Rats were observed daily for behaviour change and mortality. Body weight and food and water consumption were recorded weekly. At weeks 4, 8, 12 and 24 urinalysis for sugar, protein, bilirubin, urobilinogen and occult blood was performed. At the end of the study, plasma, erythrocyte and brain ChE activity was determined. Haematological examinations, involving erythrocyte, WBC and thrombocyte counts, differential leucocyte count, Hb, Hct and sedimentation rate were done. The following clinical chemistry parameters were examined; sodium, potassium, chloride, total protein, albumin, AP, AST, ALT, BUN, glucose and bilirubin.

Following euthanasia, gross autopsies were performed on all animals. The organs were weighed. Histopathological examination was done on the following tissues; brain, eye, spinal cord, peripheral nerve, heart, lung, spleen, bone marrow, lymph nodes, thymus, oesophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, urinary bladder, testes/ovary, prostate/uterus, pituitary, thyroid, adrenal and bronchus.

During the study there were no clinical signs of toxicity. One animal, on 15 ppm fenitrooxon, died just prior to euthanasia but the cause was not described. Only the final body weight results were presented. There were no differences in the body weights between groups at the end of the study. It was stated that there had been a slight decrease in body weight gain during initial feeding of fenitrothion at 150 ppm, however no data to support this statement were supplied. There was no treatment related changes in food or water consumption. Haematological, clinical chemistry and urinalyses did not show any treatment related changes. A Table showing ChE inhibition is shown below.

ChE inhibition (mean percentage)

Dose (ppm)	Plasma ChE males	Plasma ChE females	Erythrocyte ChE males	Erythrocyte ChE females	Brain ChE males	Brain ChE females
Fenitrothion						
10	6	55**	3	16	0	0
30	33	50**	17	29*	10	31**
150	43*	76**	69**	66**	53**	70**
Fenitrooxon						
5	26	13	0	0	0	3
15	34	33	0	8	5	13*
30	33	58*	33*	61**	2	24**

* $p<0.05$, ** $p<0.01$; 3-methyl-4-nitrophenol has no intrinsic ChE inhibitory activity in rats.

From this is can be seen that fenitrothion at 10 ppm (equal to 0.6 mg/kg bw/day) produced significant plasma ChE inhibition in females, while brain and erythrocyte ChE inhibition was seen at 30 ppm. No NOEL can therefore be established for fenitrothion, given the ChE effects seen in females at the lowest dose tested. The NOEL for brain and erythrocyte ChE inhibition was 30 ppm in the diet (equal to 1.8 mg/kg bw/day).

Sumitomo Chemical Co (1980) Six-month feeding study of Sumithion on rats. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co. Doc Code HT-20-0092. (Pre-GLP)

Fenitrothion (source and purity not specified) was fed to Wistar rats (source not specified) in the diet at 0, 10, 30 or 150 ppm (equal to 0.6, 1.8 or 9.2 mg/kg bw/day for males and 0.6, 2.0 or 11 mg/kg bw/day for females) for 6 months with 15 /sex/group. Food and water were available *ad libitum*. Animals were observed daily for clinical signs and mortality. Body weight and food and water consumption were measured weekly. Haematological tests, including erythrocyte count, WBC, Hct, Hb and sedimentation rate were carried out at the end of the study. Clinical chemistry was determined at the end of the study. The following parameters were measured: sodium, potassium, chloride, glucose, BUN, total protein, albumin, AP, AST and ALT. Urinalysis, consisting of analysis for glucose, protein, bilirubin, occult blood and urobilinogen, was done at week 4, 8, 12, and 24. Plasma, brain and erythrocyte ChE activities (pH method) were determined at the conclusion of the study. Apparently due to the lack of any dose relationship of plasma ChE inhibition a supplementary study with 15/sex/group re-examined changes in plasma ChE activity at week 2, 4, 6, 8, 12, 16, 20 and 24 for a control and 10 ppm group only.

At the conclusion of the study, the weight of the brain, lung, liver, kidney, spleen, heart, adrenal, testis and ovary were measured. Histopathological examination of brain, eye, spinal cord, peripheral nerve, bronchus, lung, heart, spleen, bone marrow, lymph node, thymus, oesophagus, stomach, small and large intestine, liver, pancreas, kidney, urinary bladder, testis, prostate, ovary, uterus, pituitary, thyroid and adrenal occurred for animals in the control and high dose group.

A supplementary study to investigate plasma ChE activity was also performed with fenitrothion being fed in the diet at 10 ppm to Wistar rats for 6 months. ChE activity was determined at weeks 2, 4, 6, 8, 12, 16, 20 and 24.

No treatment related clinical signs or mortalities were observed during the study. There were no significant difference in body weight gain between treated groups. Although rats on 150 ppm consumed consistently more food throughout the study this only reflected a difference among groups that was apparent at the beginning of the study. There were no haematological or clinical chemistry effects (with the exception of ChE effects) observed following 6-months treatment with fenitrothion. ChE inhibition measured after 6-months treatment is presented in the Table below.

ChE Inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
10	6	12 (55)	0	0	3	16
30	33	(50*)	10	31**	17	29*
150	54**	(76**)	49**	70**	69**	66**

* $p<0.05$, ** $p<0.01$; Values in parenthesis were obtained in the original study and may be suspect because of an unexpected control value. The other value shown was taken from a supplementary study that examined plasma ChE changes at intervals throughout a 6-month feeding period (low dose only examined in Supp. study).

Thus, after 6-months treatment there was no significant change of plasma ChE activity at doses less than 30 ppm in males or females. Significant inhibition of erythrocyte ChE ($p<0.01$) and brain ChE ($p<0.05$) was seen in females at 30 ppm. In the supplementary study, the mean inhibition seen throughout the study at a dose of 10 ppm was 23% (range 0-40%, with maximal inhibition seen during weeks 4-8) for males and 37% (range 12-56%, with maximal inhibition seen during weeks 4-8) for females.

Therefore, based on results seen after 6 months of treatment a NOEL for plasma, erythrocyte and brain ChE inhibition can be set at 10 ppm (equal to 0.6 mg/kg bw/day). No significant changes in organ weight or changes on histopathological examination were detected.

5.1.2 Inhalational Study

Coombs DW, Kenny TJ, Hardy CJ, Clark GC, Chanter DO & Gopinath C (1988) Sumithion TG 90-day inhalation toxicity study in the rat. Study No. SMO 300/881214. Lab: Huntingdon Research Centre Ltd, Cambridgeshire. Sponsor: Sumitomo Chemical Co Ltd. (GLP - US EPA, OECD & UK)

Fenitrothion (batch No. 60553, purity 94.6%, source Sumitomo Chem Co) was administered by inhalation to Crl(W1)BR rats (Charles River Portage, Michigan, USA) for 90 days. Rats were divided into 5 groups: air control, vehicle control, and fenitrothion at 0.2, 1 or 10 µg/L/day, with

20 rats/sex/group. Whole body exposure was used, with rats exposed for 6 h/day, 5 days/week for 90 days. The achieved fenitrothion concentration within respirable droplets (ie 100% were 6 µm) was measured during exposure and found to be almost at target concentrations. Each treatment group was further divided into 2 subgroups, each of which received the same exposure. Group A was used for all toxicological assessments during and at completion of the study, while Group B was used only for plasma and erythrocyte ChE determination during the study.

Rats were housed in groups of 5. Each treatment group was housed in a separate ventilated cabinet to prevent cross contamination. Food and water were available *ad libitum*. Clinical signs observed during exposure were recorded for all animals. Group A animals were examined twice daily, and abnormalities recorded. Body weight and food consumption for Group A animals were recorded weekly.

An ophthalmoscopic examination of all rats was done prior to the study. Group A rats were examined during week 13. Blood samples were taken from group A rats during week 12. A haematological examination determining Hct, Hb, erythrocyte count, MCHC, MCV, total and differential leucocyte count, platelet and reticulocyte count was done. Clinical chemistry examinations were also done, with glucose, ALT, AST, creatine phosphokinase, total protein, albumin, BUN, AP, bilirubin, creatinine, sodium, potassium, calcium, phosphorus, chloride, cholesterol, globulin and albumin/globulin ratio being determined.

Plasma and erythrocyte ChE were determined in group B animals prior to the study and prior to exposures 5, 10, 15, 20, 30, 40, 50 and after exposure 64. Brain ChE was determined after exposure 64.

Group A rats were killed after 66 exposures. A macroscopic examination of all animals was performed, and the following organs were weighed; brain, pituitary, thyroids, thymus, heart, lungs, liver, spleen, kidneys, adrenals, testes with epididymis and ovaries. The following tissues were preserved for histopathological examination: nasal passages, tongue, pharynx, larynx, trachea (including bifurcation), lungs, lymph nodes (tracheobronchial, mandibular, cervical, mesenteric), salivary gland, thyroids (with parathyroids), mammary gland, thymus, skin, skeletal muscle, spinal column, spinal cord (cervical, mid-thoracic and lumbar) kidneys, urinary bladder, testes, seminal vesicles, prostate, ovaries, uterus, vagina, brain (medulla/pons, cerebellar, cortex, cerebral cortex), pituitary, eyes, liver, spleen, pancreas, adrenals, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, heart, aorta, sciatic nerve, sternum/ribs, femur with joint and any gross abnormalities. The lungs from all rats were examined. All tissues from the air control, vehicle control and high dose rats was examined.

One high-dose female and 1 medium-dose male died during the study. Both deaths were related to blood sampling, and were not related to treatment with fenitrothion. As both animals were from the sub-group not scheduled for autopsy the carcases were discarded without further examination. The only clinical sign of note was a decreased responsiveness to auditory stimulation during exposure to fenitrothion in comparison to control animals. This occurred in all fenitrothion exposed groups, and was not able to be quantitatively assessed. There were no significant changes in body weight or food consumption during the study.

Ophthalmoscopic examination pretest revealed no abnormalities. At 13 weeks, a number of animals exhibited corneal opacities (2/20, 8/20, 6/20, 5/20 and 3/20). As indicated, the highest frequency was seen in vehicle controls, and there was no relationship to treatment with fenitrothion. This is an abnormality commonly seen in aging rats; the increased occurrence seen here may be related to exposure to the vehicle. Haematology and clinical chemistry examination revealed no findings of note. Mean ChE inhibition is detailed below.

ChE Inhibition (mean percentage)

Dose (µg/L/day)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
0.2	4	27	8	1	7	21
1	10	37	15	7	7	32
10	22	69	29	50	16	57

Plasma ChE inhibition occurred at the high dose in males and at all tested doses in females. Erythrocyte ChE inhibition was seen only at the high dose in both males and females, while no brain ChE inhibition was seen in males, and brain ChE inhibition was seen in all tested doses in females. Based on the effects seen in brain and plasma ChE, no NOEL can be set for this study. The NOEL for erythrocyte ChE can be set at 1 µg/L/day (1 mg/m^3/day).

There were no significant findings on macroscopic or histopathological examination, and the organ weights did not show any significant treatment related effects.

Overall, no NOEL could be established for this study, based on the effects seen in plasma and brain ChE at the lowest dose used. The LOEL is 0.2 µg/L/day (0.2 mg/m^3/day).

5.2 Other Species

Namba N et al (1966) Oral toxicity and metabolism of Sumithion on cattle, sheep and pigs. Research Bull No 89 of the Hokkaido National Agricultural Experiment Station (in Japanese) - A summarised translation.

Three per cent fenitrothion dust (source not specified) was administered orally to 3 Holstein cattle at 100 mg/kg bw/day (equal to fenitrothion technical at 3 mg/kg bw/day) for 90 days. No gross signs of abnormality were detected. Plasma ChE was decreased in 2 animals after 1 day treatment, however levels had returned to normal by day 30. After the 90-day treatment period, 1 cow was treated with 500 mg/kg bw (not specified whether active or formulation). The animal showed clinical signs including fibrillation, salivation and ataxia 1 h after treatment, however had recovered by 4 h after dosing.

Fenitrothion dust (3%, source not specified) was administered orally at 100 mg/kg bw/day (equal to 3 mg/kg bw/day of fenitrothion technical) to 2 male sheep for 60 days. No clinical signs were noted. Plasma ChE was decreased on day 7 of treatment, however, had returned to normal levels

by day 30. One animal administered a single dose at 770 mg/kg bw exhibited convulsions, salivation and an inability to stand.

Fenitrothion was administered orally at 31 mg/kg bw to a male Yorkshire pig. The animal had diarrhoea for 20 h. At 30 h, it had paralysis of the hind legs, convulsions and ataxia. All clinical signs were absent by 48 h after dosing.

6. CHRONIC TOXICITY

6.1 Mouse

6.1.1 Dietary Administration for 78 Weeks

Kudzins W (1975) 78-Week tumorigenic study in the ICR Swiss mouse using Sumithion. Project No. 343-114. Lab: Hazleton Laboratories America Inc. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: August, 1979 (Pre-GLP)

and

\Kudzins W (1979) Additional histopathology and tabular data for the 78 week mouse study with Sumithion. Project No. 343-114. Lab: Hazleton Laboratories America Inc. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: October 19, 1979

and

Kudzins W (1980) 78-Week tumorigenic study in the ICR Swiss mouse. Pathology summary groups 1 & 4. Project No. 343-114. Lab: Hazleton Laboratories America Inc. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: March, 1980

Fenitrothion (purity 97%, source Sumitomo Chem Co, batch No. not given) was administered at 0, 30, 100 or 200 ppm in the diet (equal to 0, 3.1, 11 or 21 mg/kg bw/day for males and 0, 3.6, 12 or 25 mg/kg bw/day for females) to ICR Swiss mice (Charles River Laboratories, Massachusetts) for 78 weeks. In the first 2 weeks of the study, the mice (50/sex/group) were treated at levels of 0, 10, 30 or 100 ppm before being changed to the indicated doses for the rest of the study. Mice were housed in same sex groups of 10. Food and water were available *ad libitum*.

Mice were observed daily for mortality. Body weight, food consumption, behaviour and appearance were recorded weekly from weeks 0-10, fortnightly from weeks 11-26, and every 4 weeks from weeks 27 to the end of the study. Ophthalmoscopic examination was done on all mice at weeks 28 and 78. Gross necropsies were performed on mice dying during the study and on all mice killed at the end of the study. The weights of the heart, liver, spleen, kidneys, testes with epididymis and adrenals were recorded, and organ to body weight ratios determined. The following tissues were preserved for histopathological examination: brain, pituitary, thoracic spinal cord, eye, thyroids, oesophagus, lung, heart, liver, gallbladder, spleen, kidneys, adrenals, stomach, pancreas, small intestine, large intestine, mesenteric lymph node, urinary bladder, testes

with epididymis, seminal vesicles and prostate or ovaries and uterus, skin, bone (sternum), bone marrow (femur), nerve with muscle and unusual lesions. Microscopic examination was done on all animals, where possible. There was no determination of ChE levels, or any haematological or clinical chemistry examinations.

There were no behavioural or clinical abnormalities observed during the study. No dose related changes in body weight or food consumption occurred. Individual animal results were not presented for the ophthalmological examinations. Summary results indicated that ophthalmoscopic examinations found cataracts at 28 weeks in a small number of treated mice. As the number were low (1 in each of groups 2 and 3, and 2 in group 4) this was not considered treatment related. At week 72 there was a relatively high incidence of cataracts in all groups (20/65, 17/56, 16/74 and 24/72). There was no dose-related increase in the incidence, and this finding was not considered to be related to the administration of fenitrothion. Corneal opacity was also seen at this examination, with a frequency of 7/65, 10/56, 5/74 and 7/72. This is also not considered to be treatment related, but to be a normal feature of aging mice.

Gross and histopathological examination revealed a number of abnormalities, none of which was considered to be treatment related. A number of neoplasms including thyroid cell adenoma and carcinoma, alveolar/bronchiolar adenoma and carcinoma, hepatocellular carcinoma, osteogenic sarcoma and mammary adenocarcinoma were found. Incidences of these tumours was either isolated or occurred with similar frequency in treatment and control groups and were therefore considered unrelated to treatment. There was a statistically significant decrease in the absolute and relative weight of the heart in the high dose group, however this is of questionable biological significance.

The NOEL for the study, based on the absence of any detected abnormalities was 200 ppm, equal to 21 mg/kg bw/day.

6.1.2 Dietary Administration for Two Years

Tamano S, Shibata M-A, Hagiwara A & Suzuki M (1990) Chronic dietary toxicity and carcinogenicity test of Sumithion technical in mice. Project No. 8425 & 8426. Lab: Daiyu-Kai Institute of Medical Science, Aichi, Japan. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Study date: December, 1984-1986. Report date: June 15, 1990. (GLP - US EPA)

The stability of technical fenitrothion (Sumitomo batch No. 40343, purity 96.7%) mixed with powdered feed (and 2% corn oil to prevent dusting) at 10 and 1000 ppm and freshly prepared every 4 weeks was assessed and found to be unchanged over that duration. Similarly, the homogeneity and actual concentration of fenitrothion in the feed were found to be suitable for feeding to B6C3F1 mice (Charles River Inc, Japan) at 0, 3, 10, 100 or 1000 ppm. For the purpose of assessing haematology, blood chemistry (including ChE activity) and urinalysis parameters at interim sacrifices, subgroups of 10 mice/sex were taken at weeks 13, 26, 52 and 78 from satellite groups of 50 mice/sex/group that had been treated similarly to those in the main study groups (50/sex/group). Dose selection was based on a 4-week dietary study at 0, 1, 3, 10, 100, 300 or 1000 ppm that showed a significantly reduced ChE activity at 10 ppm together with reduced food intake and weight loss in both sexes at 1000 ppm. Other findings at 1000 ppm were reduced

erythrocyte count, Hb concentration, Hct, and alkaline phosphatase in females and increased cholesterol in both males and females.

Observations in both the main and satellite groups included clinical signs and mortality (both daily), body weight, food and water consumption, and palpation (all weekly for first 14 weeks and every second week thereafter), and histopathology (all animals that survived to term, as well as those that died or were killed *in extremis*). At week 104, all mice in the main group were sacrificed for histopathological examination and some of the removed tissues ie. brain, heart, liver, spleen, kidneys, adrenals and testes or ovaries were weighed prior to fixation (except for thyroid and ovaries that were weighed after fixation). The following tissues from mice in the satellite groups (10 sex/group) at week 52 and the main group (all survivors) in week 104 were preserved in 10% formalin for histopathological examination: brain (cerebrum and cerebellum), spinal cord, sciatic nerve, eyeball, thyroid (including parathyroid), oesophagus, trachea, lung, heart, liver, gall bladder, spleen, kidney, adrenal, stomach, pancreas, small intestine (duodenum, jejunum, ileum), large intestine (caecum, colon, rectum), mesenteric lymph node, urinary bladder, prostate, testis, seminal vesicle, or ovary, uterus, bone marrow, aorta, thymus, salivary gland, mammary gland, vagina, pituitary, muscle, bone (sternum, femur, lumbar vertebrae) and any unusual lesions.

Blood and urine was collected from the main group survivors at 104 weeks for haematology (Hct, Hb, erythrocyte counts, MCV, MCH, MCHC, WBC and differential leucocyte counts), clinical chemistry (ChE activity, glucose, BUN, total protein, A/G, bilirubin, AP, ALT, AST and cholesterol) and urinalysis (specific gravity, occult blood, pH, glucose, ketones, protein, bilirubin and urobilinogen) assessment. Ophthalmoscopic examinations of mice in the control and 1000 ppm main groups were also done at week 104.

Survival in the main or satellite treatment groups were not significantly affected by dosing and no appreciable differences were reported in gross observations or behaviour. Calculated mean daily compound intake over 104 weeks in the 3, 10, 100 and 1000 ppm groups were respectively 0.37, 1.44, 12.6 and 134.3 mg/kg bw/day for males and 0.46, 1.51, 13.1 and 144.3 mg/kg bw/day for females.

No consistent treatment-related changes in haematology, urinalysis and ophthalmoscopy were observed. However, other treatment-related effects were:

- reductions in mean body weight and percent body weight gain at 1000 ppm for both sexes; at one year, body weights in both sexes were 17% lower than controls and after 2 years this difference had increased to 19% for females but reduced to 13% for males;
- reduced mean food consumption and water intake at 1000 ppm that correlated with bodyweight loss;
- alopecia among females at 1000 ppm was reduced at the terminal kill. This observation correlated well with a significantly reduced incidence of hair follicle atrophy among females at 1000 ppm (ie. 17/36 in control and 1/43 at 1000 ppm);
- an elevated plasma cholesterol ($p<0.01$-0.05) in the 100 and 1000 ppm groups of both sexes and reduced plasma glucose ($p<0.01$-0.05) at 1000 ppm of both sexes;

- an increased absolute and bodyweight-relative weight of the brain in both sexes at 1000 ppm that was significant ($p<0.01$) at the terminal kill. This finding was associated with a significant reduction ($p<0.01$) in the expected calcification (grade 1) of the brain parenchyma in 2-year old mice;
- significant reductions ($p<0.01$) in ChE activity in plasma, erythrocyte and brain at 100 and 1000 ppm in both sexes (see Table below).

ChE inhibition after 104 weeks of treatment (Mean Percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
3	0 [4]	12.5 [9]	{-9}[2]	5 [2]	4 [0]	9 [2]
10	12 [19]	14 [15]	3 [10]	8 [8]	0 [4]	6 [3]
100	62* [75]	68* [73]	80* [59]	51* [53]	64* [62]	45* [51]
1000	88* [89]	90* [89]	84* [85]	80* [81]	83* [78]	77* [77]

Values in square brackets represent the average inhibition measured at 13, 26, 52, 78 and 104 weeks of treatment; Negative values are where test levels exceed controls; * $p<0.01$.

No treatment-related change in incidence of neoplasms was observed. The numbers of mice with benign and malignant neoplasms were as follows:

No. of mice with observation		**Dosage Group**				
	Sex	**0 ppm**	**3 ppm**	**10 ppm**	**100 ppm**	**1000 ppm**
benign neoplasms	M	40	47	32	45	24
	F	23	14	18	19	16
malignant neoplasms	M	64	72	59	85	41
	F	49	44	43	50	45
all neoplasms	M	104	119	91	130	65
	F	72	58	61	69	61

In conclusion, there was no evidence of carcinogenicity in mice with fenitrothion doses that caused a significant reduction in bodyweight, elevated plasma cholesterol and an increased absolute and body-relative brain weight in both sexes. The NOEL was 10 ppm (equal to 1.44 mg/kg bw/day in males and 1.51 mg/kg bw/day in females) based on significant plasma, erythrocyte and brain ChE inhibition and plasma cholesterol concentration at the next higher dose of 100 ppm.

6.2 Rat

6.2.1 Gavage Administration for One Year

Ecobichon DJ, Comeau AM & Cameron PH (1980) Chronic toxicity of technical fenitrothion in male rats. Tox Appl Pharm 56: 409-417

Fenitrothion (purity 96.7%, Sumitomo Chem Co) in peanut oil was administered by daily gavage to male Wistar rats (Canadian Breeding Farms and Laboratories, St Constant, Quebec) at 0, 0.5, 1, 5 or 10 mg/kg bw/day for 12 months with 60 rats/group. At the end of this period, animals were maintained for a period of time to investigate the reversibility of the effects. Animals were housed in groups of 5 under controlled conditions, and food and water were available *ad libitum*.

Rats were weighed twice weekly for the first 4 weeks, once weekly for 30 weeks, then every 2nd week until the end of the study. At selected time intervals (not specified) 10 vehicle controls and 5 rats/treatment group were euthanised. Blood was collected, and the liver, kidney, brain, spinal cord and distal sciatic nerve removed from 2 rats/group and preserved for light and electron microscopic examination. Homogenates of fresh liver and kidney from each animal, and 3 brains/group were prepared for enzymatic assay. ChE activity was determined at intervals (not specified) throughout the study.

Haematological examination, consisting of Hct, Hb, erythrocyte count, WBC, total and differential leucocyte count, MCV and MCHC was done after 6 and 12 months as was determination of cholesterol, total protein, albumin, globulins, AST, ALT, AP, bilirubin, glucose, BUN and creatinine.

Body weight was significantly lower in rats on 10 mg/kg bw/day from week 20 (estimated from graph). These animals also showed clinical signs including hyperexcitability, piloerection, exophthalmos, fasciculations and chromodacryorrhea. All groups showed a decreased body weight at week 48, resulting from an inadvertent introduction of rats having murine respiratory syndrome into the holding facility and thereby necessitating tetracycline therapy for 2 weeks. In the post-treatment recovery phase, the body weight of high dose animals increased, and at 58 weeks there were no differences in body weight between groups. Other clinical signs seen during the study included corneal opacity, chronic respiratory disease and otitis media. These signs were seen in rats from all groups, and were not considered treatment related. A reflex activity test (dropping a rat from a height of approximately 23 cm onto a padded platform) performed on an unspecified number of rats did not reveal any changes.

Haematology and clinical chemistry examination did not reveal any treatment related changes. Plasma ChE activity was significantly ($p<0.05$) decreased at 5 and 10 mg/kg bw/day. At the end of month 1, the plasma ChE in the 2 lower dosage groups was decreased to <80% of control values (estimated from a graph), however this had recovered to be within 20% of control values by month 2, and is therefore not considered biologically significant. Erythrocyte ChE was significantly decreased by fenitrothion at 5 and 10 mg/kg bw/day, but not at 0.5 or 1.0 mg/kg/day. Brain ChE was also significantly decreased by the 2 highest doses, and not affected

by the lower doses of fenitrothion. Therefore, the NOEL was 1.0 mg/kg bw/day, based on effects on ChE activity seen at 5.0 mg/kg bw/day.

Histopathological examination of liver and kidney tissue revealed minor changes, such as hepatocellular swelling and vacuolisation. These signs were seen in both treatment and control groups, and were therefore not related to treatment. The brain and spinal cord appeared normal. The sciatic nerves examined by light microscopy appeared to be normal.

6.2.2 Dietary Administration for Two Years

Kadota T, Kohda H & Miyamoto J (1980) Ninety-two week feeding study of Sumithion in rats with special reference to cholinesterase activity. Ref No. HT-50-0001 Report date: Mar, 1980. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

and

\Kadota T, Kohda H & Miyamoto J (1977) Individual data on 92-week feeding study of Sumithion in rats with special reference to change of cholinesterase activity. Ref No. HT-70-1001 Report date: February 4, 1977. Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

and

Kadota T, Kohda H & Miyamoto J (1975) Subchronic toxicity studies of Sumithion, Sumioxon and p-nitrocresol in rats and 92 week feeding study of Sumithion with special reference to change of cholinesterase activity. Botyu-Kagaku 40: 38-47

Fenitrothion (purity 97.2%; Batch No. and Source not given) the diet at 0, 2.5, 5 or 10 ppm (equal to 0, 0.1, 0.3 or 0.5 mg/kg bw/day for males and 0, 0.2, 0.3 or 0.6 mg/kg bw/day for females) was fed to Wistar rats (15/sex/group, source not given) for 92 weeks. Rats were housed individually under controlled conditions. Food and water were available *ad libitum*. Behaviour was observed daily, and body weight, food intake and water intake were measure weekly. Plasma and erythrocyte ChE was measured at week 2, 4, 6, 8, 12, 16, 20, 24, 42, 68 and 92. Brain ChE was measured at the end of the study. ChE levels were determined by an electrometric method. No pathological or histopathological examination of the animals was performed.

There were no signs of toxicity observed during the study. Mortalities occurred in all groups, and were not related to dose. There were no significant changes in food consumption or body weight related to treatment during the study. ChE inhibition is present below.

ChE inhibition (Mean Percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
2.5	1	2	1	1	1	0
5	14	16	5	4	4	1
10	18	28	10	13	6	4

From the Table it is apparent that the only ChE inhibition having biological significance was that observed in the plasma of female rats at 10 ppm.

Overall, the NOEL for the study was 5 ppm, equal to 0.3 mg/kg bw/day, based on the inhibition of plasma ChE at 10 ppm seen in females.

Kudzins W (1974) Two-year dietary administration in the rat. Lab: Hazleton Laboratories Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Report No. HT-41-0006 (Pre-GLP)

and

104-Week chronic administration in rats, individual animal data Vol I & II. Lab: Hazleton Laboratories America Inc, Sponsor Sumitomo Chemical Co Ltd, Osaka, Japan. Report No. HT-01-0193. Report date: December 4, 1980 (Pre-GLP)

and

104-Week chronic administration in rats, Project No 343-107 Individual Histopathology Findings. Lab: Hazleton Laboratories America Inc, Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Report No. HT-11-0194. Report date: March 6, 1981 (Pre-GLP)

Fenitrothion (purity 97%, batch no not given; obtained from Sumitomo Chem Co) was administered to Sprague-Dawley rats at doses of 0, 10, 30 or 100 ppm (equal to 0, 0.5 1.5 or 5 mg/kg bw/day for males and 0, 0.6, 1.9 or 6.5 mg/kg bw/day for females) in the diet. Rats were selected from the F1a litters produced in a 3-generation study (see section 7.1), with 60 rats/sex for control and 50 rats/sex/treatment group selected at random from the weanlings available as F1a animals. Although rats were randomly distributed among treatment groups, the mean body weight of the high-dose group was approximately 20% lower than controls. Animals were individually housed, and food and water were available *ad libitum*.

Body weights and food consumption were recorded weekly until week 26, every second week from weeks 27 to 52, and every 4 weeks from week 53 to termination. Animals were observed daily for clinical signs. Appearance and behaviour was recorded at the time of determining body weights. The presence of any tissue masses was also recorded.

Blood samples were taken from 5 rats/sex/group at weeks 13, 52 and 104. At week 26, blood samples were taken from 5 rats in the control and 100 ppm groups. Hct, Hb, erythrocyte counts,

WBC and differential leucocyte counts were determined for all animals. Prothrombin time was determined for rats in groups 1 and 4. Fasting blood glucose, BUN, total serum protein, total serum bilirubin, AP, serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase and serum protein electrophoresis were determined on all blood samples. Additionally, at weeks 52 and 104 sodium, potassium, chloride, carbon dioxide, calcium and albumin levels were determined. Urinalysis including specific gravity, pH, glucose, ketones, protein and bilirubin levels and examination of the sediment were performed. Plasma and erythrocyte ChE were determined on 10 rats/sex/group at weeks 0, 2, 4, 8, 13, 26, 52, 78 and 104. At weeks 52 and 104, brain ChE was determined on 10 rats/sex/group. Ophthalmoscopic examination of all rats was done at weeks 81 and 104.

Gross necropsies were done on all animals that died during the study. At week 52, 10 rats/sex/group were euthanised for an interim autopsy. At week 104 all surviving rats were euthanised. The weights of the heart, liver spleen, kidneys and testes with epididymis were recorded prior to fixation. Weights of thyroid and adrenals were recorded after fixation. The following tissues were preserved in 10% formalin for histopathological examination: brain, pituitary, thoracic spinal cord, eye, thyroid, oesophagus, lung, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, small intestine, large intestine, mesenteric lymph node, urinary bladder, testes with epididymis and seminal vesicles, or ovaries and uterus, skin, costochondral junction, sternal bone marrow, nerve with muscle and any unusual lesions.

There were no treatment related clinical signs observed throughout the study, with incidences of hunching, sores on the body, red nasal discharge and laboured breathing being found in all groups. There was no treatment related change in mortality. The body weight of the high-dose animals remained between 10 to 20% lower than control animals throughout the study, which is considered to be of biological significance. There was no significant difference in the food consumption between groups. Therefore, based on body weight changes seen at 100 ppm the NOEL is 30 ppm (equal to 1.5 mg/kg bw/day),.

Haematological and clinical chemistry examinations did not find any significant differences between groups, and there were no significant findings on urinalysis. The percentage ChE inhibition is presented below.

ChE inhibition (Mean Percentage)

Dose (ppm)	Plasma ChE males	Plasma ChE females	Erythrocyte ChE males	Erythrocyte ChE females	Brain ChE males	Brain ChE females
10	18	35	9	10	7.5	3.5
30	37	66	28	39	28	18
100	54	82	54	75	38	55

Therefore, it can be seen that there is plasma ChE inhibition of biological significance (>20%) in females at 10 ppm and in males at 30 ppm. Erythrocyte ChE inhibition occurs at 30 ppm in both sexes, while brain ChE inhibition occurs at 30 ppm in males and 100 ppm in females, a reversal of the sensitivity seen in plasma ChE. The NOEL for erythrocyte and brain ChE inhibition is therefore 10 ppm (equal to 0.5 mg/kg bw/day), while no NOEL can be set for plasma ChE inhibition, given the effects seen in females at the lowest dose.

There were no significant treatment related macro- or microscopic findings at post mortem. A number of neoplasms, including interstitial cell tumours (Leydig cell tumours), gliomas phaechromocytomas, and pituitary adenomas were seen, however the occurrences were isolated and were not related to treatment. In males, 2 control animals showed signs of peripheral neuropathy. This was not seen in any treated animals, although no detailed neuropathological examination was done. The NOEL for carcinogenesis and other chronic organ changes is therefore set at 100 ppm (equal to 5 mg/kg bw/day)

The NOEL for brain and erythrocyte ChE inhibition is 0.5 mg/kg bw/day. An overall NOEL for the study cannot be established, based on the plasma ChE inhibition seen in females at the lowest dose tested.

6.3 Dog

6.3.1 Dietary Administration for One Year

Griggs LMP, Jefferson ND, Blair M, Kopplin JR, Richter WR & Spicer EJF (1984) One year dietary toxicity study in dogs. Study No. HT-41-0272. Sponsor: Sumitomo Chemcal Co Ltd, Osaka, Japan. Study Date: April 13, 1984 (GLP - US FDA)

Fenitrothion (Batch Number 00735, purity 96.8%, source Sumitomo Chem Co) was administered to purebred Beagle dogs (6/group/group; obtained from Ridglan Farms Wisconsin) at 0, 5, 10 or 50 ppm in the diet (equal to 0, 0.2, 0.3 or 1.6 mg/kg bw/day) for 12 months. Dogs were housed individually in metabolism cages under standard laboratory conditions. Water and food were available *ad libitum.* Dogs were monitored for mortality and changes in appearance or behaviour twice daily. Detailed examinations were done once weekly, and all animals were examined by a veterinarian pretest and at 3, 6 and 12 months.

Body weight and food consumption were recorded weekly. An ophthalmological examination was done on all dogs pretest and at 6 and 12 months. Haematology and clinical chemistry examinations were performed pretest and at weeks 4, 8, 12, 17, 26, 39 and 52. Haematological parameters included Hct, Hb, erythrocyte count, WBC, differential leucocyte count, platelet count, reticulocyte count, mean corpuscular volume and mean corpuscular haemoglobin concentration. Clinical chemistry parameters included lactic dehydrogenase, AST, ALT, AP, glucose, BUN, total bilirubin, cholesterol, albumin, globulin, albumin/globulin ratio, total protein, creatinine, sodium, potassium, chloride, calcium and phosphorus. Urinalysis was done pretest and at weeks 4, 13, 26, 39 and 52, and included volume colour and appearance, pH, specific gravity, protein, glucose, ketones, urobilinogen, nitrites, bilirubin, occult blood and a microscopic examination.

Plasma and erythrocyte ChE were measured pretest and at weeks 4, 8, 13, 17, 21, 26, 39 and 52. Brain ChE was measured at study termination. All animals underwent a gross necropsy at the study termination. The weights of the liver, kidneys, heart, testis with epididymis, brain, ovary, pituitary, thyroid/parathyroid and adrenal glands were determined. A standard list of tissues were preserved, however a histopathological examination was not done.

No mortalities occurred during the study. A number of animals from both treated and control groups showed signs of diarrhoea and emesis, particularly during the first weeks of the study. This was not compound related. No treatment related clinical signs were seen. On physical examination, some animals, mainly in the mid- and high-dose groups had a relatively rapid heart rate, however this was considered of little biological significance. There were no significant dose related changes in body weight or food consumption during the study. Haematology, clinical chemistry and urinalysis examinations did not reveal any significant changes, although there was a mild elevation of cholesterol in high-dose males early in the study. No changes were observed on ophthalmic examination. The mean ChE inhibition is presented below.

ChE Inhibition (mean percentage)

Dose (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
5	17	19	12	0	0	0
10	19	25	6	2	0	0
50	46	49	26	2	5	2

Therefore, it can be seen that there was biologically significant plasma ChE inhibition in both males and females at 50 ppm. Additionally, there was biologically significant inhibition of plasma ChE in females at 10 ppm. There was significant erythrocyte ChE inhibition in males at 50 ppm, although no inhibition was seen in females. No significant inhibition of brain ChE was seen in this study. Therefore, the NOEL for brain ChE was was 50 ppm, the NOEL for erythrocyte ChE was 10 ppm, and the NOEL for plasma ChE was 5 ppm. The overall NOEL for ChE inhibition was 5 ppm (equal to 0.2 mg/kg bw/day), based on the effects seen in females at 10 ppm.

No abnormalities were detected on gross post mortem examination. Organ weights did not show any significant differences between control and treated animals. No histopathological examination of tissues was done.

Overall, the NOEL for this study was 5 ppm in the diet, equal to 0.2 mg/kg bw/day, based on the plasma ChE inhibition seen at 10 ppm in female dogs.

6.4 Monkey

6.4.1 Gavage Administration for Two Years

2-Year Chronic Oral Toxicity - Monkey Ref 621-07168 Sponsor: Sumitomo Chemical Co Ltd, Osaka, Japan. Lab: Industrial BIO-TEST Laboratories Inc. Study dates: 31/12/75 - 12/1/78 (Pre-GLP) (Validated)

Fenitrothion (purity 97.3%, no batch number or source reported) was suspended in corn oil, and administered by gavage to feral Cynomolgus monkeys (*Macaca fascicularis)* (Primate Imports Corporation, Port Washington NY). Monkeys (7/sex/group) were dosed at 0, 0.1, 0.5 or 2 mg/kg bw/day. Controls received 1 mL/kg bw/day corn oil. All monkeys were individually housed in galvanised steel primate cages, and were fed a standard monkey diet supplemented with multivitamins and fresh fruit. Water was available *ad libitum*.

Body weights were determined weekly. Food consumption was not determined, due to the eating habits of the animals. Animals were checked daily for mortality and morbidity. Examination for abnormal reactions occurred at least once weekly, and abnormal reactions were recorded. Electromyograms (EMGs) and electoencephalograms (EEGs) were conducted on all surviving monkeys at 3, 6, 12, 18 and 24 months. EMGs were done on the triceps and quadriceps of each monkey. Four channel EEG recordings were evaluated for amplitude, synchrony, rhythm and frequencies.

Ophthalmoscopic examinations were done at 3, 6, 9, 12, 18 and 24 months. The examination included cornea, iris, lens, vitreous body, retina, optic nerve and conjunctiva. Intraocular pressure was also measure using a tonometer. Electroretinograms (ERGs) were conducted after 5, 6, 12, 19 and 23 months. These were obtained by placing an electrode (+) over the dilated anaesthetised eye of the sedated, dark adapted monkey, while another electrode (-) was inserted medially through the nictitating membrane. The eye was exposed to high intensity light for 1/1000 of a second, and the action potential within the retina was assessed.

Blood samples for haematology and clinical chemistry were collected by femoral puncture, and urine samples were collected by placing a metabolism pan under the cage. Samples were collected pretest and at 3,6, 12, 18 and 24 months of testing. A sample for ChE testing was collected at 1 month. Haematology parameters determined were WBC, differential leucocyte count, erythrocyte count, Hb, Hct, MCV, MCH, MCHC, platelet estimation, reticulocyte count, capillary coagulation time, prothrombin time and clot retraction time. The clinical chemistry parameters determined included blood glucose, BUN, AP, AST, ALT, bilirubin, alpha, beta and gamma globulin concentration, sodium potassium and chloride levels and serum albumin

concentration. Plasma and erythrocyte ChE levels were also determined. Urine was analysed for glucose concentration, albumin concentration, pH, specific gravity and ketones. Urine was also examined microscopically.

Complete gross necropsy was performed on all animals surviving to the end of the study, and on animals dying or euthanised during the study. A complete set of organs and tissues was preserved in formalin. The weights of liver, kidneys, spleen, heart, brain, gonads, adrenal glands, thyroid gland and pituitary gland were recorded. Organ to body and organ to brain weight ratios were determined. Histopathological examination of all tissues was done. Samples of liver, medial rectus muscle from the eye and interosseous muscle from the metatarsal region were taken for examination by electron microscope, however the results were not reported.

There were mortalities in the control, low dose and high dose groups during the course of the study that were unrelated to treatment. No clinical signs could be directly related to treatment. Hair loss was seen in monkeys in all groups in the second half of the study which was attributed to Vitamin C deficiency. The quantity of fresh fruit in the diet was increased. A number of animals in the high dose group developed necrotic injuries in the lower limbs which were related to injuries incurred during restraint and handling procedures. While this was not directly related to treatment, these injuries were only present in the high-dose group, and may reflect handling difficulties with these animals.

Male monkeys showed no treatment related changes in body weight during the study. In females, all treatment groups showed depressions of body weights greater than 10% in comparison to controls. Body weights were consistently more than 10% lower than controls from week 20 in the high dose and from week 31 in the middle dose, however body weight changes in the middle dose group were close to 10%. In the low dose group, body weight was intermittently decreased. The mean body weight of the low dose group was decreased more than 10% in comparison to control animals for weeks 37 to 46 of the study, and for weeks 50 to 55. Given the intermittent nature of the changes, this was not considered of biological significance. Based on the significant body weight changes at 2.0 mg/kg/day in the female animals, the NOEL can be set at 0.5 mg/kg bw/day, the lowest dose tested.

No changes of biological significance were seen in the haematology, clinical chemistry or urinalysis tests. The results of the ChE inhibition tests are set out below.

ChE inhibition (mean percentage)

Dose (mg/kg bw/day)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	males	females	males	females	males	females
0.1	7	0	2	6	0	13
0.5	0	0	6	0	0	2
2.0	56	64	41	47	2	25

Therefore, it can be seen that there was inhibition of biological significance of plasma and erythrocyte ChE in both male and female animals at 2.0 mg/kg bw/day. Brain ChE was

significantly inhibited in females at this dose, however no inhibition was seen in males. The NOEL for ChE inhibition can be set at 0.5 mg/kg bw/day, based on the effects seen at 2.0 mg/kg bw/day.

On ophthalmic examination there were no findings of significance. One high dose monkey presented with a retinal lesion originally presumed to be a melanoma of the choroid or pigment epithelium of the retina. The slow progression of the lesion made this diagnosis unlikely, however there was no confirmation of diagnosis at histopathological examination. Electroretinogram examination did not reveal any abnormalities.

EMG examination showed slight abnormalities in the high dose animals. These consisted of a slight increase in the duration and amplitude of insertional activity in both the triceps and quadriceps. This showed a dose related tendency, but was only greater than 10% in the high-dose group. Therefore, the NOEL for this effect is 0.5 mg/kg bw/day, based on the effects seen at 2.0 mg/kg bw/day.

EEGs showed no major abnormalities in any group throughout the study. There were minor abnormalities seen in a number of high dose animals in the examination in month 24, however these were unlikely to be related to treatment. Therefore, the NOEL for EEG effects was 2.0 mg/kg bw/day.

There were no abnormal findings on either gross or histopathological examination. There were some minor changes in the relative organ weights, although the significance of these was questionable. In females, there was a decrease in the absolute and relative weight of the kidneys, spleen and thyroid and an increase in the weight of the adrenals. For adrenal and thyroid the changes were dose related, however given the lack of histopathological signs they were not considered of biological significance. The NOEL based on necropsy examination was 2.0 mg/kg bw/day.

Overall, based on the changes seen in body weight, the ChE inhibition in plasma and erythrocytes, the ChE inhibition in the brain of female monkeys and on the EMG changes, the NOEL for the study was 0.5 mg/kg bw/day.

7. REPRODUCTIVE TOXICITY

7.1 Two-Generation Rat Dietary Study

Hoberman AM (1990) Reproductive effects of Sumithion administered orally in feed to Crl:CD(SD)BR rats for two generations. Lab: Argus Research Laboratories, Inc. USA, Report No. 1119-008, Report date: 24 October 1990, Sumitomo Chemical Co. Unpublished (GLP - US EPA, OECD & Jap MAFF).

Technical grade fenitrothion (lot 60553; purity 94.6%) was administered to 2 generations (2 litters for the first generation and 1 for the second) of weanling (or at 45 days for F0) Sprague-Dawley rats (Crl:CD(SD)BR, Charles River, Raleigh, NC, USA) in the diet at dose levels of 0,

10, 40 or 120 ppm (corrected for a.i. purity). The diet was prepared weekly by admixing fenitrothion suspended in corn oil with the normal dry food. Fenitrothion stability and homogeneity in the diet were checked and found to be suitable for at least 21 days after preparation. The concentration of fenitrothion in the diet, verified weekly for 4 weeks and monthly thereafter, indicated that actual levels were within ±10% of target doses except for sporadic deviations (10) of up 11.5% below and 61% above target doses.

Dose selection for the study was based on a prior one-generation dose-ranging study where clinical signs, reduced food consumption and body weight in rats of both sexes together with a reduced number of implantation sites in females and reduced body weight for pups at dietary concentrations in excess of 150 ppm were observed. Dosages for the main study were calculated from actual food consumption over the 202-day exposure and for F0 males ranged between 0.4 and 1.0 mg/kg bw/day at 10 ppm (mean 0.65 mg/kg bw/day, days 1-82), 1.6 and 4.3 mg/kg bw/day at 40 ppm (mean 2.67 mg/kg bw/day, days 1-82), and 4.8 and 12.8 mg/kg bw/day at 120 ppm (mean 7.99 mg/kg bw/day, days 1-82). For F0 females, dosages during the 82-day premating period ranged between 0.6 and 1.1 mg/kg bw/day at 10 ppm (mean 0.74 mg/kg bw/day), 2.5 and 4.3 mg/kg bw/day at 40 ppm (mean 3.07 mg/kg bw/day), and 7.8 and 13.5 mg/kg bw/day at 120 ppm (mean 9.63 mg/kg bw/day). Owing to the increased nutritional requirements during gestation and lactation there was an approximate 15% increase in dose during gestation and 60-80% during lactation (ie. mean dose of first and second lactation periods was 1.2, 4.95 and 13.6 mg/kg bw/day respectively).

Rats were randomly assigned to groups of 30/sex and fed fenitrothion for 82 days (or 88 days for the F1a generation) prior to a 21-day male/female (1:1) cohabitation period. Evidence of a copulatory plug after daily examination was deemed to be day 0 of gestation. All F0 (P1) generation females were allowed to deliver naturally and pups were culled to 4/sex on day 4 of lactation. Females that were not pregnant were submitted to a second cohobation period after a 2-week rest period. F1a generation pups were permitted to remain with dams till day 28 postpartum when they were randomly assigned to 30/sex/group for treatment at the same dose as their parents. F1b generation pups remained with the dams for 21 days postpartum after which dams and pups were sacrificed and submitted for necropsy. Weanling F1a (P2) rats were allowed to mate as before (sibling matings avoided). All F2 litters were sacrificed on lactation day 21. Five F2 pups/sex/group underwent necropsy.
A daily assessment of viability revealed 5 deaths among F0 females but none appeared to be treatment related because they were not dose related or coincide with the greatest daily intake of fenitrothion. Three at 10 ppm died; 2 during the first gestation and another during the second gestation (day 18). For the 2 at 120 ppm, 1 died during the second gestation while the other died during parturition (day 205). Clinical signs preceding death were chromodacryorrhea, alopecia and pale mucous membranes. Reduced food consumption with concomitant weight loss was observed in all dying 10 ppm rats but not at 120 ppm. Necropsy revealed a ruptured blood vessel of the urinary bladder in one 10 ppm rat and a red substance around nose and mouth and genitalia of another. No other gross lesions were observed in the other dead rats although 2 rats, 1 at 10 ppm and another at 120 ppm were pregnant with normal fetuses at the time of death. The pregnant 120 ppm dam died following delivery of 2/4 pups. One other death occurred in the study, namely at 120 ppm where an F1a dam died during parturition.

There were no clinical signs at any dose for males or females in the F0 generation and the F1a males. During gestation, loose stools/diarrhoea and chromorrhinorrhoea were observed in 120 ppm F1a females. Additionally, tremors were observed during lactation. Body weight and food consumption, assessed weekly throughout the study, revealed that all F0 rats at 120 ppm and F1a rats at 40 and 120 ppm had reduced mean body weight gains resulting in significantly ($p<0.05$-$p<0.01$) lower body weight relative to controls. Female F0 rats at 40 ppm also had significantly reduced body weight ($p<0.05$) during the first lactation period.
Absolute food consumption for F0 males was unaffected by treatment up to 120 ppm but for females consumption was significantly ($p<0.05$) decreased at 120 ppm in both the first and second lactation periods. In the F1a generation, males at 120 ppm and females at 40 and 120 ppm had significantly ($p<0.05$-$p<0.01$) reduced absolute food consumption.

Fertility (pregnancy rate) among females was reduced in all treated F0 groups during the first cohabitation. F0 males had significantly ($p<0.05$) reduced fertility at 10 and 40 ppm during the first cohabitation and a significant ($p<0.05$) reduction in mating performance at 120 ppm during the second cohabitation period. No significant effects on mating performance or fertility were observed at any dose for the F1 generation.

F1a and F1b pup body weights were either reduced or significantly ($p<0.01$) reduced and mortality was significantly increased ($p<0.05$-$p<0.01$) among F1a and F2 pups at 120 ppm. Viability and lactation indices were also significantly decreased at 120 ppm in the F0 and F1a generation litters. The were no changes to gestation duration, implantation averages or pup sex ratios at any dose. Similarly, there were no histopathological changes observed at any dose.

Fenitrothion at dietary concentrations up to 120 ppm did not cause any impairment in the reproductive performance of rats in this 2-generation study. The NOEL for parental toxicity was 10 ppm [between 0.65 (male) to 0.74 (female) mg/kg bw/day] based on a dose-related reduction in food consumption, body weight gain and body weight of both sexes at higher doses (40 or 120 ppm). The NOEL for reproductive toxicity was 40 ppm (3.07 mg/kg bw/day for females), on the basis of reduced pup weight, viability and lactation indices at the next higher dose of 120 ppm.

7.2 Three-Generation Rat Dietary Study

Olson WA (1972) Interim report. Three generation reproduction study - Rats: Sumithion and Neo-pynamin. Study No. 343-106. Lab: Hazleton Laboratories Inc., Vienna, Virginia, USA. Report date: November 14, 1972. Sumitomo Chemical Co Ltd, Osaka, Japan.

and

Rutter Jr HA (1973) Three generation reproduction study - Rats: Sumithion and Neo-pynamin. Study No. 343-106. Lab: Hazleton Laboratories Inc., Vienna, Virginia, USA. Report date: October 16, 1973. Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

and

Ralph JA & Pence DH (1980) Revised report - "Rutter Jr HA (1973) Three generation reproduction study - Rats: Sumithion and Neo-pynamin." Study No. 343-106. Lab: Hazleton Laboratories Inc., Vienna, Virginia, USA. Report date: December 11, 1980. Sumitomo Chemical Co Ltd, Osaka, Japan.

(Neo-pynamin data not presented in the following report)

Fenitrothion (batch or stability not stated; 97% purity) was administered to Sprague-Dawley rats (Charles River; 5-6 weeks old at treatment initiation) at concentrations of 0, 10, 30 or 150 ppm in the diet for nine weeks prior to and including mating, gestation, parturition and lactation for the F0 generation (& F1a) and at 0, 10, 30 or 100 ppm thereafter (including F1b) for the 3-generation study. For the first generation (and F1a mating), treatment groups of 15 males and 30 females were used (control; 20 males and 40 females) whereas all groups were reduced to 10 males and 20 females for the remainder of the study. Males and females co-habitated in the ratio of 1:2 and males were rotated weekly during the 3-week mating period. On post-partum day 2, litters were reduced to 8 by culling randomly selected pups.

F0, F1 and F2 generation parental rats were each mated twice and the first litter offspring (F2a, F3a) were killed at weaning while the second litter offspring (F1b, F2b, F3b) were used as the next generation parents. Weaned F1a pups were used in a subsequent chronic toxicity study (No. 343-107, see section 6.1). Necropsy and gross observations were performed on one-third of F2a, F2b and F3a pups and one-half of the F3b pups. Parents were killed after weaning F2b and F3b pups.

Although no regular analysis of the diet was performed, it was prepared at weekly intervals and actual food consumption was recorded at the commencement of the study, then at weeks 4 and 9 during the premating period. The respective group estimated daily intake of fenitrothion was between 0.5-1, 1.5-3.0 and 5-10 (7.5-15 for 150 ppm) mg/kg bw/day for rats with body weight either 0.1 or 0.4 kg (IPCS, Environmental Health Criteria No. 70, 1987).

Mortality, clinical signs of toxicity, body weight, and food consumption were assessed at 0, 4 and 9 weeks during the premating period. Maternal reproductive parameters determined were

pregnancy and lactation indices. Offspring were assessed for litter viability, live birth rate and pup survival. No histopathological examinations were performed but samples of adrenal, brain, heart, eye, intestines (small and large), kidneys, liver, lungs, pituitary, gonads, pancreas, spleen, stomach, bone, bone marrow and urinary bladder were stored for possible later use.

There were no deaths, adverse clinical signs or reductions in food consumption observed during the study. Mean body weight of the F0 generation parental rats exposed to 150 ppm was reduced at week 9 (by 7% for males and females; both not significant) and for females only at week 4 (8%). No significant body weight reduction was observed at any dose level in subsequent generations.

The fertility indices for all treated females were comparable with control groups (except for a significant reduction (p 0.05) for the F2a generation 10 ppm group; not biologically significant). However, although the mean pup weight at delivery for the 150 ppm treatment group tended to be lower than control, all other treatment groups appeared similar. Similarly, gestation index and live births ratio were comparable but the number and weight of pups surviving to weaning was reduced for the 100 ppm groups (all generations) and 150 ppm (F1a) groups. Thus, for the 150 ppm F1a generation pups, survival was significantly reduced by 51% (p 0.05) with males and females being 31% and 28% respectively lighter (both; p 0.05) than controls. For the 100 ppm F1b, F2a, F2b, F3a and F3b generation pups, survival was reduced significantly (all; p 0.05) with reductions of 28%, 22%, 11%, 25% and 24% respectively. Although the male and female pup weight losses ranged from 3-17%, only males of the F1b, and males and females of the F2a generation had significant (p 0.05) losses.

The NOEL for parental toxicity was 100 ppm (5 mg/kg bw/day) based on reduction in body weight at 150 ppm. The NOEL for reproductive effects was 30 ppm (1.5 mg/kg bw/day) based on poor pup survival and an inability to gain weight during lactation at higher doses.

8. DEVELOPMENTAL TOXICITY

8.1 Gavage Teratology Studies

8.1.1 Mouse

Kohda H & Kadota T (1975) Teratogenic studies with Sumithion. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co. Ltd. Report date: December 20, 1975. Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

Fenitrothion (neither batch nor stability specified; purity 97.2%) was administered once daily by gavage to mated female ICR-JCL mice (28/group; Nihon-Clea Co., Osaka, Japan) at dose levels of 0, 20, 70 or 200 mg/kg bw/day on days 7 to 12 post-coitum. Controls received an equivalent volume of corn oil. The day evidence of mating was found was considered to be day 0. Selection of dose levels was based on the results of a preliminary dose-ranging study (code not given) that showed the maximum tolerated dose was approximately 200 mg/kg bw/day. Mortality, body weight and clinical signs were monitored daily. Approximately 20 dams/group (ie 19, 20, 19 &

20 respectively) were killed on day 18 of gestation and macroscopic, visceral and skeletal examination of the fetuses performed. The remaining dams (6, 7, 6 & 7/group) were allowed to deliver naturally and nurse their pups. After 6 weeks, all pups were killed, examined for visceral abnormalities and selected tissues (brain, liver, kidneys, spleen and testis/ovary) macroscopically examined and weighed.

No adverse clinical signs were reported and maternal body weight gain was comparable among groups. Similarly, no differences in implantation index, fetal viability, gender ratio, mean body weight and skeletal malformations (and variations) were observed. However, there was an increased incidence (0, 3, 3 & 4 or 0%, 1.4%, 1.4% & 1.8% of the pups respectively) of external abnormalities for all 3 treatment groups, namely 1 with cleft palate and 2 with open eyelids at 20 mg/kg bw/day, 3 with open eyelids at 70 mg/kg bw/day, and 2 with cleft palate and 2 with open eyelids at 200 mg/kg bw/day. No external abnormalities were observed in the control group but it was claimed (though not shown) that historical control data had a comparable incidence of these abnormalities.

Of the naturally delivered pups, the number, weight, and viability was comparable among groups. Similarly, maternal body weight and gestation duration were also comparable. Development of the reared pups up to 6 weeks and organ weights at sacrifice were unremarkable.

The maternal NOEL may be taken as 200 mg/kg bw/day based on no adverse effects being observed at the highest dose tested. Although no historical data from the laboratory performing the experiments were submitted, published external abnormality incidences (Esaki & Tanioka, 1970) for this mouse strain are similar to that found in this study, suggesting that the NOEL for embryo/fetotoxicity can be set also be set at the highest dose tested, namely 200 mg/kg bw/day. No teratogenicity was evident.

8.1.1.1 EUP Study

Hiraoka Y, Imada A, Tanaka J & Okuda H (1989) The effects of fenitrothion emulsion (organic phosphorus pesticide) and its degraded solution on mice. Hiroshima J Med Sci 4: 209-212

This published report (same as described in section 3.3.1.2) compared a 50% fenitrothion EUP emulsion (designated MEP) with its degradation products (generated by exposure to sunlight for 8 h/day at 30 ^{0}C and pH 8, 10 or 14 until 90% degradation was achieved) for any teratogenic effects in ICR mice (5/dose/treatment except for 40 and 90 mg/kg/day at pH 8, 15/group; control group, 25). MEP or its degraded product (equivalent to 20, 40 or 90 mg/kg bw/day and calculated relative to undegraded fenitrothion concentration; pH adjusted to 5) was administered SC to female mice on days 3-15 after detection of a vaginal plug. Dams were killed on day 18 of pregnancy and the number of fetuses and implantations counted. Fetuses were then weighed, examined externally and processed for cartilage and bone histopathology.

No clinical signs were reported but all dams except 1/15 of the groups given the pH 8 degraded product survived to the terminal kill. Fetal body weight was comparable among groups except for a significantly lower weight in the pH 8 degraded product group (but this result may be

influenced by the small number of live fetuses arising from 3/4 live dams). Similarly, no differences in pregnancy rate, resorption rate, live or dead fetuses per litter, and skeletal malformations were observed.

This report is of little value for regulatory purposes because of the small number of mice tested in some treatment groups.

8.1.2 Rat

Kohda H & Kadota T (1975) Teratogenic studies with Sumithion. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co. Ltd. Japan. Report date: December 20, 1975. Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

This report is from the same study described in section 8.1.1. Fenitrothion (neither batch nor stability was specified; purity 97.2%) was administered once daily by gavage to mated female Sprague-Dawley rats (25/group; Nihon-Clea Co., Osaka, Japan) at dose levels of 0, 2, 7 or 20 mg/kg bw/day on days 9 to 14 post-coitum. Controls received an equivalent volume of corn oil. The day evidence of mating was found was considered to be day 0. Selection of dose levels was based on the results of a preliminary dose-ranging study (code not given) that showed the maximum tolerated dose was about 20 mg/kg bw/day. Mortality, body weight and clinical signs were monitored daily. Eighteen dams/group were killed on day 20 of gestation. All fetuses recovered after hysterotomy underwent macroscopic examination and then histopathological processing . Of the recovered pups, half were examined for visceral abnormalities and the remainder for skeletal abnormalities. The remaining dams (4-8/group) were allowed to deliver naturally and nurse their pups. After 6 weeks, all pups were killed, examined for visceral abnormalities and selected tissues (brain, liver, kidneys, spleen and testis/ovary) macroscopically examined and weighed.

There were no deaths or premature abortions but maternal toxicity was evident during treatment from day 12 onwards, with ataxia, piloerection and urinary incontinence at 20 mg/kg bw/day. Tremors and hypersensitivity were also observed for 3-4 days after the last dose. Dams at 20 mg/kg bw/day also lost body weight during treatment, so that on day 14 they were approximately 8% lighter (estimated from graph supplied) than controls.

There were no differences in implantation number (though not expected because treatment started after implantation), fetal viability and weight, and gender ratio. The incidence of 1 encephalocele in the 7 mg/kg bw/day group and 1 cleft palate at 20 mg/kg bw/day was in agreement with historical control data expectations. There were no significant visceral or skeletal variations among treatment groups but an apparent dose-related increase in 27th presacral vertebrae (1.4%, 3.2% & 5.3%; control - none) was within the range of historical control data.

In all treatment groups, pups were delivered at the expected time and had comparable body weight and survival during lactation. Similarly, normal development (including preyer reflex and righting reflex testing) of pups was observed in all groups. At the terminal kill, no visceral abnormalities or organ weight differences were observed.

A maternotoxicity NOEL can be set at 7 mg/kg bw/day, based on body weight loss at the highest dose tested. A NOEL for embryo/fetotoxicity could be set at 20 mg/kg bw/day, the highest dose tested. There was no evidence of teratogenicity.

Morseth SL (1987) Teratology study in rats with Sumithion. Lab: Hazleton Laboratories America, Inc. Project No: 343-194, Report date: February 26, 1987, Sumitomo Chemical Co Ltd, Osaka, Japan. (GLP - US EPA & FDA, Jap. MAFF)

Pregnant Charles River (Kingston, NY) Crl:CD(SD) BR rats (24/group) were gavaged with technical-grade fenitrothion (Sumitomo Chem Co, Osaka, Japan; batch 41208, purity 96.6%; dose volumes adjusted with corn oil) at 0, 3, 8 or 25 mg/kg bw/day from days 6-15 of pregnancy, with necropsy on day 20. A preliminary study (project No. 343-193) confirmed chemical stability of the fenitrothion for the duration of the study, but no justification for the doses selected in the teratology study was provided.

All dams survived treatment but clinical signs of toxicity (tremors (from day 6 onwards), rough coat, sores, rhinorrhoea, chromodacryorrhoea and urine stains) were evident at 25 mg/kg bw/day during the latter half of the treatment period. Other observations such as alopecia and soft faeces appeared treatment unrelated as there was a similar incidence among groups. Although food consumption was comparable among groups, dams at 25 mg/kg bw/day had reduced body weight gain for each day of treatment and 3 days post-treatment, resulting in a significant absolute mean body weight reduction from days 11 to 19 (eg 5% on day 19; p 0.05) .

There were no observed adverse effects on reproductive parameters viz. pregnancy rate, numbers of corpora lutea, implantation sites, resorptions, percentage of pre-implantation loss, live and viable fetuses. Fetus size and weight were similarly unaffected. Gravid uterine weights, although significantly greater at 3 (12%) and 8 (13%) mg/kg bw/day relative to control, were comparable at 25 mg/kg bw/day.

Necropsy findings for dams revealed sporadic and probably treatment-unrelated abnormalities such as dilated renal pelvis (1 control, 1 low-dose and 1 high-dose dam), diaphragmatic hernia (1 low-dose dam) and enlarged placentas (1 mid-dose dam). There were no gross fetal variations (except for 1 high-dose fetus with agnathia) and fetal visceral variations were mostly associated with kidneys and ureters, though not with any apparent dose-relationship or treatment-related distribution. However, at 25 mg/kg bw/day there was a significant increase in the incidence of litters (20%; p 0.05) and fetuses (3%; p 0.05) with 1 full and 1 rudimentary 13th rib; an apparent increased non-significant but possibly treatment-related litter and fetal incidence at 3 (14%, 2.5%) and 8 (10%, 2%) mg/kg bw/day (control, 0%). Similarly, an increased litter incidence of 13th rudimentary rib at high dose (2.3%; control 0%) and 7th cervical rib at all doses (14%, 10%, 20%; control 4.2%) was not significant. The unexpected absence of any incidence of 1 full and 1 rudimentary 13th rib in the control group most likely contributed to the statistical significance observed at the highest dose. The absence of attendant maternotoxicity at doses other than the highest tested suggests that the embryo/fetotoxicity observed at this dose is biologically significant.

A NOEL of 8 mg/kg bw/day for maternotoxicity is appropriate, based on body weight loss and adverse clinical signs at the highest dose tested. Similarly the skeletal variants observed at the highest dose tested indicates that the embryo/fetotoxicity NOEL can be set at the same dose, 8 mg/kg bw/day. No evidence of teratogenicity was observed.

8.1.3 Rabbit

Ladd R, Jenkins DH, Wright PL & Keplinger ML (1971) Teratogenic study with Sumithion technical in albino rabbits. Lab: Industrial BIO-TEST Laboratories Inc. Northbrook IL. USA. Report date: December 15, 1971. Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP) (Validated)

Fenitrothion technical (batch No. 601; purity 97.1%; compound stability not assessed) was administered at doses of 0.3 or 1.0 mg/kg bw/day to LH primed and artificially inseminated New Zealand White rabbits (supplier not specified; 17/group except control had 22) in gelatin capsules from gestation day 6 through to 18. The day of insemination was designated day 0 of gestation and a positive control group given 37.5 mg/kg bw/day thalidomide was included in the study. Fenitrothion doses were chosen because of observed toxicity (maternal weight loss and increased number of resorptions) at higher levels in preliminary experiments (not detailed). Does were observed daily for clinical signs and weighed on gestation days 0, 6, 9, 12, 15, 18 and at the terminal kill (day 29). Fetuses removed after hysterotomy were weighed, examined for viability, gross malformations, visceral, and skeletal changes.

There were no deaths in this study. The number of pregnant animals in each group was relatively low with 10/22, 7/17, 9/17 and 11/17 for the control, thalidomide, 0.3 and 1.0 mg/kg bw/day fenitrothion groups respectively. There was a tendency for reduced mean-weight gain with dose (5% at high dose) during treatment of pregnant does with fenitrothion. At the terminal kill, does at 1.0 mg/kg bw/day were 6% lighter than controls.

Fertility indices (corpora lutea, implantations and resorption number) were comparable among treatment groups except for an unusually large number of late resorptions (11; 100% of the litter) in 1 doe at 1.0 mg/kg bw/day fenitrothion. Similarly, fetal body weight, viability and number of gross abnormalities was comparable among groups except for an aborted litter of 4 at 1.0 mg/kg bw/day fenitrothion and 7/58 (12%) grossly abnormal fetuses (5 left and 1 right talipomanus, 1 umbilical hernia and 1 acrania) in the positive control group. Although sections revealing the incidence of an incompletely ossified sternum were similar among treatment groups other than at 1.0 mg/kg bw/day (32%; control 14.6%), the incidence of non-ossified sternum seemed to be significantly elevated in a dose-related trend, ie 2.7%, 39%, 12.7% and 26% for control, positive control, 0.3 and 1.0 mg/kg bw/day fenitrothion respectively. No historical control data was either supplied or referred to.

A NOEL for embryo/fetotoxic effects cannot be set because of a dose-related incidence of non-ossified sternum sections including the lowest dose tested (0.3 mg/kg bw/day). Results of this study indicated that fenitrothion was without teratogenic effects.

Morseth SL, Serabian MA, Lichtenberger JM, Vargas KJ, Thakur AK & Burlew PL (1986) Teratology study in rabbits with fenitrothion TG (Sumithion). Lab: Hazleton Laboratories America, Inc. Project No: 343-182, Report date: July 18, 1986. Sumitomo Chemical Co Ltd, Osaka, Japan. (GLP - US EPA)

Fenitrothion technical (batch no. 41208; purity, 96.6%) in corn oil was administered to artificially inseminated female New Zealand White rabbits (16/group; 5 months of age; Hazleton Research Labs, Denver, PA, USA) by gastric intubation on gestation days 7-19 at doses of 0, 3, 10 or 30 mg/kg bw/day. Doses were selected on the outcome of 2 pilot studies; in a 5-day PO toxicity study (project No. 343-180) using 1, 3, 10, 30 or 100 mg/kg bw/day, all rabbits at 100 mg/kg bw/day died after 3 days and those at 30 mg/kg bw/day showed signs of neurotoxicity (tremors & ataxia); a teratology study (project No: 343-181) using 5, 10 or 20 mg/kg bw/day reported no adverse findings. Although fresh solutions were prepared weekly the compound stability determined after 1 and 3 weeks storage was deemed to be adequate (range 94-102% over the 3 doses). Mortality, body weight and clinical signs were monitored with uteri weights and macroscopic, visceral and skeletal examination of fetuses being determined at day 29.

Although there were 10 deaths recorded, only 6 appeared related to compound exposure. One control rabbit and 1 rabbit at 10 mg/kg bw/day were anorexic and died on gestation day 8 and 18 respectively. Two other rabbits at 3 mg/kg bw/day died on days 11 and 12 respectively because of an incorrect gavage technique. All treatment-related deaths were observed at 30 mg/kg bw/day on gestation days 10-18. Maternal toxicity was evident for the 30 mg/kg bw/day group, with negative body weight gain during treatment that was significant (p 0.05) during gestation days 7-13. Additionally, 3 does delivered prematurely during post-treatment (days 22-29) and were sacrificed. Clinical signs noted were anorexia in all groups, together with reduced motor activity, ataxia, salivation, dyspnoea, and tremors in does during treatment with 30 mg/kg bw/day. Sporadic post-treatment signs were ataxia and slight tremors. Food consumption tended to be lower, though not significantly so, during treatment at 30 mg/kg bw/day, but significantly increased (by 1.6-fold) post-treatment. A significant reduction in food consumption (20%) was also recorded for the 3 mg/kg bw/day group (but not at 10 mg/kg bw/day) during gestation days 9-13. At their scheduled deaths no treatment-related gross pathology was observed in does.

Pregnancy rates and mean number of corpora lutea were comparable among groups and uterine examination of does surviving to the scheduled sacrifice showed no effects with respect to total resorption incidence, fetal weight and gender. There appeared to be no compound-related increase in the incidence of macroscopic, visceral or skeletal malformations among treatment groups.

There was no evidence of teratogenicity or embryo/fetotoxicity but maternotoxicity, characterised by increased mortality, adverse clinical signs and premature delivery, was evident at the highest dose tested, namely 30 mg/kg bw/day. Based on these findings, the NOEL for maternotoxicity was 10 mg/kg bw/day and for embryo/fetoxicity, 30 mg/kg bw/day.

9. GENOTOXICITY

A summary of submitted and published findings of genotoxicity studies with fenitrothion is shown in the Table below.

Summary of Mutagenicity Testing with Fenitrothion

Assay	Bacterial strain or Cell type	Dose levels	Metabolic activation	Results	Reference
Gene Mutation					
S. typhimurium	TA1535 TA1537 TA1538	10-10 000 µg/plate	+, - +, - +, -	-, - -, - -, -	Suzuki & Miyamoto (1975)
	TA98 TA100 TA1537	500-2500 µg/plate	+, - +, - +, -	-, - both weak + -, -	Kawachi (1978)*
	TA98 TA100 TA1535 TA1537 TA1538 *E.coli* WP2 hcr	<5000 µg/plate	+, - +, - +, - +, - +, - +, -	-, - both weak + -, - -, - -, - -, -	Moriya et al (1983)*
	TA98 TA100 TA100NR TA100 1,8-DNP_6 TA1535 TA1537 *E.coli* WP2 uvrA	100-5000 µg/plate	+, - +, - +, - +, - +, - +, - +, -	-, - both weak + -, - + (weak), - -, - -, - -, -	Hara et al (1989)*
	E. coli K12	13.2-300 µg/mL		-	Suzuki et al (1974)
Host-mediated	Mouse (ICR) & *S. typhimurium*	100-200 mg/kg bw		-	Suzuki & Miyamoto (1975)
Mammalian cell	Chinese Hamster V79 lung cells	0.01-0.3 mM	+, -	-, -	Hara et al (1989)*
DNA Damage and Repair					
Rec-	*B. subtilis* H17/M45	up to 20 µL	+, -	-, -	Shirasu et al (1979)*
Sister Chromatid Exchange	Human embryonic cells (HE2144)	0.5 mM		2.6 times higher SCE than control	Kawachi (1978)*
	Chinese hamster cells (Don-6)	1 mM			
	ICR mouse embryo cells	0.01- 0.1 mM	+, -	-, -	Suzuki & Miyamoto (1980)*
Unscheduled DNA synthesis *in vivo* & *in vitro*	SD rat hepatocyte	300 mg/kg bw; PO		-	Kawamoto et al (1989)*

* Studies not reviewed but cited in IPCS, EHC No. 133, 1992;
Results (+, positive; -, negative) are expressed relative to the presence (+) or absence (-) of metabolic activation

Summary of Mutagenicity Testing with Fenitrothion

Species or Cell type	Dose levels	Metabolic activation	Results	Reference
Chromosomal Aberration				
Chinese hamster lung cells	0.1 mg/mL		21% aberration	Kawachi (1978)*
Chinese hamster ovary cells-K1	3-30 µg/mL 75-300 µg/mL	- +	- -	Hara et al (1988)
Mouse (ICR) (male)	200-800 mg/kg bw; IP		-	Hara & Suzuki (1982a)
Mouse (Q) (male)	1000 mg/kg bw; IP		-	Degraeve et al (1984)
Rat (Wistar) F2b & F3b males	1-8 mg/kg bw in the diet		F2b, - F3b, -	Beneš et al (1975)
Rat (Long-Evans)	400-1000 mg/kg bw; PO 20-40 mg/kg bw; PO x 5 days		+ (weak) + (weak)	Kawachi (1978)*
Rat (SD) (male)	100-400 mg/kg bw; PO 20-80 mg/kg bw; PO x 5 days		- -	Hara & Suzuki (1982b)*
Rat (Wistar) (male)	15-60 mg/kg bw; IP x 5 days		-	Malhi & Grover (1987)*
Micronucleus Test				
Mouse (ICR)	200-800 mg/kg bw; IP		-	Hara & Suzuki (1982c)*
Mouse (ddy) (male)	200-800 mg/kg bw; IP 100-600 mg/kg bw; IP x 4 days		- -	Hayashi et al (1988)*
Mouse (CD1) (male)	22-85 mg/kg bw; Dermal		-	Schop et al (1990)
Rat (Wistar) (male)	75-330 mg/kg bw; IP		-	Grover & Mahli (1985)*
Dominant Lethal Test				
Mouse (Q) (male)	1000 mg/kg bw; IP		-	Degraeve et al (1984)
Mouse (ICR)	20-200 mg/kg bw; PO x 5 days		-	Kodha & Kadota (1975) [see sections 8.1 & 8.2.1]
Rat (SD)	2-20 mg/kg bw; PO x 5 days		-	
Rat (Wistar)	1-8 mg/kg bw in the diet		-	Beneš et al (1975)
Others				
D. melanogaster	50 & 150 ppm diet		-	Velázquez (1987)
S. cerevisiae	0.3% in DMSO/plate		-	Yadav et al (1982)

* Studies not reviewed but cited in IPCS, EHC No. 133, 1992

9.1 Gene Mutation Assays

Lab: Research Department, Pesticides Division, Sumitomo Chemical Co. Osaka, Japan.
Project No: HT-50-0142, Report date: October 17, 1975. Sumitomo Chemical Co Ltd, Osaka,

Fenitrothion (Lot no. 31184; purity 98.5%; dissolved in DMSO) was tested for the number of histidine (in strains -1535, TA-1537 and TA (in *E. coli* -3102), either on minimal growth plates at concentrations of 10-10 in broth at concentrations of 10-1000 µg/mL and compared with a positive control (N-methyl-N'-nitrosoguanidine) Fenitrothion was negative for mutagenic activity whilst the positive control gave the expected response. Fenitrothion toxicity for tester strains was reported as being negative

In the presence of either liver, lung, kidney, testis or brain homogenates from phenobarbitone (1%) induced male Sprague-Dawley rats (Nihon-Dobutsu Co., Osaka, Japan; 150 g bw) or a liver g bw), no increase in the number of revertants of (strains -1535 or TA-1538) over the range of 10-1000

with 2-aminoanthracene (20 mg/kg bw) IP 24 h before sacrifice produced the expected increase in mutant colony number.

in vivo, fenitrothion (100 or mg/kg bw) was administered PO or IM to male ICR mice (Nihon-Clea Co., Osaka, Japan; g bw). After 1 h, an inoculum of 5 x ^{8} *S. typhimurium* G46) was injected IP and

replicated trials, fenitrothion did not increase the mean mutation frequency but dimethylnitrosamine (50 or 100 mg/kg bw; positive control) gave the expected increase.

investigated using 5 DNA repair-deficient strains, namely *E. coli* *B. subtilis* *S. typhimurium* (uvr B-) and assessed relative to their respective wild-type

agar plates after 24 h, indicating that fenitrothion over the range of 10-10 000 of any DNA damaging activity in this assay. The positive control (N-methyl-N'-nitrosoguanidine)

Suzuki H, Miyamoto J & Oka A (1974) Mutagenicity and radiomimeticity screening of Sumithion. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co Ltd. Osaka, Japan. Project No: (not given), Report date: 1974. Sumitomo Chemical Co Ltd, Osaka, Japan. (Pre-GLP)

and

Interim report (Authors unknown): The mutagenicity screening of Sumithion using microbial systems. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co Ltd. Osaka, Japan. Project No: (not given), Report date: (not given).

The potential for fenitrothion (Lot number and purity not given; diluted with DMSO) to induce revertants in trpA- mutated *E. coli* K12 (W-3623 strain) following a 15-h exposure to 13.2, 132 µg/mL or a saturated solution (estimated to be 660 µg/mL) in broth or buffer prior to plating on minimal growth or complete growth agar plates, was examined. There was no positive control described in the interim report but N-methyl-N'-nitroso-N-nitrosoguanidine described in the final report gave the expected increase in mutation frequency. Bacterial survival appeared little changed for either exposure method (relative to the blank) suggesting that fenitrothion is not a mutagen.

9.2 Chromosomal Aberration Tests

Hara M, Yamada H, Kogiso S & Yoshitake A (1988) In vitro chromosomal aberration test of Sumithion in Chinese hamster ovary cells (CHO-K1) in culture. Lab: Biochemistry and Toxicology, Sumitomo Chemical Co Ltd, Osaka, Japan. Project No: HT-80-0420, Report date: August 8, 1988. Sumitomo Chemical Co Ltd, Osaka, Japan. (company QA only)

Chinese hamster ovary cells (CHO-K1) were incubated with fenitrothion (Lot no. 60553; purity 96.7%) at concentrations of 3-30 µg/mL for 8, 16 or 24 h in the absence of metabolic activation, or at concentrations of 75-300 µg/mL for 2 h then in fresh medium for a further 14 or 22 h in the presence of hepatic S9 fraction from rats pretreated with PCB (Kanechlor-400; 500 mg/kg bw IP). The incidences of chromosomal aberrations and polyploidy were not affected at concentrations up to 30 µg/mL in the 8, 16 or 24 h incubation without S9 or up to 300 µg/mL in the 14 or 22 h incubation with S9. Marked cytotoxicity in excess of 30 µg/mL in the absence of S9, and 300 µg/mL in the presence of S9, prevented cytogenetic analysis above these concentrations. Positive controls were mitomycin in the absence of S9 and benzo(a)pyrene or cyclophosphamide in the presence of S9 for the 14 and 22-h post treatment incubation respectively. These controls induced chromosomal abnormalities in 5-57% of the cells. Thus, there was no evidence that fenitrothion induces structural or numerical chromosome abnormalities in CHO-K1 cells.

Degraeve N, Chollet MC & Moutschen J (1984) Genetic and cytogenetic effects of fenitrothion. Arch. Environ. Health 39: 24-26

Technical fenitrothion (Pestanal, Riedel-de Haen; >99% purity) was administered IP (0 or 1 g/kg bw) to mature Q-strain male mice (5/group; 4 months old) followed by mating with 4

untreated females that were replaced weekly for 7 consecutive weeks. A positive control group received 60 mg/kg bw of methyl methane sulfonate. Pregnant females underwent an autopsy on day 14 after vaginal plug detection. In another group of treated males, bone marrow and spermatogonia were harvested 12, 24 and 36 h after IP dosing and examined cytogenetically.

There were no reported clinical signs in males. Spermatogonia and bone marrow cells in metaphase revealed no significant increase in chromosomal damage relative to controls. Similarly, there were no effects on pregnancy rate, the number of implantations, live embryos or embryonic deaths, indicating no fenitrothion effects in the dominant lethal assay. The positive control gave the expected significant increase in chromosomal damage and fetal mortality.

Hara M & Suzuki H (1982a) In vivo chromosomal aberration tests of Sumithion on bone marrow cells of mice. Lab: Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Project No: HT-20-0235, Report date: July 1, 1982. Sumitomo Chemical Co Ltd, Osaka, Japan. (company GLP only)

Fenitrothion (Lot no. 00106; purity 96.8%) dissolved in corn oil was administered IP to male ICR mice (6/group; Shizuoka Agricultural Cooperative Assoc., Shizuoka, Japan) at doses of 200, 400 or 800 mg/kg bw. Mice were killed 6, 24 or 48 h after dosing. Selection of the highest dose was based on the absence of deaths occurring at 800 mg/kg bw in a preliminary acute dosing study. Mitomycin (4 mg/kg bw) was used as a positive control and bone marrow smears were prepared for cytogenetic analysis.

The frequency of chromosome abnormalities among 50 well-spread cells in metaphase was not significantly increased after any post-dosing time interval. Positive controls gave the expected result.

pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. Fund Appl Toxicol 15: 666-675

insecticides (dichlorvos, aminocarb, chlordane and DDT) and a herbicide (2,4-D) only details relating to fenitrothion are summarised below. Selection of fenitrothion (in DMSO) dose levels

results of a acute dermal toxicity study, which gave an LD50 value of 690 mg/kg (estimated from supplied graph). All 12 male CD1 mice (Charles River Labs, Quebec) from each group were killed for preparation of bone marrow smears 24 h after dosing. Two controls, namely

activation dependent carcinogen CY (cyclophosphamide; dissolved in saline), were included. Skin from application sites was examined histopathologically and blood samples were collected

were no deaths, no significant increases in the frequency of micronucleated polychromatic erythrocytes and no changes in the ratio of polychromatic to normochromatic cells. Serum ChE was significantly ($p<0.05$) inhibited by 40% and CY gave the expected increase in nuclear

Beneš V, Šrám RJ & Tuscany R (1975) Fenitrothion. I. Study of mutagenic activity in rats. J Hyg Epidemiol Microbiol Immunol 2: 163-172

Wistar SPF rats from Velaz-Srbsko (Hungary; 20/group) were fed technical fenitrothion (batch not stated; 96% purity with less than 0.5% 3-methyl-4-nitrophenol) in the diet at concentrations of 0, 10, 40 or 80 ppm (approx. 1, 4 or 8 mg/kg bw) during a 4-generation reproduction study in which each 100-day-old rat was mated and allowed to deliver 2 litters/generation. A dominant lethal test was performed in 2 ways; one involved a "tentative determination" in both sexes involving single matings after the second litter weaning (ie F0b, F1b, F2b & F3b) and the other at different stages of spermatogenesis in F2a and F4b males. Matings were conducted in the ratio of 1 male:2 females with treated rats being mated with untreated females. For a positive control, tris(1-aziridinyl)phosphine oxide was administered IP once at 2.0 or 2.5 mg/kg bw to F4b and F2a males respectively.

The frequency of dominant lethals (derived from number of corpora lutea, implantations, live embryos and embryonic deaths) after single matings over 4 generations was not significantly different in either males or females over the dose range studied. Similarly, no significant increase in dominant lethals was observed in F2a or F4b male rats irrespective of either dose or duration, or stage of spermatogenesis. The positive control induced the characteristic spectrum of changes at the post-meiotic stage (week 1-4). A cytogenetic analysis of bone marrow cells from 5 F2b males at 80 ppm (200 days) or 5 F3b at 80 ppm (500 days) of fenitrothion revealed no increase in chromosomal changes (100 cells/rat in metaphase) relative to control male rats.

9.3 Other Mutagenicity Tests

Velázquez A, Xamena N, Creus A & Marcos R (1987) Mutagenicity studies on fenitrothion in Drosophila. Mutagenesis 2: 333-336

Fenitrothion (Bayer Hispania Commercial S.A., Barcelona; purity 97%) at concentrations ranging from 50 to 150 ppm (in 2% DMSO) was tested for its potential to induce gene mutations and chromosomal aberrations in germ cells of male malathion-resistant *Drosophila melanogaster* flies. Specifically, the incidence of sex-linked recessive lethals, total and partial sex-chromosome losses, and non-disjunction were studied following 3 different exposure methods, namely adult feeding, abdominal injection and larval feeding.

Fenitrothion did not induce point mutations in the sex-linked recessive lethal assay, or the frequency of recessive lethals, indicating a lack of mutagenic and clastogenic activity respectively. Similarly, after larval exposure to fenitrothion during pre-meiotic stages the chromosome loss test did not reveal any evidence of sex-chromosome loss or non-disjunction.

Yadav AS, Vashishat RK & Kakar SN (1982) Testing of endosulfan and fenitrothion for genotoxicity in Sacchromyces cerevisiae. Mutat Res 105: 403-407

Two insecticides, endosulfan and fenitrothion, were tested for their ability to induce mitotic crossing-over, mitotic gene conversion and reverse mutation in *Sacchromyces cerevisiae.* Treatment of 5 x 10^8 cells in suspension with 0.3% fenitrothion (source or purity not stated) dissolved in DMSO (9.4%) did not induce any of these events.

10. NEUROTOXICITY

10.1 Acute Studies

10.1.1 Rat

Yoshikawa H, Yoshida M & Hara I (1990) Electroretinographic changes induced by organophosphorus pesticides in rats. J Toxicol Sci 15: 87-95

Fenitrothion (Wako Pure Chemical Industries Co, Osaka, Japan; batch and purity not stated) in olive oil was administered IP (0 or 13.8 mg/kg bw) to male Wistar rats. Electroretinographic changes in anaesthetised rats were recorded 5 h and 2 days after dosing and ChE activities in brain and retinochoroid were measured after 3 days.

There was no description of any clinical signs in this published report. ChE activity was significantly reduced by 29% in brain ($p<0.05$) and by 13% in the retinochoroid (not significant). Changes in the electroretinographic profile [ie amplitude and latency of the a- and b-waves; a-wave is the portion of the curve below the baseline whereas the b-wave amplitude is calculated from the trough of the a-wave to the peak of the curve above the baseline] recorded via cornea-

successive xenon flashes (computer generated mean profile after 10 flashes) revealed a significantly increased a- and b-wave amplitude after 2 days (19% & 21% respectively; both

without any change in latency period. (Other OPs tested, ie fenthion and chlorpyriphos had significantly decreased a- and b-wave amplitude with a concomitant latency time increase,

Beyrouty P, Benjamin W, Robinson K & Noveroske J (1992) An acute study of the potential effects of orally administered fenitrothion on behaviour and neuromorphology in rats. Project

Chemical Co Ltd. Report date: November 20, 1992 with amendments on November 22, 1993. (GLP - US EPA)

oil was administered by gavage to 49-52 day old Sprague-Dawley rats (Crl:CD (SD)BR; Charles River, Quebec, Canada). Rats were randomly assigned into groups of 12
treated at 0, 12.5, 50 or 200 mg/kg bw, while females were treated at 0, 50, 200 or 800 mg/kg bw. Owing to a dosing error, a female in each of the low -dose groups and a male in the high-dose group were replaced. Treated rats were observed twice daily for clinical signs and

A quantitative and qualitative functional observation battery (FOB) test was performed before treatment then on the first day of treatment (day 0) at the presumed maximal effect time (ie. 1- -1 h post dosing for males and females respectively), and on days 7 and 14. Qualitative observations were performed with rats in the home cage, during handling and in a test

behaviour (static). For handling observations, the ease of removal from the home cage, vocalisation, lacrimation, pupil size, salivation, urinary staining, diarrhoea, body and abdominal

assessed. Arena observations involved rearing, ataxic, hypotonic or impaired gait, overall gait incapacity, bizarre behaviour (movement) palpebral closure, tremors, twitches, convulsions,

urination, auricular startle, olfactory response, air righting reflex and positional passivity when placed on top of a box. The quantitative aspects entailed grip strength (fore- and hindlimb),

At the completion of the study on day 15, 6 rats/sex/group were prepared for neuropathology assessment by whole-body perfusion with buffered paraformaldehyde/glutaraldehyde solution.

midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and 3 levels of the spinal cord (cervical, thoracic and lumbar) were examined after conventional processing with paraffin

peripheral nerves was performed with samples of sciatic nerve (at mid-thigh region; cross-section, and sciatic notch; longitudinal and cross-section), sural nerve (at knee; cross-section) and

levels of the spinal cord (left Gasserian ganglion; cross-section, lumbar dorsal root ganglion (L4); cross-section, lumbar dorsal root (L4); cross-section, lumbar ventral root (L4); longitudinal and cross-section, cervical dorsal root ganglion (C5); cross-section, cervical dorsal root (C5); cross-section, cervical ventral root (C5); cross-section) were prepared for examination after epoxy resin embedding.

For the other 6 rats/sex/group, a complete necroscopy was performed and samples of brain tissue processed and preserved for subsequent immunochemistry. Any abnormal tissues observed at necroscopy were subjected to histopathological investigation. Immunochemistry of glial fibrillary acidic protein (GFAP), performed as dot blots on nitrocellulose membrane, used brain tissue homogenates derived from free-hand dissected cerebellum, cerebral cortex, hippocampus, striatum and thalamus/hypothalamus regions, and the rest of the brain.

One male at 200 mg/kg bw and 1 female at 800 mg/kg bw were found dead on days 2 and 1 respectively after treatment. Signs observed prior to the death of the male were decreased respiration, pallor, ocular discharge, and fur staining on muzzle and ventral surface. A reduced body temperature was the only sign noted for the female rat. Necropsy revealed multiple dark areas on the stomach for both rats and some discolouration of the digesta in the female. The male also had dilatation of the renal pelvis and multiple dark areas of the thymus. Surviving males at 200 mg/kg bw had reduced weight gain (20.3 g) from days 0 to 7 relative to the control group (45.7 g) so that the mean bodyweight at day 7 was significantly lower ($p<0.01$) and a statistical significance ($p<0.05$) was maintained for the remainder of the study. No significant differences in female body weights were observed.

Significant ($p<0.05$-0.001) qualitative differences in FOB tests between treatment and control groups occurred in both sexes only on the day of dosing. All male rats (12/12) at 200 mg/kg bw and most (12/13) at 50 mg/kg bw had tremors of the head, body and limbs. Gait changes were observed at 50 mg/kg bw as a result of 10/13 having a moderate or greater degree of reduced mobility. At the highest dose it was not possible to assess meaningful gait changes because of the almost complete immobility of most (11/12) rats. Similarly, activities such as arousal, rearing and grooming were reduced in these two treatment groups. Autonomic signs such as pupil constriction and salivation were increased at 50 and 200 mg/kg bw with most to all rats being affected. In 2/13 rats at 50 mg/kg bw and 4/12 at 200 mg/kg bw, the saliva had a red discolouration. Although not significant, 2/13 rats at 50 mg/kg bw had slightly reduced extensor thrust whereas at 200 mg/kg bw only 3/12 had a normal thrust. Other significant changes at 50 and 200 mg/kg bw were decreased pinna reflex, reduced or absent tail and toe pinch test response, impaired visual placing test response, delayed postural passivity and increased failure at the air righting reflex test. Any biological significance associated with the reduced urination and defaecation observed at 200 mg/kg bw in males is unclear.

For females, all (12/12) at 800 mg/kg bw and 4/12 at 50 mg/kg bw had tremors. Gait changes were observed in all treatment groups with an apparent dose relationship. At 800 mg/kg bw, 4/12 were immobile and 1/12 had severe, 6/12 had moderate and 1/12 had slight ataxic gait. At 200 mg/kg bw, 1/12 had severe, 8/12 had moderate and 2/12 had slight ataxic gait. At 50 mg/kg bw, 1/12 had moderate and 7/12 had slight ataxic gait. Significant reductions in arousal, rearing and locomotor activity were observed at all doses whereas tail and toe pinch response and visual

placing test were reduced only at 200 and 800 mg/kg bw. Other significant changes were pupil constriction, salivation, air righting reflex failure, and increased positional passivity at 200 and 800 mg/kg. Pinna reflex response at 800 mg/kg bw was also reduced or absent.

Significant reductions ($p<0.05$ or $p<0.01$) in grip strength (forelimb at 50 mg/kg bw and fore- and hindlimb at 200 mg/kg bw), motor activity and body temperature were observed in males at 50 and 200 mg/kg bw on day 0. Similarly, significant reductions in grip strength (forelimb at 200 mg/kg bw and fore- and hindlimb at 800 mg/kg bw) were recorded at 200 and 800 mg/kg bw and motor activity and body temperature were significantly reduced at all doses.

No gross pathological or neuropathology findings were observed in survivors at the conclusion of the study. Immuno-detection of GFAP revealed a significant ($p<0.05$) increase in the cerebral cortex of females at 800 mg/kg bw. However, much of this increase was attributable to 3 rats having 143%, 116% and 119% respectively of the GFAP observed in the control group. Hence, the biological significance of this finding in the absence of any histopathological changes is unclear.

Thus, no NOEL could be established for females because of reduced motor activity, body temperature and gait changes at 50, 200 and 800 mg/kg bw. For males, the NOEL can be set at 12.5 mg/kg bw based on marked changes in FOB testing at 50 and 200 mg/kg bw.

10.1.2 Chicken

Kadota T, Kagoshima M & Miyamoto J (1974) Acute oral toxicity and delayed neurotoxicity of Sumithion in hens. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: July 3, 1974. (Pre-GLP)

and

Kadota T, Kagoshima M & Miyamoto J (1976) (Revised report) Acute oral toxicity and delayed neurotoxicity of Sumithion in hens. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: October 16, 1976.

and

Kadota T, Kagoshima M & Miyamoto J (1975) Acute oral toxicity and delayed neurotoxicity of 5 organophosphorus compounds, Salithion, Cyanox, Surecide, Sumithion and Sumioxon in adult hens. Botyu-Kagaku 40: 49-53

In a study stated to be performed according to US EPA guidelines, fenitrothion (Sumitomo Chem Co, Osaka, Japan; Lot No. 417; purity 97.2%) in Sorpol 355 (an emulsifier) was administered PO (500 mg/kg bw) to 16 White Leghorn hens (Nihon Dobutsu Co., Osaka, Japan). Amelioration of anticipated clinical signs was achieved by co-administration of atropine (20 mg/kg bw; SC) and 2-PAM (100 mg/kg bw; IP); and repeated 6, 24, 48, and 72 h later. After 3-weeks observation for signs of leg paralysis, the treatment/observation regimen was repeated. Tri-orthocresyl phosphate (TOCP) in 10% Tween 80 was administered PO (500 mg/kg bw) to 3 hens in a positive control

group and signs of delayed neurotoxicity (eg leg paralysis) were monitored daily for 4 weeks. A group of 4 control hens was used as a reference for body weight change calculations. Hens were killed at the end of their respective observation periods and their distal sciatic nerves excised and processed for histopathology. In the revised report but not in the original report it is stated that a section of the spinal cord from the lumbro-sacral region containing the intumescentia lumbalis was also examined for possible lesions.

Five hens (5/16) died 24-48 h after treatment and survivors had leg weakness, ataxia, balance loss, spontaneous motor activity loss and an irregular respiration rate that persisted for 5-7 days. For the second treatment cycle, no deaths occurred but similar clinical signs, albeit with reduced severity were observed. Although not presented in the original report, the revised report indicates that mean body weight loss after 2 treatment cycles was 7%. In the positive control group there were no deaths but by day 10-12 (12-14 in revised report), leg weakness, loss of balance, and leg coordination were apparent and by day 15 (day 18 in revised report), all hens had leg paralysis that persisted for the duration of the observation period. Body weight loss in this group relative to control was 39% immediately after treatment.

Histopathological examination of 1000 transverse sections did not reveal any sciatic nerve degeneration or demyelination in treated hens but was apparent in all (3/3) positive control hens. Similarly, in 100 transverse spinal cord sections no swelling or degradation of nerve fibre, ganglionic cells or Nissl granules were observed. No spinal cord lesions were observed in the positive control group.

10.2 Subchronic Studies

10.2.1 Rat

Lehotzky K & Ungváry G (1976) Experimental data on neurotoxicity of fenitrothion. Acta Pharmacol Toxicol 39: 374-382

This published report indicates that male CFY rats (10/group and 20 controls) failed or took longer to respond to a conditioned avoidance reflex (ie jumping onto a stick in response to an electrical stimulation) after PO administration of fenitrothion (0, 10 or 100 mg/kg bw/day) for 30 days. The number of conditioned reflex responses after a 2 h exposure to 100 mg/kg bw was reduced by 46% relative to control and was maintained (ie between 30-40%) for 14 days (no results reported for days 15-30). For controls, the number of reflex responses declined gradually over 14 days to the same level as for treated rats. The reason for this decline is unclear. Another puzzling aspect was that the time for treated rats to respond to stimuli (latency) remained relatively unchanged (range 1.2-2 sec; from supplied graph) over the first 14 days of treatment (remainder not reported) whereas control rats gradually became considerably less responsive (range 1.2-6.6 sec). Characteristic OP clinical signs of salivation, tremor, ataxia and abnormal gait were seen almost "immediately" after dosing but was reported to "disappear during treatment". Whole blood ChE inhibition ranged from 60-80% over days 3-28 for the 10 mg/kg bw/day treatment group but was not reported for the 100 mg/kg bw group.

The apparent discrepancies (see section 10.2.3) and judicious reporting of data in this study suggest that it is not suitable for regulatory purposes.

Lehotzky K, Szeberenyi MJ & Kiss A (1989) Behavioural consequences of prenatal exposure to the organophosphate insecticide Sumithion. Neurotoxicol Teratol 11: 321-324

In a short communication, fenitrothion (50% EC; Sumitomo Chem Co, Japan) emulsified in sunflower oil was administered once daily by gavage to mated (1M:4F) Lati CFY rats (6/group) at dose levels of 0, 5, 10 or 15 mg/kg bw/day from day 7 to 15 of gestation. There was no comment on dose selection. At parturition, pups were weighed and the litter size reduced to 10 by random culling. External auditory canal and eyelid opening times were monitored and pups weaned at day 22 (a sperm positive vaginal smear was deemed day 1 of gestation). At weaning, males (10/group) were selected for behavioural assessment tests that included auditory startle, righting response, open field activity, motor coordination, rotor-rod test, conditioned stimuli, and social interaction on days 26, 36 and 104.

There were no comments on maternal mortality, maternal body weights, food consumption, clinical signs or gestation duration. There were no significant differences in pup numbers per litter or in pup weight at parturition but significant ($p<0.5$) pup mortality was observed at all doses (16% at the low and mid dose, and 17.5% at the high dose; control 5%) up to day 16. There was no comment on any necropsy findings on the dead pups. Two pups, 1 being anophthalmic and another with tremors and ataxia on day 16, were excluded from the high-dose test group scheduled for behaviour testing. Opening of the external auditory canals and eyelids occurred at the expected time.

Dose-related effects seen on behavioural development (reflexes, motor coordination in the rotor-rod test and open-field activity) achieved significance ($p<0.05$) for weanlings in the high-dose group although a significant delayed response to conditioned stimuli was also observed at the mid dose.

Thus, PO administration of fenitrothion to pregnant rats during organogenesis gave rise to pups that had dose-related reductions in open field activity and motor coordination after weaning. The no-effect dose level was 5 mg/kg bw/day.

Rondeau DB, Young L, Hebert D & Trotter BL (1981) Behavioural toxicity of chronic administration of fenitrothion in rats. Neurobehav Toxicol Teratol 3: 313-319

Technical fenitrothion (Sumitomo Chem Co, Japan; purity 96.7%) in peanut oil was administered to male Sprague-Dawley rats (6/group) by gavage at doses of 0, 10, 20 or 40 mg/kg bw/day for 40 consecutive days. A series of neurobehavioural tests was conducted 24 h after the first treatment, and then at intervals of 72 h during the 40 day treatment period. These tests involved an assessment of motor activity, catalepsy, rigidity, reflex responses, gait analysis and landing foot spread.

At the end of the 40-day observation period, mortality in the groups was 0/6, 1/6, 3/6 and 5/6 respectively. Deaths at the mid and high dose occurred within 13 days while at the low dose it

was delayed until day 32. No clinical signs were observed at the low dose but at the mid and high doses, salivation, piloerection, excessive urination, chromodacryorrhea, tremors and poor fur condition were observed. These signs persisted until day 10-12 and then gradually declined so that by the end of treatment surviving animals had no clinical signs.

In low-dose rats, body weight loss was significant on days 31 and 40 (both 50%; $p<0.05$) whereas at mid (30%; $p<0.05$) and high doses (45%; $p<0.01$), significance was achieved by day 6. The series of behavioural tests showed loss of many reflexes, changes in motor activity and gait at the mid and high doses. However, it is difficult to draw any conclusions these from these tests as many rats were emaciated and/or *in extremis* during testing.

10.2.2 Chicken

Kadota T, Kagoshima M & Miyamoto J (1974) Acute oral toxicity and delayed neurotoxicity of Sumithion in hens. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: July 3, 1974. (Pre-GLP)

and

Kadota T, Kagoshima M & Miyamoto J (1976) (Revised report) Acute oral toxicity and delayed neurotoxicity of Sumithion in hens. Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: October 16, 1976.

In a study stated to be performed according to US EPA guidelines, fenitrothion (Sumitomo Chem Co, Osaka, Japan; Lot No. 417; purity 97.2%) in 10% Tween 80 was administered PO (16.7 or 33.4 mg/kg bw/day, being 1/30 and 1/15 of the LD50 respectively) to White Leghorn hens (8/group; Nihon Dobutsu Co., Osaka, Japan) daily for 4 weeks. After 3-weeks observation for clinical signs and daily body weight measurements, hens were killed and their distal sciatic nerves removed and processed for histopathology. In the revised report but not in the original report it was stated that a section of the spinal cord from the lumbro-sacral region containing the intumescentia lumbalis was also examined for possible lesions. A control group of 4 hens was used for a body weight change comparison.

One 33.4 mg/kg bw/day hen died on day 5. Reduced spontaneous motor activity, gradually returning to normal 1 week after treatment, was the only clinical sign associated with the 16.7 mg/kg bw/day group. By contrast, ataxia, tremor, and depressed appetite were apparent in the 33.4 mg/kg bw/day hens although these signs returned to normal 2 weeks after treatment. At 16.7 and 33.4 mg/kg bw/day mean body weight declined during treatment so that at the conclusion of dosing, hens were 16% and 28% lighter than controls (estimated from supplied graph). At the conclusion of the observation period, low and high-dose groups had recovered some weight to be both only 11% lighter than control. [The revised report indicates the control hens had a mean body weight of 1.64 kg at week 4 whereas the original report graph shows a weight of approximately 1.85 kg. If the "revised" weight is correct then there would appear to be no significant body weight reduction at the lower dose (16.7 mg/kg bw/day group); ie 6% and 18% respectively at the conclusion of treatment.]

Of 1000 transverse sections examined, no evidence of sciatic nerve degeneration or demyelination was apparent at either dose. Similarly, of 100 transverse sections of spinal cord, no evidence of neuropathy was observed at either dose. Positive control hens had the expected sciatic nerve degeneration or demyelination but no spinal cord lesions.

The NOEL for body weight changes can be set at 16.7 mg/kg bw/day as significant loss was observed at 33.4 mg/kg bw/day. There was no evidence of neurotoxicity at any dose tested.

10.2.3 Rabbit

Lehotzky K & Ungváry G (1976) Experimental data on neurotoxicity of fenitrothion. Acta Pharmacol Toxicol 39: 374-382

Fenitrothion (Sumitomo Chem Co, Osaka, Japan) technical (batch not given; purity >95%) was administered PO at 10 or 25 mg/kg bw/day to groups of 6 rabbits (strain, gender or source not reported) for 8 and 4 weeks respectively. An untreated control group of 6 was also included. No rationale for the dose selection was given. Sciatic nerve conduction velocity was determined by electrical stimulation (supramaximal stimuli of 1/sec, 0.2 msec duration) through the intact skin of unanaesthetised rabbits and recording the difference between 2 action potential latencies at the gastrocnemius muscle using needle electrodes. At the conclusion of treatment, the sciatic nerve was removed from anaesthetised rabbits and processed for light and electron microscopy. Neither the number of rabbits used nor the number of nerve sections examined by either light or electron microscopy was reported.

No rabbits died during the study. At 10 mg/kg bw/day the sciatic nerve conduction velocity, measured at 2-week intervals, progressively declined from 26.0 m/sec before treatment to 18.2 m/sec at the 8th week (ie 25.5, 24.5, 21.7*, 18.2* m/sec for each fortnightly recording; * $p<0.01$). At the time when reduced nerve conduction velocity achieved statistical significance, rabbits apparently had no clinical signs, although whole blood ChE activity was reduced by 50%. Rabbits at 25 mg/kg bw/day had clinical signs of excitation, salivation, mild ataxia and gait loss but there was no mention of the time of onset or duration or the degree of whole blood ChE inhibition. It was stated that nerve conduction velocity became faster (though not quantified) and rabbits responded with extensive muscle contraction after only 1 electrical stimulus.

Results from nerve histopathology at 25 mg/kg bw/day were not reported but at 10 mg/kg bw/day, swollen and dislocated axoplasm was seen at low magnification and identified under electron microscopy as intramyelinic vacuoles containing thin lammelae of split myelin sheaths claimed to be evidence for fragmentation. (It is unclear as to exactly when the histopathology was performed since the "Methods" indicate that it occurred after cessation of treatment whereas a nerve bundle photomicrograph legend (Fig 4) suggests that it was performed after 10-days treatment.) There was no indication for the incidence of the observed lesions among examined slides.

The apparent discrepancies and judicious reporting of data (see also section 10.2.1) suggest that this study it is not suitable for regulatory purposes.

Miyamoto J, Hosokawa S, Kadota T, Kohda H, Arai M, Sugihara S & Hirao K (1976) Studies on cholinesterase inhibition and structural changes at neuromuscular junction in rabbits by subacute administration of Sumithion. J Pesticide Sci 1: 171-178

Technical fenitrothion (Sumitomo Chem Co; Lot No. 993; purity 97.2%) was mixed into the diet and fed to male Japanese albino rabbits (Nihon Dobutsu Co, Osaka, Japan; 15/group) at doses of 0, 3000 or 1000 ppm (calculated to be 0, 3 or 10 mg/kg bw/day) for 6 months. Rabbits were monitored daily for behaviour and weighed weekly in the first month and bimonthly thereafter to assess body weight changes. Blood was collected once at the end of treatment for blood chemistry and haematological assessment. Plasma and erythrocyte ChE activity was measured after 1, 2, 3, 4, 6, 10, 13, 18 and 24 weeks of treatment and for brain at 24 weeks using an electrometric method (pH) whereas for ChE activity in a musculi rectus medialis homogenate at 24 weeks, a colorimetric assay (Ellman's method) was used. Histochemical localisation of ChE activity was performed on the musculi rectus medialis using frozen sections. Sections, taken from brain, eye (with optic nerve and musculi oculi), spinal cord, sciatic nerve, bronchus, lung, spleen, bone marrow, mesenteric lymph node, thymus, oesophagus, stomach, intestine, liver, pancreas, kidney, urinary bladder, testis, prostate, pituitary, thyroid and adrenals after the terminal kill, were examined with light microscopy. Transmission electron microscopy was only performed on the musculi rectus medialis. Liver, kidney, spleen, lung, brain, heart, adrenal, testis, thyroid and pituitary were weighed and compared with organ weights from the control group.

There were no treatment-related deaths although 1 died accidently (group not reported) during restraint for blood collection. There were no clinical signs or significant body weight changes observed. Similarly, no noteworthy changes in clinical chemistry or haematology relative to control were recorded. Mean ChE inhibition after 24 weeks of treatment is detailed below:

ChE Activity - (Mean % reduction)

Dose (mg/kg bw)	Plasma	Erythrocyte	Brain	M. rectus medialis
3	27	24	9	9
10	41	49	32	8

Plasma and erythrocyte ChE activity were similarly reduced at the two doses tested whereas for brain there was appreciably less inhibition and for musculi rectus medialis there was almost no inhibition. As might be anticipated from the absence of substantial ChE inhibition in musculi rectus medialis homogenates, no difference between musculi rectus medialis sections taken from control and treated rabbits was detected using a histochemical assessment of ChE activity. No treatment-related lesions were observed for any tissue under histopathological examination. Similarly, electron microscopy of the musculi rectus medialis revealed no treatment-related changes.

A NOEL for this study cannot be set because of biologically significant plasma and erythrocyte ChE inhibition at the lowest dose tested, namely 3 mg/kg bw/day. There was no evidence of neurotoxicity at any dose tested.

11. HUMAN STUDIES

11.1 Acute Oral

Shelanski MV, Levenson T & Karras C (1977) (No title given though it involves fenitrothion effects in humans). Product Investigation, Inc. Conshohocken PA, USA. Report date: November 22, 1977.

A fenitrothion emulsifiable concentrate prepared *in-house* (technical fenitrothion, 22%; Atlox 3409F, 3%; Arotex 3470, 3%, and distilled water 72%) was administered to 2 groups of adult volunteers (6/group). Fenitrothion (0.1 mg/kg bw) in a capsule was administered PO as 3 equal doses over 6-10 h on day 1 to group 1 volunteers. One week later (though for an unspecified number of volunteers it was 6 days), all group 1 members had the fenitrothion emulsion (0.1 mg/kg bw) applied topically to their left arm, then 2 days later on their right arm (0.5 mg/kg bw) and finally 1 day later onto their right cheek (0.1 mg/kg bw). Prior to PO dosing (several days ?) and during the week (or 6 days) preceding topical dosing, volunteers were given capsules containing lactose. For the 2nd group of volunteers, fenitrothion (0.5 mg/kg bw) was administered in capsules as 3 equal doses over a 6-10 h period on day 1 then repeated 2 or 3 days later (the number of volunteers at each time was not indicated). Only clinical signs and blood ChE activity (assayed before and after each treatment; exact time not reported) were monitored.

There were no treatment-related clinical signs or application-site skin irritation. ChE activity was unchanged, relative to pretest levels, in both treatment groups.

Nosál M & Hladká A (1968) Determination of the exposure to fenitrothion (O, O-dimethyl-O/3-methyl-4-nitrophenyl/thiophosphate) on the basis of the excretion of p-nitro-m-cresol by the urine of the persons tested. Int Arch Gewerbepathol Gewerbehyg 25: 28-38

Fenitrothion was given to 24 volunteers as a single PO dose ranging between 0.042 and 0.33 mg/kg bw or 2.5-20 mg/person. The excretion of a urinary metabolite, 3-methyl-4-nitrophenol, was almost complete within 24 h, and ranged from about 70% of the dose (0.042 mg/kg bw) to about 50% (0.33 mg/kg bw). Neither mean plasma nor erythrocyte ChE activities were depressed much below normal (10%) after 6 or 24 h. When repeat doses of 0.04-0.08 mg/kg bw were given 4 times PO at 24-h intervals to 5 individuals, most of the 3-methyl-4-nitrophenol excreted appeared in the urine within 12 h of administration. Plasma or erythrocyte ChE activity (measured using a electrometric method, pH) did not appear to be affected by repeat-dose treatment.

11.2 Occupational Exposure

Vandekar M (1965) Observations on the toxicity of carbaryl, folithion and 3-isopropylphenyl N-methylcarbamate in a village-scale trial in southern Nigeria. Bull WHO 33: 107-115

In a field spraying operation in a village in southern Nigeria using a 5% spray of fenitrothion, 18 villagers examined one week later did not show any clinical symptoms of toxicity or plasma ChE depression. The ChE levels in the 3 spraymen examined on the first, second, and sixth days after spraying were also not depressed compared with pre-spraying levels.

Motabar M, Sanai GH & Heidari AA (1972) Toxicological evaluation of Sumithion on operators and inhabitants in the Mamasani area, southern Iran. Iran J Pharm Health 2: 40-49

During a 30-day program of indoor spraying of fenitrothion (2 g/m^2) for malaria control in Southern Iran in August 1971, a group of 28 pest control operators and 925 inhabitants were monitored with respect to their health and ChE activity. Clinical investigations and ChE tests on 840 workers showed 42 cases of clinical symptoms, most of which were very slight and subsided after the workers had showered and rested (2-3 h). Of 20 spraymen, 8 showed decreased ChE levels, and one individual preparing the spray mixture showed a significant depression of the enzyme activity, which was reactivated after an appropriate treatment. Among 925 inhabitants, only 15 cases of very mild complaints, namely dizziness and nausea, were reported. While fenitrothion was characterized as a pesticide that is safe for the inhabitants in a subtropical region during dry, hot seasons, further investigations were recommended on the toxic effects on operators under tropical conditions.

Ueda K, Goto S, Kuroda M & Nishimura M (1977) Deposit assessment of low volume aerial spray with Sumibassa 75% EC and its effects on engaging workers (translation from Japanese). Research Department, Pesticides Division, Sumitomo Chemical Co Ltd, Osaka, Japan. Report date: not given but date for aerial spraying was July 28, 1977.

Aerial spraying of Sumibassa 75% EC {45% fenitrothion & 30% o-sec-butylphenyl N-methyl carbamate (Bassa)} from a helicopter resulted in workers being deliberately exposed to spray drift whilst attending to monitoring equipment. The maximum spray mist concentration determined directly under the spray path was 1.64 µg/L of fenitrothion and 1.26 µg/L of Bassa but this declined to undetectable levels after 30 min. Although the workers exposed to spray drift showed no clinical signs of poisoning, their mean plasma ChE activity declined by 13% and 21% after 1 and 3 h respectively. By contrast, erythrocyte ChE activity only declined by 5% and 7% respectively over the same time interval.

Usutani S, Nishiyama K, Sato I, Matsuura K & Sawada Y (1978) Studies on the amount of exposure to pesticides and blood levels of organophosphorus pesticides of farmers engaging in joint control works over apple orchards. J Jap Assoc Rural Med 27: 79-88 (Translation from Japanese)

This published report (translated from Japanese) investigated the extent of fenitrothion exposure for workers involved in manually spraying orchard apple trees. In a typical spray operation,

groups of 2 operators and an assistant, protected by rubber boots and gloves, prepared, handled, transported and sprayed a formulation daily for 3 consecutive days; spray preparation was performed by the assistant. The extent of inhaled fenitrothion was monitored using a respiratory mask connected to a water trap with the volume of inhaled air being monitored via a "Gasuhr" (air meter). Using this apparatus, 3 operators were exposed to clean air only, whilst for 9 other fenitrothion-exposed workers plasma levels of fenitrothion were measured "after task" using a GC methodology (detection limit - 1 ng/mL; mean recovery of 50 ng/mL from spiked plasma - 85%). Plasma ALT, AST and ChE levels (pH method) were also determined. Urinary excretion (24 h pooled sample) of 3-methyl-4-nitrophenol (a major fenitrothion metabolite - see metabolic pathway, section 2.1) was determined by GC in 19 workers (including those having plasma fenitrothion monitoring).

This report does not make reference to any clinical signs. The mean quantity of inhaled fenitrothion for operators was 0.011 mg/m^3. Reflecting a demarcation of activities, operators had a lower maximum fenitrothion concentration in plasma (16 ng/mL; median 8 ng/mL; 3/5 had detectable concentrations after day 1 and 1/5 after day 3 spraying; day 2 concentrations not determined because rain suspended afternoon spraying) relative to assistants (30 ng/mL). Fenitrothion clearance from plasma appeared to be relatively rapid since only 2/7 (the other 2 in the group were not tested for unknown reasons) had detectable quantities (Cmax, 13 ng/mL) the following morning (elapsed time between sampling times not reported). AST, ALT and ChE activity determinations revealed no treatment-related changes. A urine sample collected after spraying revealed a mean 3-methyl-4-nitrophenol concentration of 133 ng/mL for operators and 229 ng/mL for assistants, a result reflecting differences in plasma concentration.

Thus, a fenitrothion formulation sprayed for insect control in orchards was inhaled and found within the systemic circulation immediately after use. The amount inhaled was related to the exposure and a rapid clearance was evident by its almost complete absence in plasma after 24 h. As expected, urinary excretion of a metabolite confirmed the relationship between exposure and plasma concentration.

Nishiyama K, Sawada Y, Hosokawa Y & Usutani S (1978) Studies on the amount of exposure to pesticides and blood levels of organophosphorus pesticides of farmers engaging in fixed piping joint control works of disease and insect damage to apples. J Jap Assoc Rural Med 27: 181-186 (Translation from Japanese)

In the second of a series of reports translated from Japanese, the investigation involved examining worker exposure to fenitrothion (0.1% or 0.15% dilution of wettable powder) when the insecticide was distributed through an orchard via rigid piping to several ports from which a hand-held spray apparatus was connected. The methodology used in this study to measure the volume of fenitrothion inhaled and its concentration in plasma was the same as given in the study described above (Usutani et al, 1978). Similarly, activity of ChE, AST, ALT, LDH, CPK and LAP in plasma and urinary excretion (24 h pooled specimen) of 3-methyl-4-nitrophenol were determined. The paucity of detail in the protocol description necessitated some deduction from the results. Thus, for inhalational exposure it appears that only 1 sprayer at each fenitrothion concentration was tested at approximately 20 min intervals (range 12-20 min; 4 & 6 samples taken respectively). However, after exposure to 0.15% fenitrothion, the plasma concentration,

enzyme activity and urinary concentration determinations were performed in 5 sprayers and 1 mixer.

There was no reference to any clinical signs. The mean quantity of inhaled fenitrothion was 0.023 and 0.035 mg/m^3 at 0.1 or 0.15% respectively whereas the concentration in plasma was 16 (mean) and 5 ng/mL at 0.15% for the sprayers and the mixer respectively. Fenitrothion did not appear to have any affect on any of the enzyme activities measured before and after exposure. The concentration of 3-methyl-4-nitrophenol (a major metabolite) in urine was 2.18 µg/mL (mean) for the sprayers and 0.7 µg/mL for the mixer.

Thus, a fenitrothion formulation sprayed in orchards via a fixed pipe distribution network was inhaled and found at low concentration in the systemic circulation after use. The amount inhaled varied considerably among sprayers and was reported to be due to several factors, ie tree height, wind intensity and direction. As expected, the extent of metabolite excretion in urine corresponded with the fenitrothion concentration in plasma.

Fakhri ZI (1993) Cholinesterase assessment as a result of fenitrothion exposure: a survey in a group of public health workers exposed to an organophosphorus pesticide. Occup Med 43: 197-202

After the spraying of fenitrothion (62.5 g/L) for malaria control in the Central Region of Sudan, a group of pest control operators (2 supervisors, 3 group heads, 5 mixers, and 7 spraymen) were checked for signs of poisoning and had their whole blood ChE activity monitored (on days 1-5 then day 7, 8, 15, 20, 39, 40, 41, 42). Nine workers experienced 1 or more of the following toxicity symptoms during the course of the 42 day study; sweating, weakness, abdominal cramps, blurred vision, dizziness or salivation. Except on 3 occasions (day 4, 41 and 42), ChE activity was significantly inhibited (p 0.05) by between 12.5% and 50%. While fenitrothion was characterized as a pesticide that is safe for the inhabitants in subtropical regions, it was concluded that appropriate precautionary measures should be adopted for spray personnel required to operate under humid conditions.

11.3 Poisoning Incidents

Wadia RS, Bhirud RH, Gulvani AV & RB Amin (1977) Neurological manifestations of three organophosphorus poisons. Ind J Med Res 66: 460-468

This study examined the clinical progress of 150 patients admitted to hospital after consuming varying quantities of different insecticides; 32 had consumed fenthion, 48 with fenitrothion, 50 with malathion, 6 with carbamate and the compound was unknown in 14 cases. Of the 48 fenitrothion cases, 1 death occurred after ingestion of 3 g and paralysis was observed in 2/3 patients at 6 g, 7/20 at 3 g, 2/16 at 1.5 g and 0/9 at less than 1.5 g. For the 11 patients with paralysis, plasma ChE levels were inhibited by >80% in 7 cases, between 60-80% in 2 cases and between 40-60% in 1 case; the remaining case received no comment.

Groszek B, Pach J & Klys M (1995) Intermediate syndrome in acute fenitrothion poisoning. Przegl d Lekarski 52(5): 271-274

"Intermediate syndrome" experienced after OP poisoning can be distinguished from the characteristic muscarinic, nicotinic and CNS effects observed soon after exposure and the delayed neurotoxicity effects seen 2-3 weeks later despite apparently satisfactory clinical management. Intermediate syndrome occurs 24-96 h after exposure and is characterised by muscular weakness affecting neck, proximal limb and respiratory muscles. Since only some OPs are capable of inducing this phenomenon, this study retrospectively examined 16 fenitrothion oral poisoning cases (14M & 2F; 9 attempting suicide, 7 accidental) for the occurrence of intermediate syndrome. Patients had consumed between 50-100 mL of a 50% fenitrothion solution and 6 died within 5-22 days after exposure despite gastric lavage, atropine and oxime therapy. Fenitrothion concentration in the plasma of these fatal cases ranged between 0.47-8.35 µg/mL. Of the remaining 10 survivors, Intermediate syndrome was observed in 7. Although plasma ChE activity was not detectable in survivors that exhibited Intermediate syndrome (and 1 other) at the time of their admission, recovery time to normalisation ranged from 5 to >10 weeks whereas 2/3 symptomless patients had activities of 200 and 1200 mU/mL and all 3 had recovered within 2-4 weeks. Fenitrothion concentration in the plasma of survivors with intermediate syndrome ranged between 0.18 and 3.02 µg/mL while for others it ranged between 96 and 360 ng/mL.

Kojima T, Yashiki M, Miyazaki F, Chikasue F & Ohtani M (1989) Detection of S-methylfenitrothion, aminofenitrothion and acetylaminofenitrothion in the urine of a fenitrothion intoxication case. Forensic Sci Internat 41: 245-253

The presence of several fenitrothion metabolites including 3-methyl-4-nitrophenol, aminofenitrothion, S-methylfenitrothion and acetylaminofenitrothion were detected in the urine of 23-year old male attempting suicide by ingesting approximately 50 mL of a 50% fenitrothion emulsion. All metabolites except S-methylfenitrothion were detected up to 62 h after ingestion. The half life of fenitrothion in plasma was about 4.5 h and its concentration after 3 h (first recording) was 170 ng/mL. Plasma ChE activity at 3 h was approximately 13% that of the average population range and it gradually increased to 14%, 27% and 34% at day 1, 2 and 3 respectively. The patient recovered sufficiently to be discharged after 3 days.

Yoshida M, Shimada E, Yamanaka S, Aoyama H, Yamamura Y & Owada S (1987) A case of acute poisoning with fenitrothion (Sumithion). Hum Toxicol 6: 403-406

A 56-old male ingested about 60 mL of a 50% fenitrothion emulsion in an attempt to commit suicide. At admission and up until his death due to respiratory insufficiency on day 6, plasma ChE levels were <10% that of normal despite combined haemoperfusion and haemodialysis treatment. Urinary excretion of the metabolite 3-methyl-4-nitrophenol was maximal at about 58 mg/day on day 3 after ingestion. Measuring the concentration of unchanged fenitrothion in the organs after death revealed that most was found in fat followed in order by pancreas, muscle and lung.

Ecobichon DJ, Ozere RL, Reid E & Crocker JFS (1977) Acute fenitrothion poisoning. CMAJ 116: 377-379

Accidental inhalational and dermal exposure to fenitrothion (EC, 7.5% v/v) resulted in a 33-year-old female technician having blurred vision, nausea, abdominal cramps, muscular weakness, mental confusion and tremors 2 days later. Plasma and erythrocyte ChE levels were inhibited by 44% and 14% respectively at the time of the clinical symptoms. Despite therapy with 1 g/day praloxime chloride (but not atropine) IV for 2 days, symptoms intensified and after 5 days plasma and erythrocyte ChE were inhibited by 64.5% and 34% respectively. Her condition improved after 7 IV doses of praloxime chloride and was discharged on day 16 after exposure.

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APPENDIX I

EXTRACT OF THE REPORT OF THE 14TH ADVISORY COMMITTEE ON PESTICIDES & HEALTH (ACPH) MEETING (6 NOVEMBER,1997)

6. EXISTING CHEMICALS REVIEW PROGRAM

6.1 Fenitrothion - Public Health Assessment

The Committee:

- CONSIDERED the public health assessment of fenitrothion, conducted as part of the Existing Chemical Review Program;

- NOTED that:

  fenitrothion is of low to moderate acute toxicity by all routes of administration tested, and is structurally related to parathion-methyl with the insertion of an additional methyl group;
  fenitrothion's lower acute toxicity compared to the parent parathion-methyl is apparently due to more rapid detoxification and elimination;
  fenitrothion was negative for genotoxicity in a wide range of assays, both in vitro and in vivo;
  in the long-term dietary chronic studies in rat and mouse, no evidence was seen for treatment-related carcinogenicity;
  no potentiation with other compounds was seen when fenitrothion was administered jointly with other anticholinesterase compounds;
  single dose administration to hens did not reveal any evidence for delayed neuropathy;
  limited evidence of peripheral neuropathy was seen in one study in rats and rabbits after 4-8 weeks exposure at high doses; and
  fenitrothion is reported to be associated with the induction of 'Intermediate Syndrome'

- RECOMMENDED a minor modification of the Acceptable Daily Intake (ADI) for fenitrothion from 0.003 to 0.002 mg/kg/day, based on the lowest No Observable Effect Level (NOEL) for plasma cholinesterase inhibition in a 1-year dietary dog study, and using a 100-fold safety factor;

- NOTED that an Acute Reference Dose (ARfD) was able to be established at 0.03 mg/kg bw/day based on a No Observed Effect Level (NOEL) of 0.33 mg/kg bw/day for cholinesterase inhibition in a single-dose human study;

- DISCUSSED the proposal for requesting from the sponsor further dietary feeding studies designed to specifically examine the peripheral neuropathy potential of fenitrothion, in the light of:
  - the close structural relationship of fenitrothion to parathion-methyl;
  - the reported association of fenitrothion with 'Intermediate Syndrome'; and
  - the suggestion of peripheral neuropathy seen in rats and rabbits at high doses in one study;

- CONSIDERED that any additional information which adds to the overall knowledge database concerning the mechanisms of peripheral neuropathy should be encouraged, particularly in relation to research into the effects of individual cholinesterases in order to ascertain the full spectra of their biochemical and neurological activities;

- ACKNOWLEDGED the difficulties associated with the lack of definitive study protocols which appropriately targeted neurological effects, and with interpretation of the results of such studies for example, with dose-response relationships; and

- HIGHLIGHTED potential anomalies in the establishment of impurity limits in the technical specifications for fenitrothion, particularly with regards to stability under long-term storage conditions.