Australian Government

Australian Pesticides and Veterinary Medicines Authority

CHEMICAL REVIEW PROGRAM

Review of the mammalian toxicology

And

Metabolism/toxicokinetics

Of

Fenamiphos

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ABBREVIATIONS

bw	Body weight
cm	Centimetre
g	Gram
gd	gestational days
kg	Kilogram
L	Litre
m	Metre
mg	Milligram
mg/kg bw/day	mg/kg bodyweight/day
mL	Millilitre
mm	Millimetre
po	oral
ppb	Parts per billion
ppm	Parts per million
wt	Weight
2-PAM	Pyridine-2-aldoxime
ADI	Acceptable Daily Intake
ALP	Alkaline phosphatase
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARfD	Acute Reference Dose
BBB	Blood brain barrier
BUN	Blood urea nitrogen
ChE	Cholinesterase
DWLOC	drinking water levels of comparison
FAISD	First Aid Instructions & Safety Directions
FAO	Food and Agriculture Organisation of the UN
GIT	Gastro-intestinal tract
GLP	Good Laboratory Practice
Hb	Haemoglobin
Hct	Haematocrit
JMPR	Joint Meeting on Pesticide Residues
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LOEL	Lowest Observed Effect Level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MRL	Maximum Residue Limit or Level
MTMC	4-(methylthio)-meta-cresol
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NTE	Neurotoxic target esterase
OHS	Occupational health and safety
OP	Organophosphorus pesticide

PACC	Pesticides Agricultural Chemicals Committee
RBC	Red blood cell/erythrocyte
SD	Sprague Dawley (rats)
SPF	Specific pathogen free
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons, formerly the Standard for the Uniform Scheduling of Drugs and Poisons
TOCP	Tri-orthocresyl phosphate.
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

EXECUTIVE SUMMARY

Fenamiphos is an organophosphorus insecticide and nematicide used for the control of nematodes and sucking insects (including aphids and thrips) on food and non-food crops, and for the control of nematodes in turf.

Fenamiphos was nominated for review under the Australian Pesticides and Veterinary Medicines Authority's (APVMA) Chemical Review Program (CRP) because of concerns over its high acute toxicity and its potential to cause chronic effects on human health.

The current Australian acceptable daily intake (ADI) for fenamiphos is 0.0001 mg/kg and is based on the no observed effect level (NOEL) of 0.014 mg/kg bw/day for the inhibition of plasma cholinesterase (ChE) activity in a 2-year dog study and using a 100-fold safety factor. This value is supported by the NOEL of 0.011 mg/kg bw/day for plasma ChE inhibition in a 6-month supplementary dog study. Following a review of all submitted and archived data, the existing ADI for fenamiphos was considered to remain appropriate. Therefore, the current health value for fenamiphos in Australian drinking water of 0.0003 mg/mL also remains appropriate. The present review identified a suitable acute oral dosing study in dogs to allow the setting of an acute reference dose (ARfD) for fenamiphos for the first time. The new ARfD of 0.003 mg/kg bw/day was calculated by applying a 100-fold safety factor to the NOEL of 0.25 mg/kg bw for the inhibition of erythrocyte ChE activity.

No changes to the approval status of fenamiphos have been proposed in this review. Registration of the 50 g/kg granular fenamiphos product for use in the home garden is no longer supported on the basis that it does not comply with criteria established by the APVMA for home garden products. There is no objection on public health grounds to the continued registration of all other existing fenamiphos products.

The existing poisons schedule for fenamiphos remains appropriate. The review identified a number of additions and amendments to the existing Safety Directions for products containing fenamiphos.

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of oral absorption	Detection in all tissues 0.5 hours after dosing. Based on urinary excretion of radioactivity, oral absorption was complete (>98%)
Distribution	Highest tissue concentrations found in liver, kidneys and gastrointestinal tract
Potential for accumulation	No evidence of accumulation in fat
Rate and extent of excretion	Majority excreted in urine within 16 hours and completed by 48 hours
Metabolism	Extensive; urinary metabolites include fenamiphos sulfoxide, fenamiphos sulfone and various fenamiphos phenols
Toxicologically significant compounds (animals, plants and environment)	Fenamiphos; fenamiphos sulfoxide; fenamiphos sulfone
Acute toxicity (Racemic mixture – no data for enantiomers)	
Rat oral LD_{50} (mg/kg bw)	2-19
Worst oral LD_{50} in other species	8 (female mice) ~10 (dogs)
Rat dermal LD_{50} (mg/kg bw)	72 to >2000 (vehicle dependent)
Worst dermal LD_{50} in other species	179 (female rabbits)
Rat inhalation LC_{50} (mg/m^3)	74 (4-hour aerosol, nose-only)
Worst inhalation LC_{50} in other species	No data
Skin irritation	Slight (rabbits)
Eye irritation	Moderate (rabbits)
Skin sensitization	Non skin sensitiser (guinea pigs; maximisation test)
Short-term toxicity	
Target/critical effect	Plasma ChE inhibition
Lowest relevant oral NOEL (mg/kg bw/day)	No data
Lowest relevant dermal NOEL (mg/kg bw/day)	0.5 (15-day rabbit, females)
Lowest relevant inhalation NOEC (mg/m^3)	0.25 (15-day rat)
Genotoxicity	Non-genotoxic. Cytogenic at cytotoxic concentrations
Long-term toxicity and carcinogenicity	
Target/critical effect	Plasma ChE inhibition
Lowest relevant NOEL (mg/kg bw/day)	0.011 (6-month oral dog study)
Carcinogenicity	No evidence of carcinogenicity

Reproductive toxicity			
Reproduction target/critical effect	No evidence of reproductive toxicity		
Lowest relevant reproductive NOEL (mg/kg bw/day)	3		
Developmental toxicity			
Developmental target/critical effect	No evidence of developmental toxicity at non-maternotoxic doses		
Lowest relevant developmental NOEL (mg/kg bw/day)	0.3 (chain fusion of the sternebrae in one rabbit study)		
Delayed neurotoxicity	No evidence of delayed neuropathy		
Immunotoxicity	No data		
Dermal absorption	No data		
Summary	NOEL (mg/kg bw/day)	Study	Safety factor
ADI (0.0001 mg/kg bw/day) [plasma ChE inhibition]	0.01	6-month and 2-year oral studies in dogs	100
ARfD (0.003mg/kg/bw) [RBC ChE inhibition]	0.25	Acute oral dosing study in dogs; rat developmental study	100

Health Value in drinking water	Current: 0.0003 mg/L Proposed: 0.0003 mg/L

SUMMARY TOXICOLOGY REPORT

Introduction

Fenamiphos was nominated for review, as part of the APVMA's Existing Chemical Review Program, because of concerns over its high acute toxicity and concerns over its potential to cause chronic effects on human health. A number of additional data submissions on the toxicology of fenamiphos were received from industry following the data call-in process, which together with all previously submitted data, have been assessed in detail. A summary of this detailed toxicological assessment is provided below. Fenamiphos is a racemic mixture, however, no toxicological data were provided on the enantiomers.

Metabolism and Toxicokinetics

Metabolism

Three experiments were conducted by Gronberg (1969) to examine the metabolism of radiolabelled fenamiphos, fenamiphos sulfoxide or fenamiphos sulfone in rats following oral dosing. Complete recovery of radioactivity was achieved, with the majority eliminated within 12-15 hours of dosing. Depending on the radiolabel, the main pathways of excretion of radioactivity were via exhaled CO_2, urine and faeces. Peak urine and faecal levels occurred at 12 hours. Total tissue radioactivity was up to 12% of the administered dose. Highest tissue radioactivity was found in the liver, kidney, fat and gastrointestinal tract (GIT). The main urinary metabolites included fenamiphos sulfoxide phenol and fenamiphos sulfone phenol, with traces of fenamiphos sulfoxide also detected. Unknown metabolites accounted for up to approximately 40% of urinary radioactivity. Fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone were detected as metabolites in liver, while only fenamiphos sulfoxide was detected in plasma. Metabolism of radiolabelled fenamiphos sulfoxide or fenamiphos sulfone was the same as the parent compound.

An *in vitro* study was undertaken to examine the metabolism of radiolabelled fenamiphos by 9000 or 90,000 g rat liver preparations. Both liver fractions were reported to metabolise fenamiphos, with the 90,000 g microsomal fraction completely metabolising the parent compound. Three different metabolites were detected, with two of these identified as fenamiphos sulfoxide and ethyl 4-(methylthio)-m-tolyl phosphoramidate. The third metabolite could not be identified; based on the available data the author considered that it may have been a N-acetyl derivative of fenamiphos (Khasawinah & Flint 1972).

In a whole-body autoradiographic study in rats, radiolabelled fenamiphos was absorbed rapidly from the GIT and was detectable at 0.5 hours after dosing in all tissues except the brain, suggesting that fenamiphos or its metabolites did not cross the blood brain barrier (BBB). Relatively high radioactivity was detected in those organs responsible for metabolising or eliminating fenamiphos, namely the kidneys, liver, bladder and GIT. Relatively high levels were also present in the blood, lung, salivary gland and connective tissue. Radioactivity was also detected around hair follicles on the skin, which the author considered a possible elimination pathway. Radioactivity was also detectable in the lymphatic system. The levels of radioactivity in all tissues declined over time so that by 8 hours, no discernible radioactivity was detected. There was no evidence of tissue or fat accumulation of fenamiphos or its metabolites (Weber 1988).

Radiolabelled fenamiphos was almost completely absorbed from the GIT following oral administration to rats. Although not directly measured, the rate of oral absorption was apparently

very high. Elimination was almost complete 48 hours after oral or intravenous administration (99%), with most excreted via the urine (96-98%) and only small levels excreted via the bile (1.5-3.7%). Renal clearance was approximately 50% during the first 4 hours after dosing and 90% within the first 16 hours after dosing. Virtually no material was excreted via expired air (0.005%). The rate and ratio of excretion appeared to show some dependence on sex and dose route. There was no evidence of tissue accumulation of fenamiphos or its metabolites. Highest residue levels were found in the liver, kidneys and skin. The main metabolites (80-96%) were fenamiphos phenols (fenamiphos-sulfoxide phenol sulfate, fenamiphos-sulfoxide phenol and fenamiphos-sulfone phenol sulfate) (Ecker et al 1989).

Toxicokinetics

No toxicokinetic studies were submitted for evaluation.

Acute toxicity studies

Fenamiphos

The oral LD_{50} in female rats ranged from 2 to 19 mg/kg bw, while in males it ranged from 2 to 15 mg/kg bw. The time to death was within 90 minutes of dosing (the earliest death was approximately 7 minutes after dosing). Clinical signs (tremors, salivation, lacrimation, diarrhoea, convulsions, apathy, palmospasms, laboured breathing, piloerection and clonic cramps) developed at 5-30 minutes after dosing and typically lasted for several hours (in survivors). The severity of these signs tended to increase with dose. Macroscopic abnormalities in decedents included distended or slightly collapsed lungs and liver discolouration (darkening). The oral LD_{50} in mice was 8 and 23 mg/kg bw in females and males, respectively. The oral LD_{50} in guinea pigs was 75-100 mg/kg bw, while the oral LD_{50} in rabbits was ~5-18 mg/kg bw. The oral LD_{50} in dogs was approximately 10 mg/kg bw (Kimmerle & Lorke 1967; Kimmerle & Solmecke 1971; Crawford & Anderson 1973; Crawford & Anderson 1974 a & b; Lamb & Matzkanin 1975; Krotliner 1988 and 2000a).

In an acute oral dosing study conducted in dogs for the purpose of setting an ARfD, the NOEL was 0.25 mg/kg bw, based on toxicologically-significant inhibition of RBC ChE activity at 0.5 mg/kg bw. In the absence of inhibition of brain ChE activity, this study suggested that fenamiphos cannot cross the BBB and inhibit brain ChE activity (Detzer 2002).

The dermal LD_{50} in rabbits was 225 mg/kg bw in males and 179 mg/kg bw in females using an acetone vehicle (Crawford & Anderson 1972). The study conducted by Flucke (1980) using a polyethylene glycol vehicle, established a dermal LD_{50} in rats of 72 mg/kg bw in males and 92 mg/kg bw in females. However, a more recent study conducted by Krotlinger (2000b), established a dermal LD_{50} of greater-than 2000 mg/kg bw *without* a vehicle. This contrasts with earlier studies where the dermal LD_{50} in rats was 500 mg/kg bw in the absence of a vehicle (Kimmerle & Lorke 1967; Kimmerle & Solmecke 1971). The time to death in rabbits was 3-6 hours in males and 8 hours to 4 days in females (Crawford & Anderson 1972), while the time to death in a single female rat was 2 days (Krotlinger 2000b). Clinical signs consistent with those occurring following oral administration occurred in rabbits at 1-4 hours after dosing and lasted for an unspecified time. In rats, cholinergic signs occurred from hours after dosing and sometimes lasted for 2 days. Dark red discolouration of the liver was noted in the single female rat that died in the study of Krotlinger (2000b).

The LC_{50} in rats following one or four hours of head-only exposure to fenamiphos aerosols was 130 and 100 mg/m^3, respectively. The time to death was within the 1 or 4 hour exposure periods.

Clinical signs occurred for up to 7 hours after exposure and included muscle twitching and cramps. Other clinical signs such as inactivity, stiff gait and rough hair coats lasted for up to 5 days after exposure (Thyssen (1979a).

Four hours of nose-only exposure to fenamiphos aerosols resulted in a LC_{50} of 74 mg/m^3. Depending on the exposure concentration, the time to death was within the 4 hour exposure period or within a day after exposure. The onset and duration of clinical signs ranged up to 3 and 9 days after exposure, respectively. The types of signs observed depended on the exposure concentration and included piloerection, rough hair-coats, bradypnoea, laboured breathing, dyspnoea, irregular breathing pattern, reduced motility, limping, tremor, fasciculations, giddiness, high-legged gait, exophthalmos, miosis, corneal opacity, chromodacryorrhea, red encrusted nostrils, salivation, pallor, emaciation and periorbicular red stains. Macroscopic abnormalities detected in decedents included pale or dark red patches on the lungs, pale organs and corneal opacity (Pauluhn 2001).

In rabbits, fenamiphos was a non-skin irritant (Crawford & Anderson 1971) or a slight skin irritant (Kato 1984a). When administered as a crystalline solid, fenamiphos was a slight eye irritant in rabbits (Crawford & Anderson 1971). When administered as an undiluted liquid, fenamiphos was a moderate eye irritant and caused systemic toxicity in rabbits whose eye was unwashed (Kato 1984b). No such effects were seen in rabbits whose eye was washed 20-30 seconds after administration.

Fenamiphos was not a skin sensitiser in guinea pigs (Watanabe 1983; Stropp 1995).

Fenamiphos Metabolites

Toxicologically-significant fenamiphos metabolites include fenamiphos sulfoxide, fenamiphos sulfone, desisopropyl fenamiphos sulfoxide, desisopropyl fenamiphos sulfone and desisopropyl fenamiphos. The oral LD_{50} values for fenamiphos sulfone and fenamiphos sulfoxide are 2-25 mg/kg bw in rats (Crawford & Anderson 1974b; Thyssen 1974a & b). The oral LD_{50} for desisopropyl fenamiphos sulfoxide is approximately 4 mg/kg bw in rats (Lamb & Matzkanin 1975). The oral LD_{50} for desisopropyl fenamiphos in rats is 1 and 2 mg/kg bw in males and females, respectively (Lamb & Matzkanin 1977). No acute oral toxicity data were available for desisopropyl fenamiphos sulfone. The time to death and occurrence of clinical signs following dosing with these fenamiphos metabolites was consistent with those seen with the parent compound.

Fenamiphos Impurities

Toxicologically-significant fenamiphos impurities include aryldiamide, diarylamide, diaryl ethyl ester, diethyl ester, diethylmonamide, di-SCH_3 compound, ethyl aryl ester, ethyldiamide, 4-(methylthio)-meta-cresol (MTMC).

The oral LD_{50} values in rats for diethylmonamide, ethyldiamide, diethylester, aryldiamide, di-SCH_3 compound, diarylethylester, diarylamide and ethylarylester acid were >5 mg/kg bw in males and >4 mg/kg bw in females. No clinical signs or mortalities occurred at the tested doses (Crawford & Anderson 1973).

The oral LD_{50} for MTMC, MTMC-sulfone and MTMC-sulfoxide in rats ranged from 1175 to1854 mg/kg bw. The time to death was 4 hours to 4 days in males and 2-24 hours in females. Ataxia and decreased activity were observed in all rats treated with each of the 3 test compounds, which started from 5 minutes after dosing and lasted for 1 or 2 hours (Crawford & Anderson 1974a).

The oral LD_{50} for 4-methylmercapto-m-cresol in rats was >2500 mg/kg bw, with the time to death 5 hours to 10 days after dosing. Clinical signs (poor general condition, sedation and respiratory disturbances) reportedly occurred in all rats at every dose during the first 2 days after dosing (Thyssen 1974c).

The oral LD_{50} for 3-methyl-4-methylmercaptophenol in rats was 500-1000 mg/kg bw, with the time to death unspecified. A depression in general condition occurred an unspecified time after dosing (Thyssen 1974d).

The LD_{50} for 3-methyl-4-methanesulfonylphenol in rats was >1000 mg/kg bw, with no deaths or clinical signs reported (Thyssen 1974e).

The LD_{50} for desmethyl fenamiphos in rats was 1000-4000 mg/kg bw in males and approximately 1000 mg/kg bw in females, with the time to death 24-48 hours after dosing. Tremors, salivation, lacrimation, diarrhoea and convulsions occurred in all rats of both sexes within 30 minutes of dosing and lasting for 24 or 74 hours (Lamb & Matzkanin 1975).

Fenamiphos Products

A granular formulation containing 5% fenamiphos had an oral LD_{50} of 47 and 68 mg/kg bw in male and female rats, respectively. The time to death was 8-60 minutes after dosing, with clinical signs occurring within minutes of dosing and lasting for up to 24 hours (laboured breathing, muscle cramps, salivation, bloody tears and dyspnoea). Apathy occurred in survivors for 2 days after dosing. No treatment-related macroscopic abnormalities were detected. The dermal LD_{50} in rats following 24 hours of exposure was approximately 3600 mg/kg bw. The time to death ranged from 40 minutes to 6 days in males. Clinical signs similar to those observed during the oral dosing component of this study occurred from 1-2 hours after dosing. Apathy was reported to last for up to 9 days after dosing. No treatment-related macroscopic abnormalities were detected. The LC_{50} in rats following 1 or 4 hours head-only exposures to the dust was >125 and approximately 106 mg/m^3, respectively. The time to death was <4 hours, with clinical signs persisting for up to 8 days (sluggish movement, piloerection, sedation, staggering and slightly laboured breathing). All rats that died had pulmonary emphysema. In survivors, slight pulmonary emphysema occurred at higher concentrations. In the absence of a control group, it is unclear whether the effects seen in this study were due to fenamiphos *per se* or to the inhaled dust (Mihail & Thyssen 1980a).

A granular formulation containing 10% fenamiphos had an oral LD_{50} of 26 and 34 mg/kg bw in fasted male and female rats, respectively. In non-fasted rats, the oral LD_{50} was approximately 75 mg/kg bw. The time to death was 7-40 minutes after dosing, with clinical signs (dyspnoea, reduced mobility, muscular spasms, salivation and exophthalmos) occurring from 3-60 minutes after dosing and lasting for 1-3 days. Macroscopic findings in decedents included slightly distended lungs, slightly pale spleen and slightly reddened renal pelvis. In survivors, lung patches with small dots and rough spleen surface were noted. The dermal LD_{50} in rats was >5000 mg/kg bw; no deaths occurred although transient apathy occurred in an unspecified number of rats. The LC_{50} in rats following 1 or 4 hours of head-only exposures was >118 and >44 mg/m^3, respectively. There were no deaths, clinical signs or treatment-related macroscopic abnormalities. This formulation was a non-skin irritant and slight eye irritant in rabbits (Heimann & Thyssen 1981). In addition, this formulation was not mutagenic in a salmonella/microsome test (Herbold 1992).

An EC formulation containing 40% fenamiphos had an oral LD_{50} of 10 mg/kg bw in rats. The time to death was 7-46 minutes after dosing. Clinical signs included reduced mobility, muscular cramps, involuntary jumping, salivation and bloody tears, with laboured breathing and transient lateral

recumbency occurring at lethal doses. Survivors exhibited apathy for 1-3 days after dosing. No treatment-related macroscopic abnormalities were detected. The dermal LD_{50} in rats was 83 mg/kg bw in males and 64 mg/kg bw in females, with the time to death ranging from 1 hour to 4 days. Clinical signs consistent with those seen following oral dosing occurred from 30 minutes to 2.5 hours. Slight pulmonary emphysema and dark spots on lungs were reported in an unspecified number of rats that died during the study and those that were sacrificed at the end of the study. The LC_{50} in rats following 1 or 4 hours of head-only exposures was 330-440 mg/m^3 (females) and 132-198 mg/m^3, respectively. The time to death was one day following either exposure period. Clinical signs included restlessness and ruffled coat (few minutes to 8 days after exposure) in addition to muscle twitching, cramps and salivation (for up to one day after exposure). At lethal concentrations, rats appeared drowsy, prostrate or laterally recumbent for up to 2 hours prior to death. Gross abnormalities detected in decedents included pink-coloured lungs and slight to marked emphysema, with an unspecified number also having a pale spleen and a lobular pattern of the liver. An unspecified number of rats sacrificed at the end of the study exhibited slightly dilated lungs. In a supplementary study, exposure to product vapours for 7 hours caused central nervous system disturbances at approximately 15 minutes after exposure and lasted for a day. No cholinergic signs were reported. All survivors subsequently made a full recovery. Mucosae of the eyes and nose were reportedly irritated but only during exposure. Post-mortem examination revealed "old pink-coloured" lungs and mild emphysemas in an unspecified number of rats. The authors attributed the findings in this supplementary study to the xylene content of this product. This formulation was a severe skin and eye irritant in rabbits. A 0.7% aqueous dilution of this formulation had an oral LD_{50} in rats of 13 mg/kg bw and an LC_{50} of 90 mg/m^3 (4 hours, head-only exposure). It was a non-skin and eye irritant in rabbits (Mihail & Thyssen 1980b).

Antidote studies

Treatment of rats with various antidotal compounds (atropine sulphate, 2-PAM, toxogonin, atropine sulphate plus 2-PAM and atropine sulphate plus toxogonin) resulted in an approximate doubling of the LD_{50} for fenamiphos (Kimmerle 1972).

Short-Term Repeat-Dose Studies

Oral Exposure

There were no short-term repeat-dose oral dosing studies submitted for evaluation.

Dermal Exposure

Fenamiphos was applied to the clipped dorsal skin of rats at 0, 2.5, 10 or 40 mg/kg bw/day for 6 hours/day over 22 or 23 days for males and females, respectively. Application sites were occluded and rats were immobilised during treatment. The NOEL was 10 mg/kg bw/day, based on the inhibition of plasma ChE activity at 40 mg/kg bw/day (Krotlinger 2000c).

Fenamiphos was applied to the clipped dorsal skin of rabbits at 0, 2.5 or 10.0 mg/kg bw/day for 6 hours/day on 15 consecutive working days. For some rabbits, the application site had been abraded 12 hours prior to sample application. Rabbits were immobilised during treatment. The NOEL was 0.5 mg/kg bw/day, based on the inhibition of plasma ChE activity at and above 2.5 mg/kg bw/day in females. Inhibition of RBC ChE activity occurred in both sexes only at 10 mg/kg bw. There was no difference in the level of inhibition of plasma or RBC ChE activity between abraded and non-abraded rabbits (Mihail & Schilde 1980).

Inhalational Exposure

Rats were exposed (head-only) to aerosols of fenamiphos over five 4-hour periods. In the first experiment, rats were exposed to concentrations of 0, 4, 9 or 28 mg/m^3, while a second experiment tested concentrations of 0, 0.3, 0.6 or 3.3 mg/m^3. The no observed effect concentrations (NOEC) were 0.3 mg/m^3 in females and 0.6 mg/m^3 in males, based on toxicologically-significant inhibition of plasma ChE activity at the next highest concentrations (0.6 mg/m^3 in females and 3.3 mg/m^3 in males). RBC ChE activity was inhibited at 9 mg/m^3 in females and 28 mg/m^3 in males. Transient clinical signs (inactivity, stiff gait, rough hair coats and muscle twitching) occurred during the exposure period at and above 9 mg/m^3 in males and at and above 4 mg/m^3 in females (Thyssen 1979a).

Rats were exposed to aerosols of fenamiphos (head-only exposure) at 0, 0.1, 1.0 or 10 mg/m^3 for 6 hours/day on 5 consecutive days for 3 weeks (analytical concentrations of 0, 0.03, 0.25 and 3.5 mg/m^3, respectively). The NOEC was 0.25 mg/m^3, based on the inhibition of plasma ChE activity in both sexes at 3.5 mg/m^3 (Thyssen 1979b).

Subchronic Studies

Oral Administration

In a 3-month rat study, fenamiphos was administered in the diet at 0, 4, 8, 16 or 32 ppm (equivalent to 0, 0.4, 0.8, 1.6 and 3.2 mg/kg bw/day, respectively). The NOEL was 0.4 mg/kg bw/day, based on toxicologically-significant inhibition of plasma ChE at and above 0.8 mg/kg bw/day. Inhibition of RBC ChE activity occurred at and above 1.6 mg/kg bw. 'Typical' signs of cholinesterase inhibition occurred at 3.2 mg/kg bw/day (Löser 1968a; Mawdesley-Thomas et al 1970a).

In a 14-week dietary study in rats, which examined the effect on ChE inhibition, fenamiphos was administered at 0, 0.36, 0.6 or 1.0 ppm. The NOEL was 1 ppm (equal to 0.072 and 0.084 mg/kg bw/day in males and females, respectively), the highest dose tested, based on the absence of plasma ChE inhibition at this dose (Hayes 1986a).

In a 3-month dog study, fenamiphos was administered via the diet at 0, 2, 6 or 18 ppm (estimated to be 0, 0.05, 0.15 and 0.45 mg/kg bw/day, respectively) for 3 months. No NOEL was established. The LOEL was 2 ppm (0.05 mg/kg bw/day), based on toxicologically-significant plasma ChE inhibition at and above this dose. Toxicologically-significant inhibition of RBC ChE activity occurred at and above 6 ppm (0.15 mg/kg bw/day). Clinical signs (mild muscle tremors and vomiting) occurred only at the highest dietary level of 18 ppm (0.45 mg/kg bw/day) (Löser 1968b; Mawdesley-Thomas & Urwin 1970b).

In a follow-up dog study by Löser (1969), dietary levels of fenamiphos of 0, 1, 2 or 5 ppm were given for 3 months. The NOEL was 1 ppm (estimated to be 0.025 mg/kg bw/day), based on the occurrence of toxicologically-significant plasma ChE inhibition at and above 2 ppm (estimated to be 0.05 mg/kg bw/day). Toxicologically-significant inhibition of RBC ChE activity occurred only at the highest dietary level of 5 ppm (estimated to be 0.125 mg/kg bw/day).

In a 100-day dog study, fenamiphos was administered in the diet at 0, 0.6, 1.0 or 1.7 ppm. The NOEL was 1.0 ppm (equal to 0.025 mg/kg bw/day), based on toxicologically significant inhibition of plasma ChE activity at the next highest level of 1.7 ppm (equal to 0.042 mg/kg bw/day) (Hayes 1983).

Dermal or Inhalational Administration

There were no subchronic dermal or inhalational toxicity studies submitted for evaluation.

Chronic Studies

Mice were given fenamiphos via the diet for 2 years at 0, 2, 10 or 50 ppm. The NOEL was 10 ppm (equal to 1.4 mg/kg bw/day in males and 1.8 mg/kg bw/day in females), based on significantly reduced bodyweight at the next highest level of 50 ppm (equal to 7.4 and 8.8 mg/kg bw/day in males and females, respectively). Increased mortality at 12 months occurred in females at 50 ppm (8.8 mg/kg bw). There was no evidence that fenamiphos was carcinogenic (Hayes 1982).

In the first of two rat studies, fenamiphos was admixed in the diet and fed to rats for 2 years at levels of 0, 3, 10 or 30 ppm. The NOEL was 10 ppm in females and 3 ppm in males (equal to doses of 0.56 and 0.23 mg/kg bw/day, respectively), based on inhibition of plasma ChE activity at the next highest dose (1.72 and 0.76 mg/kg bw/day, respectively). There was no evidence that fenamiphos was carcinogenic (Löser 1972a; Cherry & Newman 1973).

In the second rat study, fenamiphos was admixed in the diet and fed to rats for 2 years at 0, 2, 10 or 50 ppm. No NOEL was established in this study. The LOEL was 2 ppm (equal to 0.098 mg/kg bw/day in males and 0.121 mg/kg bw/day females) due to inhibition of plasma ChE activity at and above this level. At and above 10 ppm (equal to 0.464 mg/kg bw/day in males and 0.603 mg/kg bw/day in females), inhibition of RBC ChE activity occurred. At the highest level of 50 ppm (equal to 2.454 mg/kg bw/day in males and 3.361 mg/kg bw/day in females), inhibition of brain ChE activity occurred. Females appeared more sensitive to the effects of fenamiphos on ChE activity than males. A range of other treatment-related effects were also evident at 50 ppm including reduced bodyweight, clinical signs in females (alopecia and rough coats), increased blindness, lens opacity (females), increased incidence of inflammatory lesions in the nasal, laryngeal and lung tissue, increased absolute lung and liver weights and increased relative brain, heart and lung weights. There was no evidence that fenamiphos was carcinogenic (Hayes 1986b).

In a 2-year dog study, fenamiphos was given in the diet at 0, 0.5, 1, 2, 5 and 10 ppm. The NOEL was 0.5 ppm in females (equal to 0.014 mg/kg bw/day) and 1 ppm in males (equal to 0.029 mg/kg bw/day), based on the toxicologically significant inhibition of plasma ChE activity at and above the next highest dose (0.036 mg/kg bw/day in females and 0.063 mg/kg bw/day in males). Toxicologically-significant inhibition of RBC ChE activity occurred at and above 5 ppm (0.150 and 0.171 mg/kg bw/day in males and females, respectively) (Löser 1972b; Thomson et al 1972).

In a one-year dog study, fenamiphos was administered in the diet at 0, 1, 3 or 12 ppm. No NOEL was established. Therefore, the LOEL was 1 ppm (equal to 0.03 mg/kg bw/day in males and females), based on inhibition of plasma ChE activity at and above this level. Inhibition of RBC ChE activity occurred in males at and above 3 ppm (equal to 0.089 mg/kg bw/day in males), and at 12 ppm in females (equal to 0.349 mg/kg bw/day). Perturbations in haematology parameters in males at 12 ppm (equal to 0.308 mg/kg bw/day) were suggestive of mild anaemia of marginal toxicological significance. At 12 ppm, chronic inflammation and vasculitis occurred in females and lymphocytic inflammation of the gall bladder occurred in males all of which were considered treatment-related findings. There occurrence of atrophy and cysts of the prostate in high-dose males showed an equivocal relationship with treatment (Rieth 1991; Jones & Greufe 1993; Van Goethem & Elcock 1997).

In the absence of a NOEL for plasma ChE inhibition in the above one-year dog study, a supplementary subchronic toxicity study was conducted. Fenamiphos was admixed in the diet and fed to dogs for 6 months at concentrations of 0 or 0.5 ppm. The NOEL was 0.5 ppm (equal to 0.0108 mg/kg bw/day in males and 0.0118 mg/kg bw/day in females), based on the absence of plasma or RBC ChE inhibition at this concentration (Jones & Loney 1993).

Reproduction Studies

In a 3-generation rat study, fenamiphos was admixed in the diet and fed to 3 parental generations their offspring throughout all phases of the study at 0, 3, 10 or 30 ppm (equal to 0, 0.3, 1 and 3 mg/kg bw/day, respectively). The NOEL for parental toxicity, pup toxicity and reproductive toxicity was 30 ppm (~3 mg/kg bw/day) (Löser 1972c; Cherry et al 1972).

In a 2-generation rat study, fenamiphos was admixed in the diet and fed to 2 parental generations and their offspring throughout all phases of the study at nominal concentrations of 0, 2.5, 10 or 40 ppm. The NOEL for reproductive toxicity was 40 ppm, the highest dose tested, based on the absence of any reproductive effects at this dose. The LOEL for maternal toxicity was 2.5 ppm (equal to 0.17 mg/kg bw/day), based on the inhibition of plasma ChE activity at and above this dose. The NOEL for paternal toxicity was 2.5 ppm (equal to 0.17 mg/kg bw/day), based on the inhibition of plasma ChE activity at 10 ppm (equal to 0.65 mg/kg bw/day). The NOEL for pup toxicity was 2.5 ppm (equal to 0.17 mg/kg bw/day), based on the inhibition of plasma ChE activity at and above 10 ppm (equal to 0.65 mg/kg bw) (Eigenberg 1991 & 1997).

Developmental Studies

Groups of inseminated rats were dosed by oral gavage with fenamiphos at 0, 0.3, 1.0 or 3.0 mg/kg bw/day from gestational days (gd) 6-15. The NOEL for maternotoxicity was 1 mg/kg bw/day, based on the occurrence of mortalities and clinical signs (whole-body tremors) at 3 mg/kg bw/day. The NOEL for developmental toxicity was 3 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic (Schlüter 1981; Renhof 1986).

Fenamiphos was administered by oral gavage to groups of pregnant rats at doses of 0, 0.25, 0.85 or 3 mg/kg bw/day from gd 6-15. The NOEL for maternotoxicity was 0.25 mg/kg bw/day, based on significant inhibition of RBC ChE activity at and above 0.85 mg/kg bw/day. At 3 mg/kg bw/day, mortalities, overt signs of toxicity, significantly lower bodyweight gain and food consumption, and inhibition of plasma ChE activity, occurred. The NOEL for developmental toxicity was 3 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic (Clemens et al 1989).

Fenamiphos was administered by oral gavage to pregnant rabbits at 0, 0.1, 0.3 or 1.0 mg/kg bw/day on gd 6-18. The NOEL for maternal toxicity was 0.3 mg/kg bw/day, based on reduced bodyweight gain to gd 20 at 1.0 mg/kg bw/day. The NOEL for developmental toxicity was 0.3 mg/kg bw/day, based on the occurrence of chain fusion of the sternebrae at 1.0 mg/kg bw/day, which is considered secondary to maternotoxicity (Lamb 1982).

Mated rabbits were given fenamiphos by oral gavage from gd 6-18 at 0, 0.1, 0.5 or 2.5 mg/kg bw/day. The NOEL for maternal toxicity was 0.5 mg/kg bw/day, based on mortalities, clinical signs (salivation, diarrhoea and ataxia), reduced bodyweight gain and significantly reduced food consumption at 2.5 mg/kg bw/day. The NOEL for developmental toxicity was 2.5 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic (Becker 1986).

Genotoxicity Studies

Fenamiphos was not mutagenic to bacteria (Herbold 1985a & b) or mammalian cells (Yang 1985). Fenamiphos was clastogenic to human lymphocytes at cytotoxic concentrations in the presence and absence of exogenous metabolic activation (Herbold 1987 & 1988). Fenamiphos did not cause sister chromatid exchanges in mammalian cells (Chen et al 1982a & b) or unscheduled DNA synthesis in rat hepatocytes (Curren 1988). Fenamiphos was negative in micronucleus (Herbold 1980a) and dominant lethal tests in mice (Herbold 1980b). A 10% granular formulation of fenamiphos was not mutagenic to bacteria (Herbold 1992).

Neurotoxicity Studies

Fenamiphos was admixed in the diet at concentrations of 0, 1, 3, 10 or 30 ppm (equal to 0, 0.002, 0.005, 0.016 and 0.026 mg/kg bw/day, respectively) and fed hens for 30 days. Clinical signs occurred only at 30 ppm but were reportedly unlike 'typical' cholinergic symptoms. No signs of neurotoxicity were reported. Average bodyweight and food consumption were significantly reduced at 30 ppm (0.026 mg/kg bw). Whole blood ChE activity was inhibited in a dose-related fashion at and above 3 ppm (0.002 mg/kg bw). Histopathological abnormalities were non-specific in nature and possibly related to a viral infection (Kimmerle 1970; Spicer 1970).

Fenamiphos was administered to hens as a single oral gavage dose of 1.0, 2.5, 3.75, 5.0, 7.5 or 10 mg/kg bw. In a parallel experiment, hens were dose orally with 3.75, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 or 25.0 mg/kg bw fenamiphos under atropine protection. Positive control hens received tri-orthocresyl phosphate (TOCP). In the absence of atropine protection the NOEL was 1.0 mg/kg bw, based on the occurrence of cholinergic signs and deaths at and above 2.5 mg/kg bw. With atropine protection the NOEL was 3.75 mg/kg bw, based on the occurrence of cholinergic signs at and above 5.0 mg/kg bw. No clinical signs consistent with delayed neuropathy occurred in fenamiphos-treated hens but "typical" signs developed in the TOCP-treated hens (unsteady gait, lameness and paralysis) (Kimmerle 1971).

Fenamiphos was administered by oral gavage to hens under atropine protection at a dose of 25 mg/kg bw. Two doses were administered, separated by an interval of 3 weeks. A negative control group received the vehicle only and a positive control group received 375 mg/kg bw of TOCP by oral gavage. Approximately half of the hens died 1-4 days after the first dose of fenamiphos. Clinical signs exhibited by all fenamiphos-treated hens included staggering gait, ruffled feathers, tachypnoea, reduced activity, flaccid/drooping wings and a spasmodic state. A few hens also displayed sternal and lateral recumbency and salivation. Following the second dose of fenamiphos, one hen died 2 days post-dose and clinical signs were observed in all hens from 45 minutes to 7 days. There was a treatment-related loss of bodyweight. There was no indication that fenamiphos caused neuropathy (Flucke & Kaliner 1987).

In the study of Flucke (1988), a single oral dose of 25 mg/kg bw fenamiphos failed to inhibit neurotoxic esterase activity (NTE) in hens.

Fenamiphos was administered as a single oral gavage dose to rats at 0, 0.4, 1.6 or 2.4 mg/kg bw. No NOEL was established in this study. The LOEL was 0.4 mg/kg bw, based on the inhibition of plasma and RBC ChE activities at and above this dose. Mortalities; cholinergic signs; FOB abnormalities; discolourations of the liver, lungs and spleen were evident at the highest dose of 2.4 mg/kg bw. There was no evidence that fenamiphos caused neuropathy (Dreist 1995).

Fenamiphos was admixed in the diet and fed to rats at nominal concentrations of 0, 1, 10 or 50 ppm for 13-14 weeks. The NOEL in males was 1 ppm (equal to 0.06 mg/kg bw/day), based on inhibition of plasma and RBC ChE activities at and above 10 ppm (0.61 mg/kg bw/day). No NOEL could be established for females due to inhibition of plasma ChE activity at every dose. Transient muscle fasciculations occurred in females at the highest dose of 100 ppm (equal to 3.98 mg/kg bw/day). There was no evidence that fenamiphos caused neuropathy up to 100 ppm (equal to 3.13 and 3.98 mg/kg bw/day in males and females, respectively) (Dreist & Popp 1996).

HAZARD ASSESSMENT

Discussion

Introduction

The current review of fenamiphos was undertaken under the auspices of the APVMA's Chemical Review Program due to concerns over its high potential acute and high chronic risks and human poisonings in the USA. In addition, reviews performed by the JMPR in 1997 and 2002, and the US EPA in 1999, indicated that there were a number of studies that had not previously been evaluated by the OCS.

The toxicological database for fenamiphos is extensive and consists of unpublished reports generated by industry, in addition to a range of published studies. Studies were conducted on products similar to those currently registered in Australia.

Mechanism of Mammalian Toxicity

In common with all organophosphate compounds the primary mode of action of fenamiphos is via the inhibition acetyl ChE activity, which causes over-stimulation of those parts of the nervous system that use acetylcholine to transmit nerve impulses. Signs of intoxication are consistent with acetyl ChE inhibition and include salivation, lachrymation, vomiting, diarrhoea and laboured breathing. If intoxication is severe, muscle twitching, loss of reflexes, convulsions and death can eventuate. Fenamiphos is a direct acting OP and does not require activation to inhibit acetyl ChE activity.

The most sensitive toxicological endpoint for fenamiphos in laboratory animals is the inhibition of RBC ChE activity for acute dosing and inhibition of plasma ChE activity for repeated dosing. The following table summarises the NOELs/LOELs for plasma, RBC and brain ChE activities in laboratory animals over various durations and routes of exposure.

Table 1 Summary of ChE activity findings in laboratory animals

Study	Plasma ChE		RBC ChE		Brain ChE		Reference
	NOEL	LOEL	NOEL	LOEL	NOEL	LOEL	
Oral dosing *(mg/kg bw/day)*							
Dog (acute)	0.063	0.125	0.25	0.5	2	NI	Detzer (2002) [GLP, QA]
Rat (dev)	0.85	3	0.25	0.85	3	NI	Clemens et al (1989) [GLP, QA]
Rat (3-months)	0.4	0.8	0.8	1.6	NM	NM	Löser (1968a); Mawdesley-Thomas & Urwin (1970a)
Rat (90-day)	0.084	NI	0.084	NI	0.084	NI	Hayes (1986a) [GLP, QA]
Dog (90-day)	0.025	0.042	0.042	NI	0.042	NI	Hayes (1983) [GLP, QA]
Dog (3-months)	NE	0.05	0.05	0.15	NM	NM	Löser (1968b)

Study	Plasma ChE		RBC ChE		Brain ChE		Reference
	NOEL	LOEL	NOEL	LOEL	NOEL	LOEL	
Dog (3-months)	0.025	0.05	0.05	0.125	NM	NM	Löser (1969); Mawdesley-Thomas & Urwin (1970b)
Dog (6-months)	0.011	NI	0.011	NI	NM	NM	Jones & Loney (1993) [GLP, QA]
Rat (2-y)	0.56/0.23 (M/F)	1.72/0.76 (M/F)	0.56/76 (M/F)	1.72/2.20 (M/F)	NM	NM	Löser (1972a); Cherry & Newman (1973)
Rat (2-y)	NE	0.098/0.121 (M/F)	0.098/0.121 (M/F)	0.464/0.603 (M/F)	0.46/0.60 (M/F)	2.45/3.36 (M/F)	Hayes (1986b) [GLP, QA]
Dog (2-y)	0.029/0.014 (M/F)	0.063/0.036 (M/F)	0.063/0.036 (M/F)	0.15/0.17 (M/F)	NM	NM	Löser (1972b); Thomson et al (1972)
Dog (1-y)	NE	0.03	0.03/0.083 (M/F)	0.089/0.35 (M/F)	0.31/0.083 (M/F)	NI/0.35 (M/F)	Rieth (1991); Jones & Greufe (1993); Van Goethem & Elcock (1997) [GLP, QA]
Rat (2-gen)	0.17/NE (M/F)	0.65/0.20 (M/F)	0.17/0.20 (M/F)	0.63/0.73 (M/F)	NI/0.7 (M/F)	NI/3.2 (M/F)	Eigenberg (1991 & 1997) [GLP, QA]
Dermal administration *(mg/kg bw/day)*							
Rat (22 or 23 days)	10	40	40	NI	40	NI	Krotlinger (2000c) [GLP, QA]
Rabbit (15 days)	2.5/0.5 (M/F)	10/2.5 (M/F)	2.5	10	10	NI	Mihail & Schilde (1980)
Inhalational exposure *(mg/m^3)*							
Rat (5 x 4 hours)	0.3/0.6 (M/F)	0.6/3.3 (M/F)	4/9 (M/F)	9/28 (M/F)	NM	NM	Thyssen (1979a)
Rat (15 days)	0.25	3.5	0.25	3.5	NM	NM	Thyssen (1979)

NM = not measured; NI = no inhibition; NE = not established; M = males; F = females; QA = quality assured study; GLP = study conducted according to principles of good laboratory practice

Unlike the majority of OPs, fenamiphos was not found to inhibit brain ChE activity (despite the occurrence of overt signs of toxicity) in rats, dogs and rabbits following acute, short-term repeat-dose or subchronic dosing via the oral, dermal or inhalational routes (Thyssen 1979a; Mihail & Schilde 1980; Hayes 1983; Hayes 1986a; Detzer 2002). However, chronic oral toxicity studies conducted in rats and dogs detected some brain ChE inhibition (Hayes 1986b; Rieth 1991; Jones & Greufe 1993; Van Goethem & Elcock 1997). Collectively these data suggest that fenamiphos or its metabolites have minimal ability to cross the BBB following subchronic or lower durations of exposure in laboratory animals. This conclusion is supported by a whole-body autoradiographic study in rats that detected no radioactivity in the brain following acute oral dosing (Weber 1988). In addition, an acute oral dosing study (which incidentally is the basis of the new ARfD for fenamiphos) found that fenamiphos was unable to inhibit brain ChE activity in dogs despite the fact that it could inhibit ChE activity in brain homogenates (Detzer 2002).

Metabolism

Fenamiphos is almost completely absorbed from the GIT in rats following oral dosing, with the majority excreted via the urine within the first 16 hours of dosing (Gronberg 1969; Ecker et al

1989). There was no evidence that fenamiphos or its metabolites accumulate in any tissues (Gronberg 1969; Weber 1988; Ecker et al 1989). The primary metabolites of fenamiphos were various phenols in different stages of oxidation at the sulphur atom, and their respective sulfate conjugates (Gronberg 1969; Ecker et al 1989). There are no unique metabolites generated in plants (or animals) that are of toxicological significance.

Differences in the results of rat metabolism studies largely reflect improvements in techniques rather than any significant discrepancies in the results. In an earlier metabolism study for example up to 50% of the administered radioactivity was excreted as CO_2, presumably due to the location of the label in a metabolically labile position (Gronberg 1969). In the study of Ecker et al (1989), with the phenyl ring labelled, very minimal radiolabel was excreted as CO_2.

Dermal absorption

There were no *in vitro* or *in vivo* percutaneous absorption studies submitted or available for evaluation. Therefore the actual level of dermal absorption of fenamiphos is not known.

Acute toxicity

Fenamiphos has high acute oral toxicity [LD_{50} 2 to 19 mg/kg bw (Kimmerle & Lorke 1967; Kimmerle & Soomecke; Crawford & Anderson 1973; Crawford & Anderson 1974 a & b; Lamb & Matzkanin 1975; Krotliner 1988 and 2000a)] and high acute inhalational toxicity in rats [LC_{50} 74 mg/m^3; 4-hour nose-only exposure to aerosols (Pauluhn 2001)]. The acute dermal toxicity in rats was low to high depending on the vehicle and sex [LD_{50} 72 to >2000 mg/kg bw (Crawford & Anderson 1972; Flucke 1980; Krotlinger 2000b)]. Fenamiphos has moderate acute oral toxicity in guinea pigs and high acute oral toxicity in mice, rabbits and dogs (Kimmerle & Lorke 1967; Kimmerle & Solmecke 1971). Fenamiphos was a slight to moderate eye irritant in rabbits (Crawford & Anderson 1971, Kato 1984a & b). Fenamiphos was not a skin sensitiser in guinea pigs (Watanabe 1983; Stropp 1995). Systemic toxicity occurred in rabbits following ocular administration (Kato 1984b).

Clinical signs following acute exposures were generally consistent with muscarinic and/or nicotinic effects (e.g. salivation, lacrimation, diarrhoea, apathy, palmospasms, laboured breathing, piloerection, muscle twitching/cramps, inactivity and rough coats) (Crawford & Anderson 1972; Lamb & Matzkanin 1975; Thyssen 1979a; Krotlinger 1988, 2000a & 2000b; Pauluhn 2001; Detzer 2002). Signs unique to inhalational exposure included exophthalmos, miosis, corneal opacity, chromodacryorrhea, red encrusted nostrils, pallor, emaciation and periorbicular red stains (Pauluhn 2001). The time to death and the onset/duration of clinical signs varied depending on the dose route (see table below). In a few studies, some of the clinical signs observed were more indicative of a central effect [e.g. tremors, dyspnoea and convulsions (Crawford & Anderson 1972; Lamb & Matzkanin 1975; Krotlinger 1988 & 2000a; Pauluhn 2001]. However, in light of data indicating the poor ability of fenamiphos (or its metabolites) to cross the BBB, it is possible that these signs were elicited by a mechanism not involving the inhibition of brain acetyl ChE activity.

Table 2 Time to death and onset of clinical signs following acute dosing

Dose route	Time to death	Clinical signs	
		Onset	Duration
Oral	Within 90 minutes	5-30 minutes	Several hours
Dermal	3-6 hours (males) 8 hours to 4 days (females)	1-4 hours	Unspecified
Inhalational	Within 4 hours	Within 3 days	Up to 9 days

In those studies where animals were necropsied, macroscopic abnormalities occurring in decedents but not survivors following oral dosing included distended or slightly collapsed lungs, reddened glandular stomach and discolouration of the liver (Krotlinger 1988 & 2000a). In some cases, ulcer-like foci in the stomach, a pale or patchy dark spleen, a reddened small intestine or dark patches on the lungs were also observed (Krotlinger 1988). Following dermal administration, dark red discolouration of the liver was noted in the single female decedent (Krotlinger 2000b). Following inhalational exposure, abnormalities occurring only in decedents were lungs appearing pale and/or having a few dark red foci, bloated stomachs (and intestines in some rats) and pale organs (particularly the spleen) were observed. Most also had a discharge from the muzzle. Corneal opacity (uni/bilateral) was noted in a smaller proportion of rats (Pauluhn 2001).

The acute oral toxicity in rats of a number of fenamiphos metabolites (fenamiphos sulfone, fenamiphos sulfoxide, desisopropyl fenamiphos sulfoxide and desisopropyl fenamiphos) was the same as the parent compound, including LD_{50} values, the time to death and occurrence of clinical signs (Crawford & Anderson 1974b; Thyssen 1974a & b; Lamb & Matzkanin 1975 & 1977).

The acute oral toxicities in rats of a number of impurities present in the technical material were less than fenamiphos (Crawford & Anderson 1973& 1974a; Thyssen 1974c, d & e; Lamb & Matzkanin 1975). For those compounds that caused mortalities (MTMC, MTMC-sulfone, MTMC-sulfoxide, 4-methylmercapto-m-cresol and 3-methyl-4-methylmercaptophenol), clinical signs included ataxia, decreased activity, poor general condition and respiratory disturbances, with only desmethyl fenamiphos causing clinical signs consistent with ChE inhibition (eg. tremors, salivation, lacrimation, diarrhoea and convulsions).

In rats, a 5% granular formulation of fenamiphos had moderate to high acute oral toxicity (LD_{50} of 47 and 68 mg/kg bw in male and female, respectively), low dermal toxicity (LD_{50} ~3600 mg/kg bw) and high inhalational toxicity (LC_{50} of 106 mg/m^3 following 4-hour head-only exposure to the dust). However, the acute inhalational toxicity of this formulation is not viewed with confidence because in the absence of a control group, it is unclear whether toxicity was due to fenamiphos or to the inhaled dust.

A 10% granular formulation had high acute oral toxicity in fasted rats (LD_{50} of 26 and 34 mg/kg bw in males and females, respectively) and moderate acute oral toxicity in non-fasted rats (LD_{50} ~ 75 mg/kg bw). It had low acute dermal toxicity (LD_{50} in rats >5000 mg/kg bw) and possibly high inhalational toxicity (>44 mg/m^3) in rats. This formulation was a non-skin irritant and slight eye irritant in rabbits.

In rats, an EC formulation containing 40% fenamiphos had high acute oral (LD_{50} of 10 mg/kg bw), dermal (LD_{50} of 83 mg/kg bw in males and 64 mg/kg bw in females) and inhalational (LC_{50} 132-198 mg/m^3) toxicity. This formulation was a severe skin and eye irritant in rabbits. A 0.7% aqueous dilution of this formulation was still highly acutely toxic via the oral and inhalational routes (LD_{50} of 13 mg/kg bw and an LC_{50} of 90 mg/m^3). The diluted formulation was a non skin and eye irritant in rabbits.

Repeat-dose and chronic toxicity

Dose-related inhibition of plasma and RBC ChE activities was the most common manifestation of fenamiphos toxicity in short-term, subchronic, chronic and reproduction studies in mice, rats, rabbits and dogs. At sufficiently high doses, reduced bodyweight, clinical signs and deaths

occurred. There was little indication that repeated exposure had any effect on haematology, clinical chemistry or urinary parameters, or on organ weights or gross pathology or histopathology.

Carcinogenicity and genotoxicity

Chronic feeding studies in mice, rats and dogs found no evidence that fenamiphos was carcinogenic (Hayes 1982; Löser 1972a; Cherry & Newman 1973; Hayes 1986b; Löser 1972b; Thomson et al 1972; Reith 1991; Jones & Greufe 1993; Van Goethem & Elcock 1997). *In vitro* genotoxicity assays indicated that fenamiphos was not mutagenic (Herbold 1979, 1985a & b; Yang 1985). Some *in vitro* assays indicated that fenamiphos was damaging to genetic material at cytotoxic concentrations (Herbold 1987 & 1988), while other assay did not (Chen et al 1982a & b; (Curren 1988). There was no evidence of genotoxicity *in vivo* (Herbold 1980a & b). Collectively these data indicated that fenamiphos is not genotoxic. A 10% granular formulation of fenamiphos was not mutagenic to bacteria (Herbold 1992)

Reproductive and Developmental Toxicity

Multi-generation studies conducted in rats revealed no evidence that fenamiphos caused reproductive toxicity (Löser 1972c; Cherry et al 1972; Eigenberg 1991 & 1997). There was no evidence that fenamiphos was teratogenic in rats or rabbits (Schluter 1981; Renhof 1986; Clemens et a 1989; Lamb 1982; Becker 1986). Chain fusion of the sternebrae occurred in one of two rabbit studies but only at maternotoxic doses (Lamb 1982)

Neurotoxicity

Fenamiphos is neurotoxic in chickens and rats by virtue of its ability to inhibit acetyl ChE activity but there was no evidence that it caused delayed neuropathy in either species (Kimmerle 1970; Spicer 1970; Kimmerle 1971; Flucke & Kaliner 1987; Flucke 1988; Driest 1995; Driest & Popp 1996).

DOSE LEVELS RELEVANT FOR RISK ASSESSMENT

Dose levels relevant for dietary risk assessment

To identify the lowest NOELs for the establishment of an ADI and ARfD, a summary of the NOELs determined in those oral dosing studies considered suitable for regulatory purposes are shown in the following Tables.

Table 3 Studies relevant for the establishment of an ADI

Species	NOEL (mg/kg bw/day)	LOEL (mg/kg bw/day)	Toxicological Endpoint	Reference
Subchronic Studies				
Rats (12-week dietary)	0.4	0.8	Plasma ChE inhibition	Löser (1968); Mawdesley-Thomas & Urwin (1970a)
Rats (14-week dietary)	0.072	-	Plasma ChE inhibition	Hayes (1986a) [GLP; QA]
Dogs (100-day dietary)	0.025	0.042	Plasma ChE inhibition	Hayes (1983) [GLP; QA]
Dogs (supplementary 6-month dietary)	0.0108	-	Plasma & RBC ChE inhibition (males)	Jones & Loney (1993) [GLP; QA]
Chronic studies				
Mice (20-month dietary)	1.4	7.4	Reduced bw (males)	Hayes (1982) [QA]
Rats (2-year dietary)	0.56		Plasma ChE inhibition (females)	Löser (1972a); Cherry & Newman (1973)
Rats (2-year dietary)	-	0.098	Plasma ChE inhibition (males)	Hayes (1986b) [GLP; QA]
Dogs (2-year dietary)	0.014	0.036	Plasma ChE inhibition (females)	Löser (1972b); Thomson et al (1972)
Dogs (1-year dietary)	-	0.03	Plasma ChE inhibition	Reith (1991); Jones & Greufe (1993); Van Goethem & Elcock (1997) [GLP; QA]
Reproduction studies				
Rat (3-gen dietary)	3	-	Parental, pup & reproductive toxicity	Löser (1972c); Cherry et al (1972)
Rats (2-gen dietary)	~3	-	Reproductive toxicity	Eigenberg (1991 & 1997) [GLP; QA]
	-	0.17	Maternotoxicity; plasma ChE inhibition	
	0.17	0.65	Paternal toxicity; plasma ChE inhibition	
	0.17	0.65	Pup toxicity; plasma ChE inhibition	
Neurotoxicity studies				
Rats (14-week dietary)	0.06	0.61	Plasma and RBC ChE inhibition	Dreist & Popp (1996) [GLP; QA]

QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice

Table 4 Studies relevant for the establishment of an ARfD

Species	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Acute studies				
Dogs (po gavage)	0.25	0.5	RBC ChE inhibition	Detzer (2002) [GLP, QA]
Developmental studies				
Rats (po gavage)	1	3	Maternal rats: Deaths & clinical signs	Schluter (1981); Renhof (1986)
	3	-	Development/foetal toxicity	
Rats (po gavage)	0.25	0.85	Maternal rats: Plasma and RBC ChE inhibition	Clemens et al (1989) [GLP; QA]
	3	-	Development/foetal toxicity	
Rabbits (po gavage)	0.3	1.0	Dams: Reduced bw gain	Lamb (1982) [GLP; QA]
	0.3	1.0	Developmental toxicity: chain fusion of the sternebrae	
Rabbits (po gavage)	0.5	2.5	Dams: Deaths, cholinergic signs & reduced bw gain & food consumption	Becker (1986) [GLP, QA]
	2.5	-	Development/foetal toxicity	
Neurotoxicity studies				
Rats	-	0.4	RBC ChE inhibition	Dreist (1995) [GLP; QA]

QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice

Dose levels relevant for occupational health and safety risk assessment

The most sensitive toxicological endpoint for fenamiphos in laboratory animals following repeated dosing was the inhibition of plasma ChE activity; it is therefore the most appropriate toxicological endpoint for occupational health and safety (OHS) risk assessment purposes. In terms of the duration of exposure, the inhibition of plasma ChE activity is maximal within days to weeks of dosing. Therefore repeat-dose studies ranging from several weeks to months are appropriate for establishing NOELs for OHS risk assessment purposes. The following table summarises the NOELs/LOELs in laboratory animal studies deemed suitable for OHS risk assessment purposes (noting that there were no human studies available in the database).

Table 5 Studies relevant for OHS risk assessment purposes

Study	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Rat 4-week dermal	10	40	Plasma ChE inhibition	Krotlinger (2000c) [GLP, QA]
Rabbit 15-day dermal	0.5/2.5 (F/M)	2.5/10 (F/M)	Plasma ChE inhibition	Mihail & Schilde (1980)
Rat 3-week inhalation	0.25 mg/m^3	3.5 mg/m^3	Plasma ChE inhibition	Thyssen (1979b)
Rat 90-day dietary	0.084	No inhibition	Plasma, RBC & brain ChE inhibition	Hayes (1986a) [GLP, QA]
Dog 100-day dietary	0.025	0.045	Plasma ChE inhibition	Hayes (1983) [GLP, QA]
Dog 6-month dietary	0.011	No inhibition	Plasma & RBC ChE inhibition	Jones & Loney (1993) [GLP, QA]

QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice; F = females; M = males

As fenamiphos is a direct acting OP and does not require activation to inhibit acetyl ChE activity there is unlikely to be any intraspecies differences in dermal toxicity due to differences in metabolism. The lowest dermal NOEL for plasma ChE inhibition is 0.5 mg/kg bw/day in a 15-day rabbit study (Mihail & Schilde 1980). However, based on the uncertainty regarding the dosing regimen, this study is not considered to be reliable for dermal OHS risk assessment purposes. Therefore, the NOEL of 10 mg/kg bw/day for plasma ChE inhibition in the 4-week rat study of Krotlinger (2000c) is the most appropriate comparator for dermal risk assessment purposes. The NOEC of 0.25 mg/m^3 for plasma ChE inhibition in a 3-week inhalation study in rats (exposure for 5 days/week) is the most appropriate comparator for inhalational risk assessment purposes (Thyssen 1979).

EXPOSURE ASSESSMENT

Residues in Food and Drinking Water

In Australia, fenamiphos is registered for use as a nematicide and for the control of sucking insects on a number of food crops grown in the home garden or via commercial agriculture. Fenamiphos is applied to the soil prior to seeding or planting or in some cases may be applied directly to the plants (e.g. strawberries and pineapples). Following soil application, fenamiphos is incorporated into the soil via raking or some other tillage method and then irrigated to disperse fenamiphos through the soil and to remove any material from plant foliage.

Maximum Residue Levels (MRLs) for fenamiphos have been established for aloe vera, banana, brassica [(cole or cabbage) vegetables, head cabbages, flowerhead brassicas], celery, citrus fruits, edible offal (mammalian), eggs, fruiting vegetables (cucurbits), ginger root, grapes, leafy vegetables (except lettuce head and leaf), lettuce head, lettuce leaf, meat (mammalian), milks, mushrooms, onion bulb, peanut, pineapple, poultry (edible offal of), poultry meat, root and tuber vegetables, strawberry, sugar cane and tomato.

Chronic Dietary Intake

Both the 19th and 20th Australian Total Diet Surveys (ATDS) (2002 and 2003, respectively) performed under the auspices of Food Standards Australia New Zealand (FSANZ) detected no fenamiphos in any of the foods surveyed. Therefore, the dietary exposure for the population (including infants, children and adults) was estimated by FSANZ to be zero as the concentration of fenamiphos was less than the limit of detection.

Residues in Drinking Water

Based on its current pattern of use, exposure of the general population to fenamiphos residues in drinking water is considered negligible.

Home-garden use

At the time of this assessment, there was one fenamiphos product registered for home-garden use in Australia. This product contained 50 g/kg fenamiphos as the active constituent and was used to control root-knot nematode and sugar-beet nematode in tomatoes, crucifers or ornamentals in the home garden. Granules are sprinkled over soil prior to seeding, planting or transplanting and again each spring and autumn. The general application rate was 25 g/m^3, with the product applied at 50 g/m^3 for heavy infestations. Granules are then lightly raked into the soil and the treated area watered.

It was likely that a home gardener applying the product would be exposed to fenamiphos via the inhalational and dermal routes through contact with the dust or the actual granules. There was also some potential for exposure via soil at some time after treatment. Factors such as the area of soil treated, the application rate and re-entry interval into the treated garden bed would all impact on the level of exposure. However, there are no data available to quantify the level of such exposures.

Re-entry exposure of the general public

The general public may be exposed to fenamiphos following re-entry onto treated turf areas (e.g. golf courses and bowling greens). At the time of this review, there were two liquid products registered for professional use as turf nematicides that contained 435 g/L fenamiphos as the active constituent; Nemacur Turf Nematicide Liquid (APVMA Product No. 33296) and Chipco Nemacur Turf Nematicide Liquid (APVMA Product No. 57185). Both products are applied at a rate of 110 mL/100 m^2 or 1.5 L/bowling green (i.e. 44 g fenamiphos/m^2).

The level of exposure to fenamiphos would depend on the time after application that re-entry occurred, and the types and duration of activities undertaken on the turf. Other factors such as the properties of the turf and underlying soil could also be important. The most likely exposure route is dermal due to direct contact with turf or through using equipment coming into contact with the turf (e.g. bowls or golf clubs). As a consequence of dermal exposure there is also the potential for hand-to-mouth transfer. In addition, volatilisation could lead to inhalational exposure.

There are only limited data on the levels of fenamiphos that the general public could be exposed to following re-entry onto treated turf. Snyder and Cisar (1995) examined the amount of fenamiphos in clippings and percolate following application of a 10% granular formulation to golf greens at a rate of 1.123 g/m^2. Total recovery in turf clippings 10 days after application was 0.38% for fenamiphos and 0.14% for fenamiphos metabolites (fenamiphos sulfone and sulfoxide) of the applied amount. Relatively low levels were also measured in percolate (0.06 and 0.04% for fenamiphos at days 0 and 10, respectively; 17.7 and 1.10% for fenamiphos metabolites at days 0 and 10, respectively). The majority of fenamiphos was retained in the thatch layer.

Snyder & Cisar (2002) summarised a series of studies they had conducted to examine the dislodgeability of residues following the spray application of fenamiphos to turf. Fenamiphos was dislodged using a number of different techniques (a damp cheese cloth rubbed on the surface of the turf; a damp cotton cloth or damp leather pressed on the surface; putting a golf ball over the surface; rolling golf grips on the surface and by swinging a golf club through grass and wiping the club surface with a damp cheese cloth). The following table summarises the dose of fenamiphos received by a golfer under a number of scenarios. The data indicates that exposure is mainly due to dislodgeable residues rather than the inhalation of volatilised fenamiphos.

Table 6 Dose of fenamiphos received by a golfer (μg/kg bw/day)

Scenario	Dislodged dose	Volatilised dose	Total dose
Golfer plays on 18 greens within 1 hour of application	38.00	0.45	38.45
Golfer plays on 1 greens within 1 hour of application and on the remaining 17 greens after application and irrigation every day	4.26	0.03	17.16
Golfer plays on 18 greens after application and irrigation every day	2.27	0.003	2.27
Golfer plays on 18 greens the day after application and irrigation every day	0.21	0.001	0.21

HUMAN RISK ASSESSMENT

Dietary risk assessment

The dietary risk assessment for fenamiphos will performed by the APVMA and FSANZ.

Home-garden risk assessment

As mentioned above, there was one fenamiphos granular formulation currently registered for use in the home-garden but there are no data available to quantify the level of exposure from its recommended use. As part of the current review, studies were submitted on a granular formulation ostensibly the same as this product. Nemacur 5 GR, had an oral LD_{50} in rats of 47 mg/kg bw in males and 68 mg/kg bw in males (Mihail & Thyssen 1980a). Mortalities occurred at and above 40 mg/kg bw in both sexes, with clinical signs occurring in *all* rats (15/group) at and above 1 mg/kg bw. The dermal LD_{50} in rats following 24 hours of exposure was approximately 3600 mg/kg bw. The LC_{50} in rats following 1 or 4 hours head-only exposures to the dust was >125 and approximately 106 mg/m^3, respectively. However, these LC_{50} values are not viewed with confidence given that they may have been due to the inhaled dust rather than fenamiphos *per se*.

The National Drugs and Poisons Schedule Committee (NDPSC) has previously considered the home-garden use of such a granular formulation of fenamiphos noting that it had similar acute oral, dermal and inhalational toxicity to the technical grade material. The Committee noted that in one unspecified Australian state, a 5-year old child (sex unspecified) had died following ingestion of the home garden granules. It was also noted that 0.5 g (of the granules) would be lethal for a 10 kg child, while a teaspoonful would be lethal for a 70 kg adult.

The APVMA's Guidelines for Pesticides Used by Householders (Ag Requirements Series, Part 3, Toxicology, Appendix 3-1) indicate that pesticides for household, home garden or domestic use should be relatively harmless or capable of causing only mild illness if poisoning occurs. As a guide, domestic pesticide formulations should not be expected to be acutely orally toxic up to doses of 1500 mg/kg bw in children or acutely dermally toxic up to 100 mg/kg bw. The eye and skin irritancy of the formulation should be low and they should not cause irreversible toxicity on repeated exposure. Further, they should not require the use of safety equipment that is not readily available to the householder.

Based on an assessment of the toxicity of Nemacur 5 GR, the ongoing home-garden use of the fenamiphos home-garden product is inappropriate because of its moderate to high acute oral toxicity in rats (LD_{50} 47-68 mg/kg bw) and the small amount of granules that would be lethal to a child if accidentally ingested. Therefore, this product should be removed from the home garden market because it does do not comply with criteria established by the APVMA for home garden products. This product would need to be diluted approximately thirty-fold[1] to fulfil the APVMA criteria for home-garden products.

Re-entry risk assessment

The risk assessment for re-entry of the general public onto treated turf areas has been reported in the occupation health and safety assessment component report for fenamiphos, prepared by the OCS.

[1] LD_{50} of 1500 ÷ LD_{50} of 47 mg/kg bw

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There is no objection on toxicological grounds to the ongoing approval of fenamiphos active constituent.

Impurity Limits

An integral part of the safety assessment of an active constituent is a consideration of the chemical composition of the material. Technical-grade active constituents will contain measurable levels of impurities, which can arise during manufacture and/or from subsequent degradation during storage. The chemical identity of these impurities is generally well characterised. The impurities present in the technical-grade material are usually of no particular concern since health standards are established on the basis of toxicology studies conducted using the mixture. However, for those which have high acute toxicity, genotoxicity or teratogenic potential, concentration limits need to be set, so that the toxicological profile of the technical-grade active constituent does not appreciably alter in the event of slight changes in the proportions of the impurities.

The active constituent fenamiphos contains no impurities of toxicological concern.

Acceptable Daily Intake (ADI)

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without appreciable risk to health. It is calculated by dividing the overall NOEL for the most sensitive toxicological endpoint from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans, intraspecies variation, the completeness of the toxicological database and the nature of the potential toxicologically-significant effects.

The current Australian ADI for fenamiphos of 0.0001 mg/kg and is based on the NOEL of 0.014 mg/kg bw/day for the inhibition of plasma ChE activity in a 2-year dog study and using a 100-fold safety factor (Loser 1972b; Thomson et al 1972). This value is supported by the NOEL of 0.011 mg/kg bw/day for plasma ChE inhibition in a 6-month supplementary dog study (Jones & Loney 1993), which followed a one-year dog study where the LOEL for plasma ChE inhibition was 0.03 mg/kg bw/day (Reith 1991; Jones & Greufe 1993; Van Goethem & Elcock 1997). Following a review of all submitted and archived data, a more suitable study was not identified. Therefore, the existing ADI for fenamiphos remains appropriate.

Acute Reference Dose (ARfD)

The ARfD is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram body weight basis, that can be ingested over a short period of time, usually one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation. There is currently no Australian ARfD for fenamiphos.

There were a number of acute or short-term oral dosing studies conducted in laboratory animals that were identified as relevant for the establishment of an ARfD. The acute oral dog study of Detzer (2002) established a NOEL of 0.25 mg/kg bw for RBC ChE inhibition (with a LOEL of 0.5 mg/kg bw). This NOEL is supported by the same value in a developmental study for the same endpoint in dams and a LOEL of 0.85 mg/kg bw (Clemens et al 1989). The LOEL for RBC ChE inhibition in a rat acute neurotoxicity study was 0.4 mg/kg bw (Dreist 1995).

Therefore the most suitable NOEL for the establishment of an ARfD was 0.25 mg/kg bw, based on RBC ChE inhibition at 0.5 mg/kg bw in the acute dog study by Detzer (2002). Application of a 100-fold safety factor to this pivotal NOEL (10-fold intra and 10-fold interspecies safety factors) yields an ARfD of 0.003 mg/kg bw. This ARfD is supported by the NOEL of 0.25 mg/kg bw in a rat developmental study, based on maternal RBC ChE inhibition at 0.85 mg/kg bw (Clemens et al 1989).

Water Quality Guidelines

The Australian Drinking Water Guidelines (ADWG) are a joint publication of the National Health and Medical Research Council (NHMRC) and the Agricultural and Resource Management Council of Australia and New Zealand. The ADGW are not legally enforceable but rather provide a standard for water authorities and State health authorities to ensure the quality and safety of Australia's drinking water.
The *guideline value* (mg/L) is analogous to an MRL in food and is generally based on the analytical limit of determination. If a pesticide is detected at or above this value then the source should be identified and action taken to prevent further contamination. The *health value* (also expressed as mg/L) is intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The health values are derived so as to limit intake *from water alone* to approximately 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg having a daily water consumption of 2 L over a lifetime.

Given that the ADI for fenamiphos is 0.0001 mg/kg bw/day, the Health Value may be calculated as:

$$\frac{0.0001 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.1}{2 \text{ L/day}}$$

$$= 0.00035 \text{ mg/L}$$

Hence, the current Health Value for fenamiphos of 0.0003 mg/L is supported.

Poisons Scheduling

Fenamiphos is in Schedule 7 of the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) **except** when included in Schedule 6. There is a cut-off into Schedule 6 for granular preparations containing 5 per cent or less of fenamiphos.

The oral LD_{50} for fenamiphos in female rats ranged from 3 to 19 mg/kg bw, while in males it ranged from 2 to 15 mg/kg bw. Fenamiphos has moderate acute oral toxicity in guinea pigs (LD_{50} of 75-100 mg/kg bw) and high acute oral toxicity in mice, rabbits and dogs (LD_{50} ~5-20 mg/kg bw). Dermal LD_{50} values in rats ranged from 72 to >2000 mg/kg bw depending on the vehicle, while the dermal LD50 in rabbits was 225 mg/kg bw in males and 179 mg/kg bw in females. The LC_{50} in rats

following one or four hours of head-only exposure to fenamiphos aerosols was 130 and 100 mg/m^3, respectively. Four hours of nose-only exposure to fenamiphos aerosols resulted in a LC_{50} of 74 mg/m^3. In rabbits, fenamiphos was a non-skin irritant or a slight skin irritant. When administered as a crystalline solid, fenamiphos was a slight eye irritant in rabbits. When administered as an undiluted liquid, fenamiphos was a moderate eye irritant and caused systemic toxicity in rabbits whose eye was unwashed. Fenamiphos was not a skin sensitiser in guinea pigs. Based on the high acute oral and inhalational toxicities of fenamiphos in rats (LD_{50} <50 mg/kg bw and LC_{50} <500 mg/m^3, respectively), the existing Schedule 7 entry remains appropriate.

A granular formulation containing 5% fenamiphos had an oral LD_{50} of 47 and 68 mg/kg bw in male and female rats, respectively. The dermal LD_{50} in rats following 24 hours of exposure was approximately 3600 mg/kg bw. The LC_{50} in rats following 1 or 4 hours of head-only exposures to the dust was >125 and approximately 106 mg/m^3, respectively. However, these LC_{50} values are not viewed with confidence given that they may have been due to the inhaled dust rather than fenamiphos *per se*. Based on the moderate acute oral toxicity of this granular formulation of fenamiphos and its low dermal toxicity, the existing cut-off into schedule 6 for granular preparations containing 5 per cent or less of fenamiphos remains appropriate.

First-Aid Instructions

At the time of this assessment, the first aid instructions for fenamiphos existing in the First Aid Instruction and Safety Directions (FAISDs) Handbook, as of June 2006, were as follows:

Code	***First Aid Instruction***
a	If poisoning occurs, contact a doctor or Poisons Information Centre. *Phone Australia* 131126
h	If swallowed, give one atropine tablet every 5 minutes until dryness of the mouth occurs - if poisoned by skin absorption or through lungs, remove any contaminated clothing, wash skin thoroughly and give atropine tablets as above. Get to a doctor or hospital quickly

First Aid Instruction 'h' (and 'x'), which related to the treatment of OP poisoning with atropine following oral, dermal and inhalational exposures have now been deleted from the FAISD handbook and replaced with the following instruction:

Code	***First Aid Instruction***
m	If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131 126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

Therefore, First Aid Instruction 'h' should be removed from all commercial fenamiphos product labels and replaced with statement 'm'.

Warning Statements and General Safety Precautions

There are no Warning Statements or General Safety Precautions for fenamiphos.

Safety Directions

At the time of this assessment, the safety directions for Australian products containing fenamiphos are shown in the table below.

Table 7 Existing Safety Directions for fenamiphos

EC 400 g/L or less

Codes	*Safety Directions*
100 101	Very dangerous. Particularly the concentrate
130 131 132 133	Poisonous if absorbed by skin contact or inhaled or swallowed
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 223	Do not inhale spray mist
279 280 281 282 290 292 294 301 303	When opening the container, preparing spray and using the prepared spray wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves and full facepiece respirator with combined dust and gas cartridge or canister
340 342	If product on skin, immediately wash area with soap and water
350	After use and before eating drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each day's use, wash gloves and respirator and if rubber wash with detergent and warm water and contaminated clothing

GR 50 g/kg or less

Codes	*Safety Directions*
100	Very dangerous
120 130 131 132 133	Product is poisonous if absorbed by skin contact or inhaled or swallowed
210 211	Avoid contact with eyes and skin
220 221	Do not inhale dust
351	Wash hands after use

HG GR

100	Very dangerous
120 130 131 132 133	Product is poisonous if absorbed by skin contact or inhaled or swallowed
210 211	Avoid contact with eyes and skin
220 221	Do not inhale dust
351	Wash hands after use

GR 100g/kg or less and more than 50 g/kg

Codes	*Safety Directions*
100	Very dangerous
120 130 131 132 133	Product is poisonous if absorbed by skin contact or inhaled or swallowed
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 221	Do not inhale dust
373	Obtain an emergency supply of atropine tablets 0.6 mg
279 280 283 290 292 294 300 302	When opening the container and using the product wear cotton overalls buttoned to the neck and wrist and a washable hat and elbow-length PVC gloves and half face respirator with dust cartridge or canister
340 342	If product on skin, immediately wash area with soap and water
350	After use and before eating drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 364 366	After each day's use, wash respirator and if rubber wash with detergent and warm water and contaminated clothing

EC = emulsifiable concentrate; GR = granular; HG = home-garden

At the time of this review, there were 7 registered fenamiphos products but only four of these were the subject of the current review because the data call-in preceded their registration. Two products are granular formulations and contain 50 or 100 g/kg fenamiphos as the active constituent; the lower strength formulation is a home-garden product. The remaining five products are emulsifiable concentrates and contain 435 g/L fenamiphos.

Granular formulations (GR)

There are two granular fenamiphos products that contain either 50 or 100 g/kg fenamiphos as the active constituent.

The lower strength formulation, Bayer Nemacur Granular Nematicide (APVMA Product No. 33291), is used to control root-knot nematode and sugar-beet nematode in tomatoes, crucifers or ornamentals in the home garden. As mentioned above, given the moderate to high acute oral toxicity of this product and that only a small amount of granules would be lethal to a child if ingested, this product is inappropriate for home garden use based on criteria established by the APVMA. Therefore its registration as a home garden product can no longer be supported. On this basis, the safety directions for HG GR should be deleted from the FAISD handbook.

The second granular formulation, Nemacur® 100G (APVMA Product No. 33293) contains 100 g/kg fenamiphos as the active constituent and is used to control nematodes and sucking insects on various food and non-food crops. In most cases the product is applied to the soil and then mechanically incorporated in to the soil followed by watering. For some crops (strawberries and pineapples) the product is applied to the plant. Granules are applied by hand or machine at rates of 1.2-2.5 kg/100 row or 10-25 g/stool (bananas), 40-200 kg/ha (carrots, parsnips, crucifers, duboisia, ginger, bulbs, ornamentals, potatoes, tomatoes, sugar cane), 1 kg/100 m (pineapples) or 1 kg/1000 m (strawberries). Withholding periods of 6 (strawberries) and 12 weeks (carrots, parsnips and potatoes) are specified on the product label.

On the basis of acute toxicity studies on Nemacur GR 10 (a product ostensibly the same) Nemacur® 100G is considered to have high oral toxicity (LD_{50} of 26 and 34 mg/kg bw in fasted male and females, respectively), low dermal toxicity (LD_{50} >2000 mg/kg bw) and high inhalational toxicity in rats (LC_{50} of the dust of >44 mg/m^3 for a 4 hour exposure). This formulation was a non-skin irritant and slight eye irritant in rabbits. It is unlikely to be a skin sensitiser. As all fenamiphos products are in Schedule 7 of the SUSDP (with the exception of a HG product), the statement "very dangerous" duplicates the signal heading already contained on the product label. On this basis, the following amended hazard-based safety directions for all granular formulations are appropriate:

Table 8 Amendment to existing entry

GR ~~100~~ 120 g/kg or less ~~and more than 50 g/kg~~

Codes	*Safety Directions*
~~100~~	~~Very dangerous~~
120 130 131 132 133	Product is poisonous if absorbed by skin contact or inhaled or swallowed
161 162	**Will irritate the eyes**
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 221	Do not inhale dust
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
350	After use and before eating drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: With the exception of products intended for home garden use, any changes that are necessary to personal protective equipment will be covered in the OHS review of fenamiphos.

In the absence of any GR products containing 50 g/kg or less of fenamiphos (except for the single HG product) and given that the above amended entry for GR 120 g/kg or less would cover any lower strength GR products, the entry for GR 50 g/kg or less should be deleted from the FAISD handbook.

Emulsifiable concentrates (EC)

There are five products that are emulsifiable concentrates and contain 435 g/L fenamiphos as the active constituent. However, only two of these products are the subject of the current review, namely Nemacur® 400 Nematicide Liquid (APVMA Product No. 33295) and Nemacur Turf Nematicide Liquid (APVMA Product No. 33296).

Nemacur® 400 Nematicide Liquid is used to control nematodes and sucking insects in various food and non-food crops cultivated by commercial agriculture. The product is generally applied undiluted to soil prior to planting and then as a soil maintenance treatment up to three additional times over a year. It can also be diluted in water and used as a bulb or planting material treatment or to treat entire plants (e.g. Chrysanthemums and pineapples) or to treat mushroom compost. Application rates vary considerably depending on the crop and situation. When used as a soil treatment, the product is applied by boom spray or by trickle irrigation. Following spray application, the product is mechanically incorporated into soil and the area irrigated.

Nemacur Turf Nematicide Liquid is used to control nematodes in turf and is applied at a rate of 110 mL/100 m^2 or 1.5 L/bowling green. The product is applied as a spray to wet turf or damp soil each spring and then 5 weeks later. Following application, turf is immediately irrigated with a minimum of 15 mm water and avoiding the formation of pools and puddles.

Both products have identical formulations and are very similar to an EC formulation tested in a number of acute toxicity studies. Studies conducted on Nemacur 400 EC containing 40% fenamiphos as the active constituent indicated that it had an oral LD_{50} of 10 mg/kg bw in rats. The dermal LD_{50} in rats was 83 mg/kg bw in males and 64 mg/kg bw in females. The LC_{50} in rats following 1 or 4 hours head-only exposures was 330-440 mg/m^3 (females) and 132-198 mg/m^3, respectively. Exposure to product vapours for 7 hours caused CNS disturbances at approximately 15 minutes after exposure and lasted for a day. Mucosae of the eyes and nose were reportedly irritated but only during exposure, which was attributable to the hydrocarbon content of this product

(~30%). This formulation was a severe skin and eye irritant in rabbits. A 0.7% aqueous dilution of this formulation had an oral LD_{50} in rats of 13.2 mg/kg bw and an LC_{50} of 90 mg/m^3 (4 h, head-only exposure). It was a non-skin and eye irritant in rabbits.

On the basis of these data on a similar product, Nemacur® 400 Nematicide Liquid and Nemacur Turf Nematicide Liquid are likely to have high acute oral, dermal and inhalational toxicities. Product vapours (due to the hydrocarbon content) are likely to irritate mucosae. The products are likely to be severe skin and eye irritants and unlikely to be a skin sensitiser. On this basis, the following amended hazard-based safety directions are appropriate:

Amendment to existing entry

EC ~~400~~ 450 g/L or less

Codes	*Safety Directions*
~~100 101~~	~~Very dangerous. Particularly the concentrate~~
130 131 132 133	Poisonous if absorbed by skin contact or inhaled or swallowed
190	Repeated minor exposure may have a cumulative poisoning effect
207 211	**Will damage eyes and skin**
161 163	**Will irritate nose and throat**
210 211	Avoid contact with eyes and skin
220 **222** 223	Do not inhale **vapour or** spray mist
330 331 332	**If clothing becomes contaminated with product or wet with spray remove clothing immediately**
340 342	If product on skin, immediately wash area with soap and water
340 343	**If product in eyes, wash it out immediately with water**
350	After use and before eating drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: With the exception of products intended for home garden use, any changes that are necessary to personal protective equipment will be covered in the OHS review of fenamiphos.

RECOMMENDATIONS

1. Approval Status

No change is recommended to the approval status of fenamiphos.

2. Acceptable Daily Intake

The present review reaffirmed the current ADI for fenamiphos of 0.0001 mg/kg, based on the NOEL of 0.014 mg/kg bw/day for the inhibition of plasma ChE activity in a 2-year dog study and using a 100-fold safety factor

3. Acute Reference Dose

The present review identified a suitable acute oral dosing study in dogs to allow the establishment of an ARfD for fenamiphos. The new ARfD of 0.003 mg/kg bw was calculated by applying a 100-fold safety factor to the NOEL of 0.25 mg/kg bw for RBC ChE inhibition.

4. Water Quality Guidelines

The existing NHMRC Health Value for fenamiphos in drinking water of 0.0003 mg/L is supported.

5. Poisons Scheduling

The existing poisons Schedule for fenamiphos remains appropriate.

6. Product Registration

Registration of the 50 g/kg granular formulation for use in the home garden is no longer supported on the basis that does not comply with criteria established by the APVMA for home garden products.

There is no objection on public health grounds to the continued registration of all other existing fenamiphos products.

7. First Aid and Safety Directions

First Aid Instruction ‘h’ should be removed from all commercial fenamiphos product labels and replaced with statement ‘m’ [If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131 126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed]

The following recommendations on hazard based Safety Directions in conjunction with Safety Directions related to PPE have been included in the FAISD Handbook, and should be included on the product label.

Deleted entries

Given that the home garden use of the 50 g/kg fenamiphos product is no longer supported, the safety directions for HG GR should be deleted from the FAISD handbook.

As changes have been recommended to the existing safety directions for EC 400 g/L or less and GR 100 g/kg or less and more than 50 g/kg, these entries should be deleted from the FAISD handbook. Given that the new entry for GR 120 g/kg or less would cover any lower strength products, the entry for GR 50 g/kg or less should also be deleted from the FAISD handbook.

New Entry

FORMULATION	*SAFETY DIRECTIONS*
GR 120 g/kg or less	120 131 132 133 161 162 190 210 211 220 221 340 342 340 343 350

The above hazard statement codes translate into the following safety directions:

Product is poisonous if absorbed by skin contact or inhaled or swallowed. Will irritate the eyes. Repeated minor exposure may have a cumulative poisoning effect. Avoid contact with the eyes and skin. Do not inhale dust. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water. After use and before eating drinking or smoking, wash hands, arms and face with soap and water.

New Entry

FORMULATION	*SAFETY DIRECTIONS*
EC 450 g/kg or less	130 131 132 133 160 190 207 211 161 163 210 211 220 222 223 330 331 332 340 342 340 343 350

The above hazard statement codes translate into the following safety directions:

Product is poisonous if absorbed by skin contact or inhaled or swallowed. Repeated minor exposure may have a cumulative poisoning effect. Will damage eyes and skin. Will irritate nose and throat. Avoid contact with the eyes and skin. Do not inhale vapour or spray mist. If clothing becomes contaminated with product or wet with spray remove clothing immediately. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water. After use and before eating drinking or smoking, wash hands, arms and face with soap and water.

8. Additional Data

No additional toxicological data is required.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Fenamiphos is the ISO approved common name for (R,S)-ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate (IUPAC nomenclature). The technical active is a racemic mixture. It is an organophosphorus insecticide and nematicide used for the control of nematodes and sucking insects (e.g. aphids and thrips) on food and non-food crops, and for the control of nematodes in turf.

The primary mode of action of fenamiphos is via the inhibition of the enzyme acetyl ChE, which results in over-stimulation of those parts of the nervous system that use acetylcholine to transmit nerve impulses. Signs of fenamiphos intoxication are consistent with acetyl ChE inhibition and include salivation, lachrymation, vomiting, diarrhoea and laboured breathing. If intoxication is severe, muscle twitching, loss of reflexes, convulsions and death can eventuate.

In 1995, the OCS ranked fenamiphos as a high priority candidate for review under the APVMA's Chemical Review Program. The rationale for this prioritisation was a high potential acute risk (Schedule 7 chemical), a high potential chronic risk and human poisonings in the USA. In addition, reviews performed by the JMPR in 1997 and 2002, and the US EPA in 1999, indicated that there were a number of studies that had not previously been evaluated by the OCS. A review would determine the appropriateness of the existing ADI and drinking water standard, and permit the establishment of an ARfD (for the first time). In addition, a review would provide an opportunity to assess the existing poisons schedule and examine the adequacy of the FAISDs for all registered fenamiphos products.

1.1 History of Public Health Considerations of Fenamiphos in Australia

The NDPSC and the Pesticide and Agricultural Chemicals Committee (PACC) have independently considered fenamiphos on a number of occasions from 1971 to 1991. Most of the PACC discussions related to MRL issues.

The NDPSC first considered fenamiphos in February 1971, where ethyl 4-(methylthio)-m-tolyl-isopropyl-phosphoramidate was placed in Schedule 7 of the SUSMP. This entry was amended to "fenamiphos" in November 1974, while in November 1975, a new Schedule 6 entry was included for granular preparations containing 5% or less fenamiphos.

The PACC examined an application from a registrant in May 1981 for the use of fenamiphos on sugar cane and peanuts. The Committee recommended MRLs in sugarcane, peanuts and meat at 0.05 mg/kg, and MRLs in milk at 0.005 mg/kg. In June 1988, the PACC was provided with an update on the toxicology and metabolism of the active constituent. The committee noted that the only significant toxicology was ChE inhibition and they endorsed the proposed ADI of 0.0001 mg/kg bw/day based on the application of a 100-fold safety factor to the NOEL of 0.0125 mg/kg bw/day in a 2-year dog study. A new rabbit developmental study was apparently available and it was recommended that it be requested from the company. MRLs were also set for eggs (poultry) and poultry meat at 0.05 mg/kg.

In August 1988, the NDPSC considered a review of toxicological data provided by the registrant to support technical grade active constituent clearance of fenamiphos. The Committee noted that fenamiphos is highly acutely toxic and that inhibition of plasma ChE activity, with consequent cholinergic signs, was typical of repeat-dose studies in laboratory animals. There was no evidence

that fenamiphos was carcinogenic, mutagenic, teratogenic or was reproductively toxic. The Schedule 6 and 7 entries were confirmed, however, the Committee was concerned that such an acutely toxic chemical was available in granular form for domestic use. The granular formulation had similar acute oral, dermal and inhalational toxicity to the technical grade material. It was recommended that a review of all home garden products be undertaken.

The Committee noted that in one unspecified Australian state, a 5-year old child (sex unspecified) had died following ingestion of the home garden granules. It was also noted that 0.5 g (of the granules) would be lethal for a 10 kg child, while a teaspoonful would be lethal for a 70 kg adult. FAISDs were set for GR 50 g/kg HG pack and GR 100 g/kg and less.

In February 1989, the PACC considered an extension of the MRLs for fenamiphos to artichokes, but it was decided that the applicant should supply details of the proposed use pattern(s) or residue data. In August 1989, the Committee noted that the technical grade active constituent clearance of fenamiphos had still not been approved even though it was submitted in September 1986. The Committee noted that a review of the new rabbit developmental study was still unavailable. MRLs for aloe vera (1 mg/kg) and root and tuber vegetables (0.2 mg/kg) were recommended. MRLs for carrots, potatoes and beetroots were deleted.

In February 1990, the NDPSC considered the quarantine use of unregistered chemicals (such as fenamiphos) but noted that the issue related to registration, efficacy and residues rather than to poisons scheduling. In February 1990, the PACC recommended MRLs for tomatoes at 0.5 mg/kg and that a withholding period of 14 days should apply. In August 1990, the PACC agreed to that it was appropriate to delete the separate MRL of 0.1 mg/kg for sweet potato.

Fenamiphos was last considered by the NDPSC in November 1990, when an evaluation of rabbit developmental studies, an *in vitro* mutation assay in Chinese hamster ovary cells and an *in vitro* cytogenetic assay in human lymphocytes, did not reveal any evidence of teratogenicity or genotoxicity.

In February 1991, the PACC considered that the submitted toxicology data was adequate and agreed to the clearance of the technical grade active constituent. This decision was reconfirmed at the June 1991 meeting.

Acceptable Daily Intake

The current Australian ADI is 0.0001 mg/kg bw/day, based on a NOEL of 0.0125 mg/kg bw/day (inhibition of plasma cholinesterase) in a 2-year dog study and using a 100-fold safety factor.

Acute Reference Dose

At the time of this assessment no Australian ARfD for fenamiphos had been established.

Poisons Scheduling

Fenamiphos is in Schedule 7 of the Standard for Uniform Scheduling of Drugs and Poisons (SUSMP), with a cut-off into Schedule 6 for granular preparations containing 5% or less fenamiphos.

Drinking Water Guidelines

Where a pesticide is registered for use in water or water catchment areas, the Joint Committee of the Agricultural and Resource Management Council of Australia and New Zealand and the NHMRC set Guideline and Health Values for the chemical in drinking water. A Guideline Value is generally based on the analytical limit of determination, and is set at a level consistent with good water management practice and that would not result in any significant risk to the consumer over a lifetime of consumption. Exceeding the Guideline Value indicates undesirable contamination of drinking water and should trigger action to identify the source of contamination and prevent further contamination. However, a breach of the Guideline Value does not necessarily indicate a hazard to public health. No guideline value in drinking water has been established.

Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The values are derived so as to limit intake *from water alone* to about 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. At present, the Health Value for fenamiphos is 0.0003 mg/L (*Australian Drinking Water Guidelines - Summary*, NHMRC, Canberra, Australia,1996; ISBN 0 642 24462 6 or
http://www.nhmrc.gov.au/publications/pdf/eh20.pdf).

1.2 International Toxicology Assessments

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluated the toxicology of fenamiphos in 1974, 1985, 1987, 1997 and 2002. An ADI of 0.0006 mg/kg bw/day was set in 1974, but this was reduced to a temporary ADI of 0.0003 mg/kg bw/day in 1985, due to concerns regarding foetotoxicity in rabbits. Following consideration of results of an oncogenicity study conducted in rats, and additional data from a rabbit developmental study, the JMPR established an ADI of 0.0005 mg/kg bw/day in 1987. Fenamiphos was re-evaluated by the JMPR in 1997; an ADI of 0.0008 mg/kg bw/day was set by applying a 100-fold safety factor to the NOEL of 0.083 mg/kg bw/day for inhibition of brain acetyl ChE activity and anaemia in a one-year dog study. The available data did not allow the establishment of an ARfD different from the ADI and therefore the JMPR requested data on the acute effects in dogs (the most sensitive test species) to assist in the establishment of an ARfD for fenamiphos. At its 2002 meeting, the JMPR established an ARfD of 0.003 mg/kg bw, based on the NOAEL of 0.25 mg/kg bw for inhibition of erythrocyte acetyl ChE activity in a single-dose dog study and using a 100-fold safety factor. This was supported by an acute neurotoxicity study in rats, for which the NOAEL was 0.37 mg/kg bw for cholinergic signs.

US EPA

The US EPA completed a human health risk assessment of fenamiphos in September 1999. An initial risk assessment had been prepared in 1994 and was subsequently revised and/or updated in 1995, 1996, 1998 and 1999. While both the acute and chronic dietary risk from food treated with fenamiphos was considered to be low, it was concluded that the risk from drinking water (based on limited ground water monitoring data) was high. An ARfD of 0.0012 mg/kg bw/day was set, based on a LOAEL of 0.37 mg/kg bw for inhibition of plasma ChE and RBC acetyl ChE activities in an acute neurotoxicity study in rats and using a 300-fold uncertainty factor (analogous to a safety

factor). Dietary intake estimates indicated that none of the population exceeded the ARfD. A chronic reference dose (analogous to an ADI) was set at 0.0001 mg/kg bw/day, based on a NOAEL of 0.1 mg/kg bw/day for inhibition of plasma ChE activity in a subchronic dog study and using a 100-fold uncertainty factor. Chronic dietary intake estimates were 4% of the ADI for the US population (adults and children) and 14% of the ADI for non-nursing infants less than one-year old. Residues of toxicological concern for both the acute and dietary risk assessment included the parent compound and 2 metabolites (fenamiphos sulfoxide and fenamiphos sulfone).

While there did not appear to be any concern with regard to the dietary intake of fenamiphos residues in food, the US population was considered to be at high risk of exposure to residues in drinking water. Acute dietary risk estimates indicated that acute exposure to fenamiphos in ground water exceeded the ARfD by 170, 250 and 750% for adult males, females and children, respectively. Similarly, chronic exposure to fenamiphos in ground water exceeded the ADI by 300, 300 and 1000% for adult males, females and children, respectively. Risk estimates for surface water were also performed and these also indicated an exceedence (by an unspecified amount) of safe levels. However, in the absence of high-quality monitoring data, drinking water levels of comparison (DWLOCs) were used as surrogates for the ARfD and ADI. The $DWLOC_{acute}$ for adult males, females and nursing infants less than one-year old is 37, 28 and 4 ppb, respectively. The $DWLOC_{chronic}$ for adult males and females is 3 ppb, and for children 1-6 years old it is 1 ppb.

An Interim Re-registration Eligibility Decision identified additional data requirements to assist the risk assessment. While the toxicology database was judged to be complete, additional product chemistry, residue chemistry and occupational exposure data were considered necessary to support re-registration. Rather than generating this additional data, the sole registrant requested voluntary cancellation of all US-registered fenamiphos products. The US EPA announced this voluntary cancellation in September 2002.

1.3 Chemistry –Active Constituent

Approved common name: Fenamiphos (ISO)

Alternative names: Methaphenamiphos

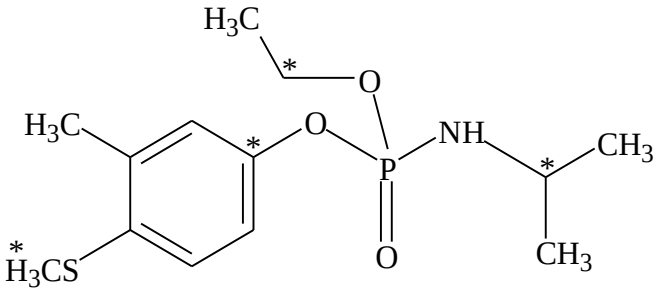
Chemical name: (R,S)-Ethyl 4-methylthio-*m*-tolyl isopropylphosphoramidate (IUPAC)
Ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl) phosphoramidate (CAS)

CAS Registry number: 22224-92-6

Empirical formula: $C_{13}H_{22}NO_3PS$

Molecular weight: 303.4

Chemical structure:



Isotope label: The position of the radiolabel (^{14}C or ^{3}H) is indicated by *

Chiral atom: Phosphorus

Chemical class: Organophosphate

Structural analogues: None

Chemical and physical properties

Colour:	Colourless
Odour:	None
Physical state:	Crystalline solid
Melting point (°C):	49.2
Density (20°C):	-
Partition coefficient: (log K_{ow})	3.30
Vapour pressure:	0.12 mPa (20°C); 4.8 mPa (50°C)
Solubility:	
in water:	0.4 g/L (20°C)
in organic solvents:	>200 g/L (20°C) (dichloromethane, isopropanol & toluene) 10-20 g/L (20°C) (hexane)
Stability:	Hydrolysis DT_{50} at 22°C = 1 y (pH 4), 8 y (pH 7) & 3 y (pH 9)

Impurities of Toxicological Concern

The active fenamiphos contains no impurities of toxicological concern, with the APVMA's minimum compositional standard specifying a minimum fenamiphos content of 920 g/kg.
There is currently no FAO specification for plant protection products containing fenamiphos.

1.4 Products

At the commencement of the review of fenamiphos, there were four products registered. As of September 2011, there were 14 products registered. The continued registration of the 12 products registered after the commencement of the review is subject to the outcomes of the review.

There is one granular formulation (GR) containing 100 g/kg fenamiphos. The remaining 13 products are emulsifiable concentrates (EC) and contain 400 g/L fenamiphos. Fenamiphos products are used to control nematodes and sucking insects on various crops or to control nematodes in turf.

2. METABOLISM AND TOXICOKINETICS

2.1 Metabolism

Gronberg RR (1969) The metabolic fate of ethyl-4-(methylthio)-m-tolyl isopropylphosphoramidate (BAY 68138), ethyl 4-(methyl-sulfinyl)-m-tolyl isopropylphosphoramidate (BAY 68138 sulfoxide) and ethyl 4-(methylsulfonyl)-m-toly-isopropylphosphoramidate (BAY 68138 sulfone) by white rats. Report No. 26759. Lab: Chemagro Corporation, Research Department, unspecified location. Sponsor: Bayer AG, unspecified location. Study duration: unspecified. Report date: 27th October 1969.

Materials and Methods

[^{14}C]fenamiphos, [^{14}C]fenamiphos sulfoxide or [^{14}C]fenamiphos sulfone (>99% radiochemical purities; Chemagro Corporation, unspecified location) were administered by oral gavage to white rats (strain unspecified; 150-200 g bw; sourced from Holtzman Company, Madison, Wisconsin). In the first of three experiments, one male and one female rat were dosed at 2 mg/kg bw using a mixture of [ethyl-^{14}C] and [methythio-^{3}H]fenamiphos and placed in metabolism chambers. An additional female rat was dosed at 2 mg/kg bw with a mixture of [isopropyl-^{14}C] and [methythio-^{3}H]fenamiphos. Ethanolamine or sulfuric acid traps were used to collect any exhaled radiolabelled compounds at unspecified times. Urine and faeces were collected at 12, 24 and 48 hours. Rats were sacrificed at 48 hours by an unspecified means. Blood and tissues (unspecified) were collected following sacrifice. In the second experiment, [ethyl-^{14}C]fenamiphos-sulfoxide or [ethyl-^{14}C]fenamiphos-sulfone were administered to female rats (one/treatment) by oral gavage in a similar manner except that no sulfuric acid trap was used. In the third experiment, female rats (numbers unspecified) were dosed with [ethyl-^{14}C]fenamiphos. One rat was sacrificed at 0.5, 1, 2 and 4 hours and 9 days and the liver, kidney, fat and blood samples obtained; brain, heart, muscle and the GIT were also taken from the rats sacrificed at 9 days. Radioactivity was quantified by liquid scintillation counting (LSC) directly for liquid samples or following solubilisation for solid samples. Metabolites were analysed by thin layer chromatography (TLC) and liquid chromatography (i.e. size exclusion chromatography). In some cases, samples were pre-treated with β-glucuronidase, aryl sulfatase or maltase or hydrolysed with sulfuric acid.

Results

Experiment 1: Total recovery of radioactivity ranged from 93.4 to 104.6%, with the majority of radioactivity eliminated within 12-15 hours of dosing. Approximately 40-50% of ethyl-^{14}C or isopropyl-^{14}C was detected in the ethanolamine trap indicating its exhalation as $^{14}CO_2$. Very little (<1%) ethyl-^{14}C was detected in the sulfuric acid trap, while 17% of isopropyl-^{14}C was detected. No methylthio-^{3}H was detected in the ethanolamine or sulfuric acid traps suggesting no exhalation as $^{3}H_2O$. Peak urinary levels of radioactivity occurred at 12 hours post-dose (23-32% with ethyl-^{14}C, 60-80% with methylthio-^{3}H and 7.7% with isopropyl-^{14}C). Total urinary excretion over 48 hours was approximately 40% of ethyl-^{14}C, 80-96% methylthio-^{3}H and 12.2% isopropyl-^{14}C. Faecal excretion of radioactivity was between 4.5-11.8% over 48 hours and generally highest at 12 hours post-dose. Total tissue radioactivity was approximately 12% for ethyl-^{14}C or isopropyl-^{14}C and only 1.4-2.3% for methylthio-^{3}H. The highest levels of methylthio-^{3}H or isopropyl-^{14}C occurred in the liver (3.5-7.3%), GIT (1.8-2.9%) and carcass (2.7-4.0%). Very little radioactivity was detected in erythrocytes or plasma (<0.1 and 0.3%, respectively).

TLC of urine identified fenamiphos sulfoxide phenol (19.6-31.4% of total urinary radioactivity) and fenamiphos sulfone phenol (6.0-8.5%) as metabolites of [methylthio-^{3}H]fenamiphos, with traces of fenamiphos sulfoxide also detected. Unknown metabolites accounted for 29.3-44.3% of total urinary radioactivity. The only identified metabolite of ethyl-^{14}C and isopropyl-^{14}C was fenamiphos sulfoxide (<2%) with 6.1-31.7% of radioactivity associated with a/an unknown metabolite/s. Enzymatic treatment released very little radioactivity (<6%) suggesting the presence of low levels of conjugated metabolites. Size exclusion chromatography of urine indicated that approximately half of the radioactivity was eluted as high molecular weight compounds and the remainder as smaller compounds. No radioactivity was eluted in the void volume.

Experiment 2: Recovery of radioactivity following dosing with [ethyl-^{14}C]fenamiphos [ethyl-^{14}C]sulfoxide or [ethyl-^{14}C]sulfone was 95.7 and 88.5%, respectively. The pattern of excretion was similar to that occurring following dosing with the parent compound (see experiment 1). Approximately 44% was detected in the ethanolamine trap (as exhaled $^{14}CO_2$), 33% in the urine (over 48 hours) and 10-18.4% in tissues. The highest tissue levels were found in the liver, GIT and carcass (5.5 and 5.5%, 2.9 and 2.0% and 6.0 and 2.0% for fenamiphos sulfoxide and sulfone, respectively).

Experiment 3: Tissue levels of radioactivity over time are summarised in the table below. Over the first 4 hours after dosing, radioactivity was only detected in the liver kidney and fat, with the highest level detected in liver. At 2 and 9 days after dosing, radioactivity was detectable in all tissues examined, with the liver still having the highest level. TLC of liver extracts revealed fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone, while only fenamiphos sulfoxide was detected in plasma (at 1, 2 and 4 hours). In liver, no parent compound was detected after 1 hour, while no fenamiphos sulfoxide or fenamiphos sulfone were detected after 4 hours.

Table 9 Tissues radioactivity levels (ppm) over time

Tissue	30 minutes	1 hour	2 hours	4 hours	2 days	9 days
Brain	-	-	-	-	<0.1	0.3
Heart	-	-	-	-	0.1	0.3
Liver	4.1	17.7	6.7	11.1	0.8-1.7	1.4
Kidney	0.6	4.6	1.5	3.1	0.4-0.5	0.6
Fat	0.1	0.3	0.1	0.4	0.2-0.4	0.6
Muscle	-	-	-	-	<0.1	0.2
GIT	-	-	-	-	0.2	0.5

Results expressed as the mean (or range) of 6 female rats

Conclusion: Complete recovery of radioactivity was achieved following oral dosing in rats, with the majority eliminated within 12-15 hours of dosing. Depending on the radiolabel, the main pathways of excretion of radioactivity were via exhaled CO_2, urine and faeces. Peak urine and faecal levels occurred at 12 hours. Total tissue radioactivity was up to 12% of the administered dose. Highest tissue radioactivity was found in the liver, kidney, fat and GIT. The main urinary metabolites included fenamiphos sulfoxide phenol and fenamiphos sulfone phenol, with traces of fenamiphos sulfoxide also detected. Unknown metabolites accounted for up to approximately 40% of urinary radioactivity. Fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone were detected as metabolites in liver, while only fenamiphos sulfoxide was detected in plasma. Metabolism of radiolabelled fenamiphos sulfoxide or fenamiphos sulfone was the same as the parent compound.

Khasawinah AM & Flint DR (1972) Metabolism of ®Nemacur [ethyl 4-(methylthio)-m-tolyl isopropylphosphoramidate) by rat liver microsomes in vitro. Report No. 34217. Lab: Chemagro, Division of Baychem Corporation, unspecified location. Sponsor: unspecified. Study duration: unspecified. Report date: 21st July 1972.

This *in vitro* study was undertaken to examine the metabolism of fenamiphos by rat liver preparations. A mixture of [^{14}C-ethyl] and [^{3}H-methylthio]fenamiphos (^{3}H/^{14}C ratio of 9; >99% radiochemical purity; prepared by Chemagro) at 25-110 μg was incubated with NADP (2 μmoles), glucose-6-phosphate (50 μmoles) and $MgSO_4$ (75 μmoles) for 5 minutes at 34°C prior to the addition of either 9000 g or 90,000 g (microsomal fraction) preparations of rat liver. These fractions had been prepared from both sexes of an unspecified rat strain by homogenation and centrifugation. Following an unspecified incubation period, metabolites were extracted with acetone and then analysed by TLC, infrared analysis, mass spectrometry and evaluation of ^{3}H/^{14}C ratios. Both liver fractions were reported to metabolise fenamiphos, with the microsomal fraction completely metabolising the parent compound. Three different metabolites were detected, with two of these identified as fenamiphos sulfoxide and ethyl 4-(methylthio)-m-tolyl phosphoramidate. The third metabolite could not be identified; based on the available data the author considered that it may have been an N-acetyl derivative of fenamiphos.

Weber H (1988) [Phenyl-1-^{14}C]Nemacur: whole-body autoradiographic distribution of the radioactivity in the rat. PF Report No. 3055. Study No. M1810188-7. Lab & Sponsor: Bayer AG, Agrochemicals CE, Institute for Metabolism Research, Monheim, Leverkusen-Bayerwerk, Germany. Study duration: unspecified. Report date: 26th August 1988.

GLP & QA: Statement of compliance with GLP standards [US EPA, 40 CFR Part 160 (29th November 1983); OECD, C (81) 30 (final, 12th May 1981)]; no QA statement

Fenamiphos labelled with ^{14}C/^{13}C in the C1 position of the phenyl ring ([phenyl-1-^{14}C]fenamiphos; >99% radiochemical purity; synthesised by Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, USA) was administered to five male wistar rats (Bor:WISW, SPF) as a single oral gavage dose of 3 mg/kg bw in physiological saline. A single control rat was dosed similarly with unlabelled fenamiphos (Batch No. APF 29038501; 97% purity; sourced from Bayer AG, unspecified location). The control rat and one of the rats treated with [phenyl-1-^{14}C]fenamiphos were replaced as they died at 15 and 60 minutes, respectively, after dosing. Rats were sourced from Winkelmann (Borchen, Germany) and weighed approximately 200 g at the time of dosing. Food and water were available *ad libitum*, except the day before treatment when they received only half of their normal food ration. Following dosing, rats were housed individually in metabolism cages. One rat was sacrificed by CO_2 asphyxiation at 0.5, 2, 8, 24 and 48 hours and prepared for whole-body autoradiography.

Radioactivity was absorbed rapidly from the GIT and detectable at 0.5 hours after dosing in all tissues except the brain, suggesting that fenamiphos or its metabolites did not cross the blood brain barrier (BBB). Relatively high radioactivity was detected in those organs responsible for metabolising or eliminating fenamiphos, namely the kidneys, liver, bladder and GIT. Relatively high levels were also present in the blood, lung, salivary gland and connective tissue. Radioactivity was also detected around hair follicles on the skin, which the author considered a possible elimination pathway. Radioactivity was also detectable in the lymphatic system. The levels of radioactivity in all tissues declined over time so that by 8 hours after dosing no discernible radioactivity was detected. There was no evidence of tissue or fat accumulation of fenamiphos or its metabolites.

Ecker W, Weber H & Brauner A (1989) [Phenyl-1-^{14}C]Nemacur: General metabolism study in the rat. Report No. 3175. Study No. M1820189-9. Lab: Bayer AG, Monheim, Institute for Metabolism Research, Leverkusen, Bayerwerk, Germany. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Research and Development, Mobay Research Park, South

Metcalf, Stilwell, Kansas, USA. Study duration: 14th July to 22nd December 1987. Report date: 21st February 1989.

GLP & QA: Statement of compliance with GLP standards [US EPA, 40 CFR Part 160 (29th November 1983); OECD, C (81) 30 (final, 12th May 1981)]; no QA statement

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision F, 40 CFR 158, 85-1 (October 1982)

Materials and Methods

Fenamiphos labelled with $^{14}C/^{13}C$ in the C1 position of the phenyl ring ([phenyl-1-^{14}C]fenamiphos; >99% purity; synthesised in the Radiosynthesis Laboratory of Mobay Corporation, Kansas City, USA) was administered to single male and female wistar rats (BOR:WISW, SPF Cpb) via the oral (gavage) (0.3 or 3 mg/kg bw) or intravenous routes (0.3 mg/kg bw). The vehicle was physiological saline and the dose volume was 10 mL/kg bw. Separate groups of rats were pre-treated by oral gavage for 14 days with 0.3 mg/kg bw/day unlabelled fenamiphos (Reference code No. APF 29038501; 97.0% purity, sourced from Bayer AG, unspecified location) followed by a single oral gavage dose of 0.3 mg/kg bw [phenyl-1-^{14}C]fenamiphos. It was stated that the dose levels were chosen based on the high toxicity of fenamiphos and on recommendations contained in the US EPA pesticide assessment guidelines. Rats were dosed immediately after preparation of the dosing solutions, which were reportedly stable for at least 2 hours based on TLC analysis. The concentration of each dosing solution was verified by LSC.

Rats were sourced from Winkelmann (Borchen, Germany) and were acclimatised for at least one week prior to experimentation. Males and females were approximately 8 and 12 weeks old, respectively, and weighed approximately 200 g. Rats were housed under standard conditions, with food and water available *ad libitum*, except the day before treatment when they received only half of their normal food ration. Following dosing, rats were housed individually in metabolism cages.

Urine was collected at 0-4, 4-8, 8-24, 24-32 and 32-48 hours after dosing. Faeces were collected at 0-24 and 24-48 hours after dosing. In males pre-treated for 14 days with unlabelled fenamiphos then dosed with a single oral gavage dose of [phenyl-1-^{14}C] fenamiphos, CO_2 was collected at 0-8, 8-24, 24-32 and 32-48 hours by exhalation into ethanol: ethanolamine (1:1). Rats were sacrificed after 48 hours by exsanguination under CO_2 anaesthesia. The following tissues were collected for analysis: liver, spleen, kidney, perirenal fat, ovaries, uterus, muscle (femur), bone (femur), skin, plasma, erythrocytes, heart, brain, lung and the carcass.

Liquid samples were analysed directly by LSC (i.e. urine, plasma, $^{14}CO_2$ and perirenal fat). Tissue samples were also analysed by LSC following combustion (samples <500 mg) or solubilisation. The biokinetic behaviour of fenamiphos was determined by statistically analysing excretion or residue data using the Mann-Whitney U-test. Metabolites were analysed by high performance liquid chromatography and gas liquid chromatography and mass spectrometry following extraction with a suitable solvent, digestion with arylsulfatase or β-glucuronidase (to analyse conjugates) or derivitisation.

Results

Absorption: The level of radioactivity (expressed as a % of administered radioactivity) recovered after 48 hours is summarised in the table below. Recovery ranged from 97-104 % of the administered radioactivity. Based on the level of radioactivity in urine plus the amount in the body (excluding the GIT) the oral absorption of fenamiphos was almost complete (98-99% of the administered dose).

Table 10 Level of radioactivity excreted over 48 hours in rats

Sample	Treatment							
	0.3 mg/kg bw, intravenous		0.3 mg/kg bw po		0.3 mg/kg bw po (14 day pre-treatment)		3 mg/kg bw po	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
$^{14}CO_2$	0	NA	0	NA	0	NA	<0.1	NA
Urine	95.3	97.8	100.0	100.2	101.5	95.6	92.8	99.3
Faeces	1.5	1.5	3.8	2.7	2.3	2.1	3.6	3.0
Body (less GIT)	0.2	0.2	0.1	0.2	0.2	0.2	<0.1	0.1
GIT	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
TOTAL	96.9	99.5	104.0	103.2	104.1	97.9	96.5	102.4

NA = not analysed

Elimination: Elimination was almost complete 48 hours after oral or intravenous administration (99%), with almost all material excreted via the urine (96-98%) and only small levels excreted via the faeces (1.5-3.8%). Renal clearance was approximately 50% during the first 4 hours after dosing and 90% within the first 16 hours after dosing. Virtually no material was excreted via expired air (0.005%). Statistical analysis revealed significantly higher ($p<0.01$-0.05) urinary excretion in males *versus* females dosed orally with [phenyl-1-^{14}C]fenamiphos. There was no such difference following intravenous administration. There was also a significant dose-related difference in relative renal excretion after 48 hours in males. However, the authors attributed this difference to the different recoveries in each group. Other significant dose-related effects were based on values below or close to the limit of detection and therefore were not considered biologically significant by the authors. Pre-treating rats orally for 14 days with unlabelled fenamiphos had no significant effect on excretion in either sex. Excretion rates following intravenous dosing were significantly higher ($p<0.01$-0.05) than after oral dosing in females but not in males. Significantly higher ($p<0.01$) radioactivity was excreted via the faeces following oral dosing compared to intravenous dosing.

Tissue distribution: The table below summarises the relative concentrations of radioactivity detected in rat tissues 48 hours after oral or intravenous administration. Very little radioactivity was detected in any tissues (0.045-0.23% of the administered radioactivity excluding the GIT). Quantifiable residues were predominantly confined to rats treated with the high oral dose. These results indicate that fenamiphos and its metabolites do not preferentially distribute to, or accumulate in, any tissues.

Table 11 Relative concentrations of radioactivity in rat tissues[1]

Tissue	Treatment							
	0.3 mg/kg bw intravenous		0.3 mg/kg bw po		0.3 mg/kg bw po (14 day pre-treatment)		3 mg/kg bw po	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Liver	<0.00257	0.00985	<0.00337	<0.00318	<0.00325	0.00373	0.00117	0.00280
Kidney	<0.00301	0.00580	<0.00191	<0.00159	<0.00248	<0.00159	0.00069	0.00053
Uterus	-	0.00153	-	0.00802	-	0.00326	-	0.00038
Skin	<0.00212	<0.00195	<0.00206	<0.00223	0.00481	<0.00287	0.00117	0.00053
Erythrocytes	<0.00124	0.00955	<0.00112	<0.00192	<0.00113	<0.00115	0.00030	0.00040
Lung	<0.00105	0.00458	<0.00080	<0.00156	<0.00158	<0.00099	0.00028	0.00036
Carcass	<0.00152	0.00168	<0.00098	<0.00155	<0.00123	<0.00123	<0.00026	0.00051
GIT	<0.00103	<0.00168	<0.00127	<0.00155	<0.00168	<0.00123	0.00048	0.00051

Results expressed as the % of the administered radioactivity; < values below the limit of quantification

Metabolism: The following table summarises the metabolites identified in excreta (urine plus faeces) following dosing with ([phenyl-1-^{14}C] fenamiphos (Note: faecal metabolites were only detected in males dosed orally at 3 mg/kg bw and included fenamiphos sulfoxide, fenamiphos-sulfoxide phenol, fenamiphos-sulfone phenol and fenamiphos phenol sulfate. The metabolite profiles were reasonably consistent across all groups and both sexes. The main metabolite was fenamiphos-sulfoxide phenol sulfate (40-54%) followed by fenamiphos-sulfoxide phenol (4-22%) and fenamiphos-sulfone phenol sulfate (4.2-15.1%). Three unidentified metabolites were detected (M1, M2 & M3) accounting for 0.7-4.4% of the administered radioactivity. The following toxicologically-significant metabolites were *not* detected: fenamiphos, fenamiphos-sulfone, desisopropyl fenamiphos and desisopropyl fenamiphos sulfone.

Table 12 Metabolite profiles in rat excreta

Metabolite	**Treatment**							
	0.3 mg/kg bw intravenous		**0.3 mg/kg bw po**		**0.3 mg/kg bw po (14 day pre-treatment)**		**3 mg/kg bw po**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Fenamiphos-sulfoxide	2.3	6.6	0	11.6	2.9	0	0.3	1.3
Desisopropyl fenamiphos-sulfoxide	0	0	0.2	0.1	0	1.7	0.4	0.7
Fenamiphos phenol	8.4	3.5	9.6	0.8	5.3	9.8	4.6	4.0
Fenamiphos-sulfoxide phenol	11.8	19.3	11.8	18.5	4.0	21.8	21.5	12.7
Fenamiphos-sulfone phenol	4.5	2.6	3.8	3.0	1.9	4.9	10.8	6.5
Fenamiphos phenol sulfate	19.3	15.8	6.9	8.2	5.3	4.9	6.1	5.7
Fenamiphos-sulfoxide phenol sulfate	40.2	44.0	53.7	42.5	48.4	45.3	43.4	40.3
Fenamiphos-sulfone phenol sulfate	7.8	4.2	7.9	7.9	15.1	7.5	10.0	11.5
Fenamiphos-sulfone phenol sulfate hydroxylated in the 3-methyl group	0.6	0.1	0	1.0	10.0	0	0.0	11.3
TOTAL IDENTIFIED	94.9	96.1	93.0	93.6	92.9	95.9	97.1	94.0
M1	1.4	0.7	0.7	1.0	0.5	0.1	0.4	1.7
M2	0.3	0.0	0.3	0.7	3.6	0	0.1	1.6
M3	1.4	1.3	1.0	1.1	0.3	0.9	0.2	0.8
UNASSIGNED	1.6	1.4	3.2	2.7	2.0	2.4	1.7	1.5
Solids	0.2	0.3	0.8	0.7	0.5	0.5	0.4	0.30
Body	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1
TOTAL	100	100	100	100	100	100	100	100

Results expressed as the % of total recovered radioactivity

Conclusions: Fenamiphos was almost completely absorbed from the GIT following oral administration. Elimination was almost complete 48 hours after oral or intravenous administration (99%), with almost all material excreted via the urine (96-98%) and only small levels excreted via the bile (1.5-3.7%). Renal clearance was approximately 50% during the first 4 hours after dosing and 90% within the first 16 hours after dosing. Virtually no material was excreted via expired air (0.005%). The rate and ratio of excretion appeared to show some dependence on sex and dose route. There was no evidence of tissue accumulation of fenamiphos or its metabolites. Highest residue levels were found in the liver, kidneys and skin. The main metabolites (80-96%) were fenamiphos

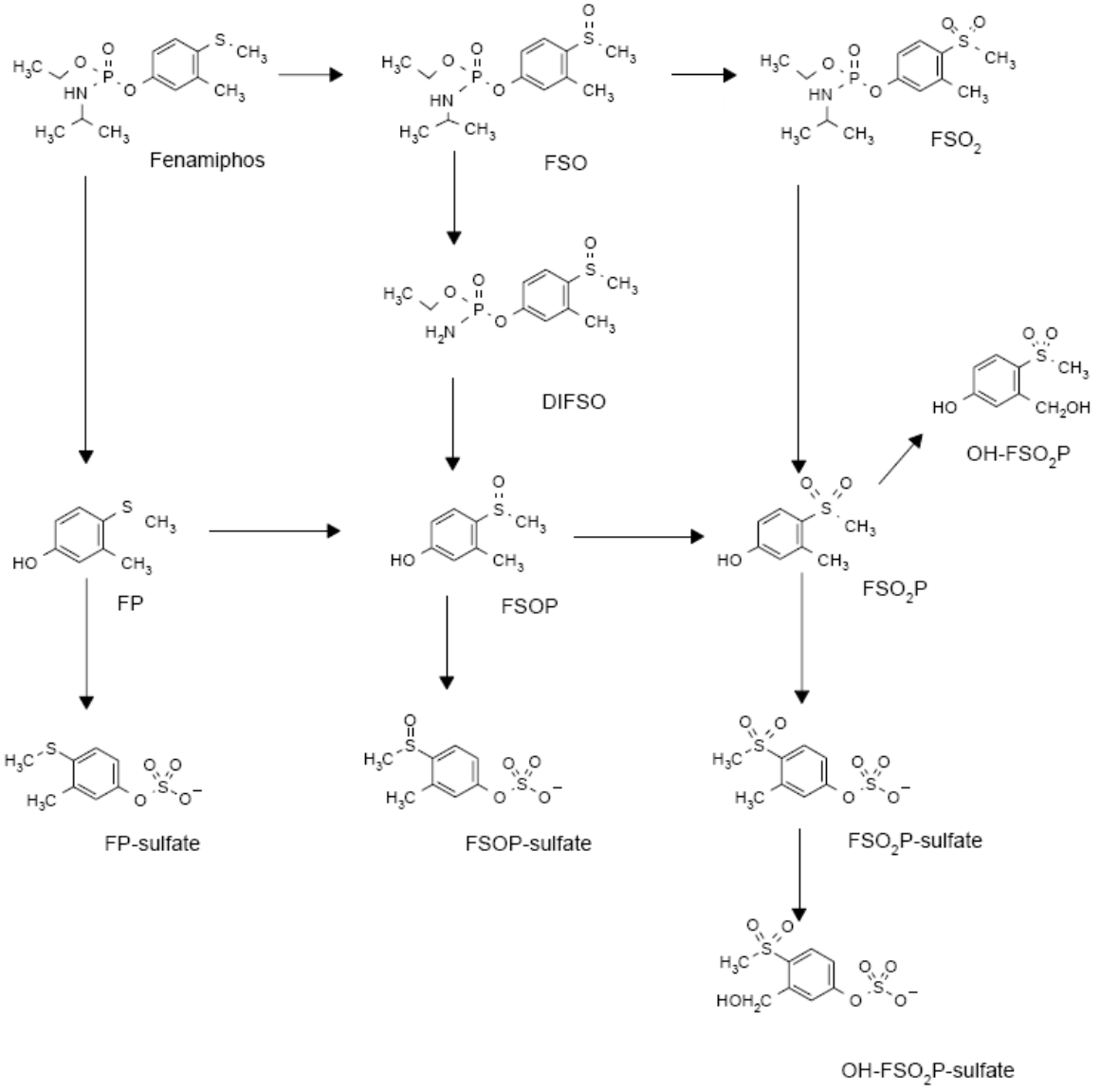
phenols (fenamiphos-sulfoxide phenol sulfate, fenamiphos-sulfoxide phenol and fenamiphos-sulfone phenol sulfate).

2.2 Toxicokinetics

No toxicokinetic studies were submitted for evaluation

The major metabolic pathways for fenamiphos in rats are summarised in the figure overleaf.

Figure 1 Proposed metabolic pathway of fenamiphos in rats



KEY: FP = fenamiphos phenol; FSO = fenamiphos sulfoxide; FSOP = fenamiphos sulfoxide phenol; FSOP-sulfate = fenamiphos sulfoxide phenol sulfate; DIFSO = desisopropyl fenamiphos sulfoxide; OH-FSO_2P = fenamiphos sulfone phenol, hydroxylated in the 3-methyl group; OH-FSO_2P-sulfate = fenamiphos sulfone phenol sulfate, hydroxylated in the 3-methyl group; FP-sulfate = fenamiphos phenol sulfate; FSO_2 = fenamiphos sulfone; FSO_2P = fenamiphos sulfone phenol; FSO_2P-sulfate = fenamiphos sulfone phenol sulfate

3. ACUTE TOXICITY STUDIES

3.1 Active Constituent

A summary of the results of acute toxicity studies conducted on fenamiphos is provided in the table below

Table 13 Summary of acute toxicity of fenamiphos

Study	Species	Results	Reference
Oral	Mice	LD_{50} 8 mg/kg bw (females)	Kimmerle & Lorke (1967)
Oral	Mice	LD_{50} 23 mg/kg bw (males)	Kimmerle & Solmecke (1971)

Study	Species	Results	Reference
Oral	Rat	LD_{50} 8 mg/kg bw (males) LD_{50} 5 mg/kg bw (females)	Kimmerle & Lorke (1967)
Oral	Rat	LD_{50} 15 mg/kg bw (males) LD_{50} 19 mg/kg bw (females)	Kimmerle & Solmecke (1971)
Oral	Rat	LD_{50} 3 mg/kg bw (males) LD_{50} 2 mg/kg bw (females)	Crawford & Anderson (1973)
Oral	Rat	LD_{50} 2 mg/kg bw (males) LD_{50} 3 mg/kg bw (females)	Crawford & Anderson (1974a)
Oral	Rat	LD_{50} 2 mg/kg bw (males) LD_{50} 3 mg/kg bw (females)	Crawford & Anderson (1974b)
Oral	Rat	LD_{50} 2 mg/kg bw (males) LD_{50} 3 mg/kg bw (females)	Lamb & Matzkanin (1975)
Oral	Rat	LD_{50} 6 mg/kg bw	Krotlinger (1988)
Oral	Rat (female)	LD_{50} <25 mg/kg bw	Krotlinger (2000a)
Oral	Guinea pigs	LD_{50} 75-100 mg/kg bw	Kimmerle & Lorke (1967)
Oral	Guinea pigs	LD_{50} 75-100 mg/kg bw (males)	Kimmerle & Solmecke (1971)
Oral	Rabbits	LD_{50} ~5 mg/kg bw	Kimmerle & Lorke (1967)
Oral	Rabbits	LD_{50} 10-18 mg/kg bw(males)	Kimmerle & Solmecke (1971)
Oral	Dog	LD_{50} ~ 10 mg/kg bw (males)	Kimmerle & Solmecke (1971)
Oral	Dog	NOEL of 0.25 mg/kg bw for RBC ChE inhibition	Detzer (2002)
Dermal	Rat	LD_{50} 500 mg/kg bw (4-hour exposure) LD_{50} 500 mg/kg bw (7-day exposure)	Kimmerle & Lorke (1967); Kimmerle & Solmecke (1971)
Dermal	Rabbit	LD_{50} 225 mg/kg bw (males) LD_{50} 179 mg/kg bw (females)	Crawford & Anderson (1972)
Dermal	Rat	LD_{50} 72 mg/kg bw(males) LD_{50} 92 mg/kg bw (females)	Flucke (1980)
Dermal	Rat	LD_{50} >2000 mg/kg bw	Krotlinger (2000b)
Inhalational (1-hour, aerosol, head-only)	Rat	LC_{50} 130 mg/m^3	Thyssen (1979a)
Inhalational (4-hour, aerosol, head-only)	Rat	LC_{50} 100 mg/m^3	Thyssen (1979a)
Inhalational (4-hour, aerosol, nose-only)	Rat	LC_{50} 74 mg/m^3	Pauluhn (2001)
Skin irritation	Rabbit	Non skin irritant	Crawford & Anderson (1971)
Skin irritation	Rabbit	Slight skin irritant	Kato (1984a)
Eye irritation	Rabbit	Slight eye irritant	Crawford & Anderson (1971)
Eye irritation	Rabbit	Slight eye irritant (systemic toxicity in unwashed rabbits)	Kato (1984b)
Skin sensitisation	Guinea pig	Non sensitiser	Watanabe (1983)
Skin sensitisation	Guinea pig	Non sensitiser	Stropp (1995)

3.1.1 Oral toxicity

Kimmerle G & Lorke D (1967) Toxicological studies with the compound Bay 68138. Report No. 272. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 4th April 1967.

Test Compound: Bay 68138 (fenamiphos); purity & source unspecified
Batch: Not specified

Test Species: Rats (15/sex/dose), mice (15 females/dose); guinea pigs (5/group); rabbits (3/sex/group); age, bw & strains unspecified
Study Duration: 14 days
Laboratory: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage	Emulsifier W (aqueous emulsion)	14 days	Male rats: 2.5, 3.5, 5.0, 7.5, 8.5, 10.0, 15.0 & 20.0	8.1 (7.4-8.9)
			Female rats: 2.5, 3.5, 5.0, 7.5 & 10.0	4.75 (3.9-5.8)
			Female mice: 2.5, 5.0, 7.5, 10.0 & 15.0	8.3 (6.9-9.9)
			Guinea pigs: 5.0, 10.0, 25.0, 50.0, 75.0 & 100.0	75-100
			Rabbits: 1.0, 2.5 & 10.0	5.0

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred in male and female rats at and above 5.0 and 3.5 mg/kg bw, respectively. The time to death was reported to be within hours of dosing. Clinical signs (spasms and red tears) occurred in all females, while they occurred in all male rats at and above 3.5 mg/kg bw. Signs occurred 5-15 minutes after dosing and had resolved by 24 hours in survivors. In female mice, deaths and clinical signs (unspecified) occurred at and above 5.0 mg/kg bw. In guinea pigs, deaths occurred at and above 75 mg/kg bw, while clinical signs (unspecified) occurred in all animals at and above 25.0 mg/kg bw. Deaths and clinical signs (unspecified) occurred in rabbits at and above 5.0 mg/kg bw.

Necropsy findings: Not recorded.

Kimmerle G & Solmecke D (1971) Bay 68138 toxicological studies Report No. 2767. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 5th May 1971.

Test Compound: Bay 68138 (fenamiphos); purity & source unspecified
Batch: Not specified
Test Species: Male NMRI mice (15/dose; 18-22 g bw); male & female Wistar rats (15/dose; 160-200 g bw); male Pirbright guinea pigs (5/dose; 500-800 g bw); rabbits (3/dose; 2.5-3.5 kg bw); male beagle dogs (2/dose; 11-12 kg bw)
Study Duration: 14 days
Laboratory: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage	Lutrol [polyethylene glycol (PEG)]	14 days	2.5, 5, 10, 17.5, 20, 25, 27.5 & 30 male mice	22.7 (20.7-24.9)
			2.5, 5, 10, 12, 15, 20 & 25 males rats	15.3 (13.6-17.2)
			2.5, 5, 10, 15, 20, 25 & 35 female rats	19.4 (16.5-22.8)
			5, 10, 25, 50, 75 & 100 guinea pigs	75-100
			5, 10, 17.5 & 25 male rabbits	10-17.5
			5, 10 & 17.5 male dogs	~10

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred across all species within one day of dosing (at and above 17.5 mg/kg bw in male mice; at and above 12 and 15 mg/kg bw in male and female rats, respectively; at and above 75 mg/kg bw in guinea pigs and at and above 10 mg/kg bw in rabbits and dogs). Unspecified clinical signs occurred across all species from 5 minutes after dosing and lasted for up to 5 days, depending on the dose (at and above 5 mg/kg bw in mice and rats; at and above 75 mg/kg bw in guinea pigs; at and above 10 mg/kg in male rabbits and dogs).

Necropsy findings: Not recorded.

Crawford CR & Anderson RH (1973) Comparative oral toxicity in rats of several impurities and a technical compound of ®Nemacur with analytical grade Nemacur. Report No. 3446. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 7th February 1973.

Test Compound: Nemacur technical (fenamiphos); 99.7% purity; unspecified source
Batch: 68-105-48
Test Species: SD rats (4/sex/dose); 200-250 g bw; unspecified age & source
Study Duration: 14 days
Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage Fasted for 20 hours	20% ethanol:80% propylene glycol	14 days	1.9, 2.9, 4.3 & 6.5 (males) 1.9, 2.3, 2.8 & 3.4 (females)	3.15 (2.3-4.3) males 2.38 (2.1-2.7) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred at and above 2.9 mg/kg bw in males (2, 3 & 4/4 at 2.9, 4.3 and 6.5 mg/kg bw, respectively) and 2.3 mg/kg bw in females (2, 3 & 4/4 at 2.3, 2.8 and 3.4 mg/kg bw, respectively). The time to death was approximately 30 minutes. All rats were reported to develop cholinergic signs within 10 minutes of dosing. In survivors, all signs had resolved by 24 hours.

Necropsy findings: Not recorded.

Crawford CR & Anderson RH (1974a) The acute oral toxicity of two ®Nemacur phenolic metabolites and MTMC to male and female rats. Report No. 39700. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 27th February 1974.

Test Compound: Nemacur technical (fenamiphos); 88% purity; unspecified source
Batches: 3050053
Test Species: Male and female rats (4/sex/group); unspecified strain, age & source; 225-286 g bw for males and 190-225 g bw for females
Study Duration: 14 days
Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.
GLP & QA: None

Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage Fasted for 20 hours	20% ethanol:80% propylene glycol	14 days	1.0, 2.0, 4.0 & 8.0 (males) 2.0, 3.0, 4.5 & 6.8 (females)	2.4 (1.4-4.0) males 3.3 (2.7-4.1) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred one hour after dosing at and above 2.0 mg/kg bw in males and 3.0 mg/kg bw in females (2, 3 and 4/4 at 2.0, 4.0 and 8.0 mg/kg bw, respectively, in males and 1, 4 and 4/4 at 3.0, 4.5 and 6.8 mg/kg bw, respectively, in females). Clinical signs (ataxia and poor general condition) began at 15 or 30 minutes after dosing and lasted for one hour.

Necropsy findings: Not recorded.

Crawford CR & Anderson RH (1974b) The acute oral toxicity of ®Nemacur-sulfone and ®Nemacur-sulfoxide to rats. Report No. 40215. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 3rd April 1974.

Test Compound: Nemacur technical (fenamiphos); 97.4% purity; unspecified source
Batch: Not specified
Test Species: Male and female rats (4/sex/group); 230-300 g bw for males and 190-250 g bw for females; unspecified strain, age & source
Study Duration: 14 days
Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage Fasted for 20 hours	20% ethanol:80% propylene glycol	14 days	1.7, 1.9, 2.1 & 2.3 (males) 2.0, 2.5, 3.1 & 3.9 (females)	2.1 (1.8-2.3) males 3.1 (2.8-3.6) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred at and above 1.9 and 3.1 mg/kg bw in males and females, respectively (1, 3 and 3/4 at 1.9, 2.1 and 2.3 mg/kg bw, respectively in males and 2 and 4/4 at 3.1 and 3.9 mg/kg bw, respectively, in females). The time of death was up to 25 minutes after dosing. Clinical signs (described as "typical' of ChE inhibition) began at 5-30 minutes after dosing and lasted for 4 or 6 hours.

Necropsy findings: Not recorded.

Lamb DW & Matzkanin CS (1975) The acute oral toxicity of Nemacur technical, desisopropyl Nemacur sulfoxide and desethyl Nemacur. Report No. 44531. Lab: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 4th April 1975.

Test Compound: Nemacur technical; 88% purity; unspecified source
Batches: 5030001

Test Species: Male and female SD rats (5/sex/group); 200-255 g bw for females & 276-346 g bw for males; unspecified age & source
Study Duration: 14 days
Laboratory: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral (gavage) Fasted for 22-24 hours	20% ethanol:80% propylene glycol	14 days	2.4, 2.8, 3.2 & 3.7 females 2.2, 2.4, 2.8 & 3.2 males	3.0 (2.7-3.3) females 2.7 (2.4-3.0) males

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred at and above 2.4 and 2.8 mg/kg bw in males and females, respectively (2, 4 and 4/5 at 2.4, 2.8 and 3.2 mg/kg bw, respectively, in males, and 2, 3 and 5/5 at 2.8, 3.2 and 3.7 mg/kg bw, respectively, in females). The time of death was within 60 minutes of dosing. Clinical signs (tremors, salivation, lacrimation, diarrhoea and convulsions) occurred in all dosed females, and in all males at and above 2.4 mg/kg bw. Clinical signs began within 30 minutes of dosing and lasted for 2 hours.

Necropsy findings: Not recorded.

Krotlinger F (1988) SDF 1291 (c.n. Fenamiphos) Study for acute oral toxicity in rats. Report No. 16744. Study No. T0027669. Lab & Sponsor: Bayer AG Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, Wuppertal, Germany. Study dates: January to February 1988. Report date: 27th May 1988.

Test Compound: SDF 1291 techn. (c.n.Fenamiphos); ~97% purity; Bayer AG, Wuppertal, Germany
Batch: Not specified
Test Species: Wistar rats [strain Bor:WISW (SPF-Cpb)]; 5/sex/dose; 163-184 g bw and approximately 8-weeks old (males); 174-191 g bw and approximately 11-weeks old (females); sourced from Winkelmann Experimental Animal Breeders, Borchen, Kreis Paderborn, Germany
Study Duration: 14 days
Laboratory: Bayer AG Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, Wuppertal, Germany
GLP & QA: None
Guidelines: OECD Test Guideline No. 401 "Acute Oral Toxicity" (adopted May 12, 1981)

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50}* (mg/kg bw)
Oral gavage	Polyethylene glycol E400	14 days	1.0, 4.0, 5.0, 5.6, 6.3, 10.0 & 100.0 (males); 1.0, 5.0, 6.3 & 8.0 (females)	6.0 (4.1-9.1) males 6.1 (5.1-7.5) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred at and above 5.6 and 5.0 mg/kg bw in males and females, respectively (3, 4, 4 and 5/5 at 5.6, 6.3, 10.0 and 100.0 mg/kg bw, respectively, in males and 1, 3 and 4/5 at 5.0, 6.3 and 8.0 mg/kg bw, respectively, in females). Deaths occurred at 7-90 minutes after dosing. Apathy, palmospasms, laboured breathing and diarrhoea occurred in all males at and above 4.0 mg/kg bw, with piloerection, clonic cramps and dyspnoea occurring at higher doses. The severity of clinical signs (ranging from slight to moderate) increased with dose, with their duration ranging from 6 minutes to 3 days after dosing. In females, diarrhoea occurred at every dose, while signs consistent with those observed in males occurred at and above 5.0 mg/kg bw. The

bodyweights of survivors increased during the 14-day recovery period suggesting no effect on bodyweight gain.

Necropsy findings: The majority of rats that died during the study exhibited distended lungs, a darkened liver and a reddened glandular stomach. Other macroscopic abnormalities noted in a smaller number of rats included ulcer-like foci in the stomach, a pale or patchy dark spleen, a reddened small intestine or dark patches on the lungs. In contrast, there were no macroscopic abnormalities detected in any survivors sacrificed after 14 days.

Krotlinger F (2000a) SRA 3886 (c.n. Fenamiphos) Study for acute oral toxicity in rats. Report No. PH-29985. Study No. T2068710. Lab & Sponsor: Institute for Toxicology, Department of Short-Term Rodent Studies and Neurotoxicology, Bayer AG, 42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany. Study dates: 9th November 1999 to 21st June 2000. Report date: 30th June 2000.

Test Compound: SRA 3886 techn. (c.n.Fenamiphos); purity unspecified, Bayer AG, Wuppertal, Germany
Batch: NCH-031*T
Test Species: Female Wistar rats (strain HsdCpd:Wu); 3 rats/dose; 165-173 g bw and approximately 9-weeks old; sourced from Harlan Winkelmann GmbH Borchen, Kreis Paderborn, Germany
Study Duration: 14 days
Laboratory: Institute for Toxicology, Department of Short-Term Rodent Studies and Neurotoxicology, Bayer AG, 42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany.
GLP & QA: GLP compliant [OECD Principles of GLP (as revised in 1997); Annex 1 German Chemical Act (Bundesgesetzblatt Part 1 of the 29th July 1994); FIFRA GLP Standards (40 CFR Part 160); Japanese Ministry of Agriculture. Forestry and Fisheries (JMAFF, 59 NohSan No. 3850)]
QA statement
Guidelines: OECD Test Guideline No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted May 22, 1996); Annex IV B, Part B, B.1 tris (Acute toxicity [oral] – Acute Toxic Class Method) to Directive 67/548/EEC of the Council of the European Communities of June 27, 1967 and its subsequent amendments; Health Effects Test Guidelines (OPPTS 870.1100), Acute Oral Toxicity (US EPA, 712-C-98-190)

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	2% (v/v) Cremophor EL in demineralised water	14 days	25 mg/kg bw	<25 mg/kg bw

Clinical signs: All rats died within 10 minutes of dosing. Palmospasms, dyspnoea and swollen buccal regions were observed in all rats, with single animals also exhibiting chromodacryorrhoea or salivation. These signs occurred from 5-10 minutes after dosing and all were graded as weak in intensity, with the exception of palmospasms, which were moderate/severe.

Necropsy findings: All rats had dark red discolouration of the liver and slightly collapsed lungs.

Detzer K (2002) SRA 3886 Acute toxicity study in beagle dogs (acute gavage study). Study No. T6069380. Report No. PH31934. Lab: Department of Toxicology-Pharma, Institute of Toxicology, Bayer AG, Friedrich-Ebert-Strasse, Wuppertal, Germany. Sponsor: Bayer Institute of Toxicology, Friedrich-Ebert-Strasse, Wuppertal, Germany. Study duration: 22nd March to 31st October 2000. Report date: 4th September 2002

[*The aim of this study was to establish a NOEL for the purposes of setting an ARfD*]

GLP & QA: OECD Principles of GLP (as revised in 1997); Annex 1 German Chemical Act (Bundesgesetzblatt Part 1, No. 21, May 14, 2001); FIFRA GLP Standards (40 CFR Part 160); GLP standards of Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11-NohSan-No. 6283)]; QA statement

Materials and Methods

Fenamiphos (Batch No. NCH-031*T; 95.4% purity; unspecified source) was formulated in 2% Cremophor and water and administered as a single oral gavage dose to male and female beagle dogs. In the first of 3 trials, 2 dogs/sex were dosed at 0.500, 0.250, 0.125, 0.063 and 0.500 mg/kg bw, with a 3 week washout period between in each dose (note: dogs were dosed in a descending manner). In the second trial, 2 dogs/sex/group were given a single dose of 0.500 or 2.00 mg/kg bw. In the third trial, 2 dogs/sex were given a single dose of 1.00 mg/kg bw. It was stated that the dose selection was based on the results of previous studies conducted in rats and dogs. No concurrent control group was included in this study (control brain ChE activity data was obtained from study No.s T1069880 and T6070009).

Dogs were sourced from Harlan Winkelmann (Borchen, Germany). Male bodyweights ranged from 8.1-9.6 kg and female bodyweights ranged from 6.2-10.2 kg. The age of the dogs was not specified. Dogs were housed individually through all phases of the study under standard conditions. Dogs were offered 300-350 g feed per day, while water was available *ad libitum*.

Observations for clinical signs were made each day, with additional examinations made on treatment days (20, 30, 60, 90, 120, 180, 240, 300 and 360 minutes for Trial 1, 30, 60, 90 and 120 minutes for Trial 2 and 0.5, 1, 2, 4, 7 and 24 hours for Trial 3). Atropine was administered if convulsions occurred. Food consumption was recorded on a daily basis and bodyweights were recorded weekly (Trial 1). Plasma and RBC ChE activities were analysed at various times after dosing. In Trial 1, plasma and RBC ChE activities were measured at 0, 20, 60, 90, 120, 180, 240 and 360 minutes, at 24 hours, and at 7, 14, and 21 days after dosing. In trial 2, plasma and RBC ChE activities were measured at 0, 90 and 120 minutes after dosing. In Trial 3, plasma and RBC ChE activities were measured at 0, 20, 120 and 240 minutes, and at 24 hours after dosing.

At the end of the observation period (21 days in Trial 1, two hours in Trial 2 and three or four hours in Trial 3), dogs were sacrificed by exsanguination under Narcoren® anaesthesia. Brain tissue was taken from the forebrain and stored frozen at -70°C prior to analysis of brain ChE activity. In Trial 1, brain ChE activity was measured 1 hour after administration of the final dose (i.e. the repeat dose with 0.500 mg/kg bw). In Trial 2, brain ChE activity was measured at 2 hours after dosing. In Trial 3, brain ChE activity was measured at 3 or 4 hours after dosing.

No statistical analysis was performed due to the small number of animals tested.

Results

Mortalities and clinical signs: In trial 1 (0.063-0.5 mg/kg bw), there were no mortalities and no apparent treatment-related clinical signs, although in the absence of a concurrent control it was unclear whether the occurrence of mushy faeces was treatment-related. In trial 2 (0.5 & 2 mg/kg bw), vomiting occurred in one female from 60-90 minutes after administration of the 0.5 mg/kg bw dose. Clinical signs occurred in all dogs for up to 2 hours after dosing at 2 mg/kg bw, which required the administration of 0.2 mg atropine at approximately 30 minutes after dosing. These clinical signs included reeling gait (4/4 dogs), vocalisation (3/4), cramp-like biting (1/4), lateral position (4/4), shivering (3/4), emesis (2/4), diarrhoea (1/4), tonic cramps (3/4), salivation (1/4),

vehement breathing (1/4) and restlessness (1/4). In trial 3 (1 mg/kg bw) there were no mortalities, with clinical signs observed in all dogs from 30 minutes to 240 minutes after dosing. These clinical signs included vocalisation, lateral position, reeling gait, salivation, slight shivering and vomiting.

Bodyweights and food intake: It was reported that there was no reduction in food consumption in Trial 1. Bodyweight gain in dogs in Trial 1 appeared unremarkable, although the absence of a concurrent control limited the value of the bodyweight data.

ChE activity measurements: In Trial 1, there was dose-related inhibition of plasma ChE activity (up to 21, 48, 50 and 67% at 0.063, 0.125, 0.250 and 0.500 mg/kg bw, respectively), with the effect at 0.063 mg/kg bw considered to be of borderline toxicological significance as the level of inhibition was only just above 20% at one time point (120 minutes) (see table below). Inhibition was maximal at approximately 60-120 minutes after dosing. By 24 hours, there was no toxicologically-significant inhibition of plasma ChE activity. For RBC ChE activity, slight inhibition (up to 19% relative to pre-treatment activity) occurred only at the highest dose (0.5 mg/kg bw) at approximately 60-90 minutes after dosing. However, as the level of inhibition was below 20%, it was not considered toxicologically-significant. It should be noted that in the second Trial (see table below), toxicologically-significant inhibition of RBC ChE activity occurred at 0.5 mg/kg bw. There was no inhibition of brain ChE activity based on a comparison with control groups from two previous studies.

Table 14 Results of plasma ChE activity measurements (Trial 1)

Time after dosing	Dose (mg/kg bw)			
	0.500	**0.250**	**0.125**	**0.063**
0	1.92±0.4 (0%)	2.00±0.53 (0%)	1.94±0.50 (0%)	1.91±0.48 (0%)
20 minutes	0.70±0.42 (64%)	1.40±0.63 (30%)	1.15±0.44 (41%)	1.66±0.42 (13%)
60 minutes	0.64±0.14 (67%)	1.05±0.42 (48%)	1.00±0.26 (48%)	1.57±0.34 (18%)
90 minutes	0.69±0.19 (64%)	1.01±0.39 (50%)	1.01±0.23 (48%)	1.62±0.43 (15%)
120 minutes	0.74±0.18 (61%)	1.01±0.39 (50%)	1.04±0.25 (46%)	1.51±0.38 (21%)
180 minutes	0.80±0.22 (58%)	1.07±0.38 (47%)	1.08±0.22 (44%)	1.59±0.39 (17%)
360 minutes	1.09±0.33 (43%)	1.31+0.43 (35%)	1.20±0.33 (38%)	1.58±0.43 (17%)
24 hours	1.61±0.41 (16%)	1.67±0.40 (17%)	1.67±0.44 (14%)	1.82±0.50 (5%)
7 days	1.92±0.46 (0%)	1.87±0.47 (7%)	1.88±0.47 (3%)	2.07±0.47 (-8%)
14 days	2.07±0.52 (-7%)	1.92±0.47 (4%)	1.91±0.49 (2%)	2.06±0.52 (-8%)
21 days	2.00±0.53 (-4%)	1.94±0.50 (3%)	1.91±0.48 (2%)	1.97±0.45 (-3%)

Results expressed as the mean ± 1 SD (kU/L), with the % inhibition relative to pre-treatment activity (0) contained in parentheses

In Trials 2 and 3, toxicologically-significant inhibition of plasma ChE activity occurred from 60 minutes after administration of each dose (see table below). The level of inhibition (relative to pre-treatment activity) was up to 81, 97 and 98% at 0.5, 1 and 2 mg/kg bw, respectively. Toxicologically-significant inhibition of RBC ChE activity occurred at each dose (up to 42, 90 and 96% at 0.5, 1 and 2 mg/kg bw, respectively) and peaked at 60 minutes. At 1 mg/kg bw, RBC ChE activity had recovered by 24 hours after dosing. There was no apparent inhibition of brain ChE activity at any dose based on a comparison of findings with control groups from two previous studies.

Table 15 Results of plasma and RBC ChE activity measurements (Trials2 &3)

Time after dosing	Dose (mg/kg bw)		
	0.500	1	2
Plasma ChE activity			
0	1.96 (1.66-2.43; 0%)	1.86 (1.56-2.24; 0%)	1.54 (1.47-1.58; 0%)
60 minutes	0.38 (0.17-0.55; 81%)	0.06 (0.05-0.07; 97%)	0.03 (0.02-0.04; 98%)
120 minutes	0.56 (0.12-0.77; 71%)	0.13 (0.08-0.16; 93%)	0.04 (0.03-0.05; 97%)
240 minutes	-	0.48 (0.14-0.86; 74%)	-
24 hours	-	1.43 (1.22-1.77; 23%)	-
RBC ChE activity			
0	1.29 (1.17-1.44; 0%)	1.58 (1.17-1.97; 0%)	1.21 (0.77-1.66; 0%)
60 minutes	0.75 (0.51-0.95; 42%)	0.17 (0.09-0.30; 90%)	0.05 (0.03-0.06; 96%)
120 minutes	0.84 (0.44-1.17; 35%)	0.21 (0.14-0.31; 87%)	0.08 (0.05-0.09; 77%)
240 minutes	-	0.53 (0.41-0.76; 66%)	-
24 hours	-	1.46 (1.06-1.73; 8%)	-

Results expressed as the mean (n=4), with the range and mean % inhibition relative to pre-treatment activity (0), contained in parentheses; - not measured at these times

Supplementary in vitro study: In the absence of the inhibition of brain ChE activity in dogs showing severe cholinergic signs, an *in vitro* study was performed to investigate the ability of fenamiphos to inhibit ChE activity in dog brain homogenates. Paraoxon, which is the oxygenated toxicologically active metabolite of parathion, was used as a positive control compound. Samples from 4 dogs were incubated in 0.9% saline and 1% Cremophor and containing 3 x 10^{-8} mol paraoxon, 1 x 10^{-7} mol paraoxon or 2 x 10^{-4} mol fenamiphos for 1, 2, 4 and 24 hours at 37°C. The table below summarises the experimental findings. The saline control group had a 20% loss of activity at 24 hours relative to the activity at 1 hour, indicating that it was only stable for 4 hours at 37°C. Fenamiphos induced a progressive inhibition of brain ChE activity to a level of approximately 78% of the control over 4 hours. The positive control compound, paraoxon, caused a constant level of inhibition (~30%). These findings indicate that fenamiphos can inhibit brain ChE activity in dogs, despite the absence of any inhibition in the main study. This suggests that fenamiphos cannot cross the BBB.

Table 16 Results of *in vitro* brain ChE activity measurements

Treatment	Time (hours)			
	1	2	4	24
0.9% saline	1.67 (1.59-1.70; 0%)	1.65 (1.62-1.70; 0%)	1.65 (1.56-1.78; 0%)	1.32 (1.21-1.35; 20%)
1% Cremophor	1.71 (1.59-1.88; 0%)	1.71 (1.56-1.78; 0%)	1.64 (1.53-1.75; 1%)	1.47 (1.37-1.53; 0%)
Paraoxon [3 x 10^{-8} mol]	1.55 (1.43-1.67; 7%)	1.54 (1.48-1.62; 6%)	1.56 (1.48-1.62; 6%)	1.24 (1.21-1.29; 6%)
Paraoxon [1 x 10^{-7} mol]	1.19 (1.10-1.35; 29%)	1.21 (1.10-1.37; 27%)	1.21 (1.16-1.29; 27%)	0.94 (0.83-1.00; 28%)
Fenamiphos [2 x 10^{-4} mol]	1.03 (0.92-1.10; 38%)	0.69 (0.59-0.78; 58%)	0.36 (0.30 & 0.38; 78%)	0.06 (0.03 & 0.08; 96%)

Results expressed as the mean U/g (n=4), with the range and % inhibition relative to the saline control group, contained in parentheses

Conclusions: The NOEL in dogs following a single oral gavage dose was 0.25 mg/kg bw, based on the toxicologically-significant inhibition of RBC ChE activity at and above 0.5 mg/kg bw. Toxicologically-significant plasma ChE inhibition occurred at and above 0.125 mg/kg bw. This study suggested that fenamiphos cannot cross the BBB and inhibit brain ChE activity. The absence of a concurrent control group was a deficiency of this study.

3.1.2 Dermal toxicity

Kimmerle G & Lorke D (1967) Toxicological studies with the compound Bay 68138. Report No. 272. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 4th April 1967.

Kimmerle G & Solmecke D (1971) Bay 68138 toxicological studies Report No. 2767. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 5th May 1971.

Test Compound: 25% emulsion of Bay 68138 (fenamiphos); purity & source unspecified
Batch: Not specified
Test Species: Male rats (5 or 10/dose); age, bw & strain unspecified
Study Duration: 7 days
Laboratory: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Doses tested (mg/kg bw)	Dermal LD_{50} (mg/kg bw)
Dermal application to clipped dorsal or abdominal skin under occluded conditions for 4 hours or 7 days	None	7 days	100, 250 & 500	500 (4-hour exposure) 500 (7-day exposure)

Clinical signs: Deaths occurred only at the highest dose following 4 hours of exposure and at and above 250 mg/kg bw after 7 days of exposure. The time to death was not reported. Clinical signs (unspecified) occurred at every dose following either four hours or 7 days of exposure.

Necropsy findings: Not recorded

Crawford CR & Anderson RH (1972) The acute dermal toxicity of ®Nemacur Technical to rabbits. Report No. 34216. Lab: Chemagro Division of Baychem Corporation Research and Development, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 20th July 1972.

Test Compound: Nemacur technical (fenamiphos); 81% purity; unspecified source
Batch: 0050136
Test Species: Male and female NZW rabbits (4/sex/dose); 1.9-2.5 kg bw; unspecified age & source
Study Duration: Not specified
Laboratory: Chemagro Division of Baychem Corporation Research and Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Doses tested (mg/kg bw)	Dermal LD_{50}* (mg/kg bw)
Dermal application to clipped dorsal skin under occluded conditions for 24 hours	acetone	14 days	112.5, 225.0, 450.0 & 900.0 (males); 56.3, 112.5, 225.0 & 450.0 (females)	225 (151-336) males 179 (72-404) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred at and above 225 mg/kg bw in males (2, 4 and 4/4 at 225, 450 and 900 mg/kg bw, respectively) and 112.5 mg/kg bw in females (2, 2 & 3/4 at 112.5, 225 & 450 mg/kg

bw, respectively). The time to death in males was 3-6 hours after exposure and from 8 hours to 4 days in females, depending on the dose. Clinical signs occurred in all rabbits at all doses and included tremors, salivation, diarrhoea and inconsistent miosis. These signs developed at 1-4 hours after exposure depending on the dose, and persisted for an unspecified period of time.

Necropsy findings: Not recorded

Flucke W (1980) Determination of acute toxicity (LD_{50}) Report & Study No. Unspecified. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal, Germany. Study dates: unspecified. Report date: 18th July 1980.

Test Compound: SRA 3886 (c.n. fenamiphos); 92.2% purity; unspecified source
Batch: Not specified
Test Species: Rats (strain unspecified); 5, 10 or 20/sex/dose; bw, age and source unspecified
Study Duration: 14 days
Laboratory: Institute of Toxicology, Bayer AG, Wuppertal, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Doses tested (mg/kg bw)	Dermal LD_{50} (mg/kg bw)
Dermal application to occluded skin for 24 hours. No other details given	Lutrol (PEG)	14 days	25, 35, 40, 45, 50, 65, 75, 100, 125 &1000 (males); 25, 50, 75, 100, 125 & 1000 (females)	72 (range 62-83) Males 92 (range 79-108) Females

Clinical signs: Not recorded

Necropsy findings: Not recorded.

Krotlinger F (2000b) SRA 3886 (c.n. Fenamiphos) Study for acute dermal toxicity in rats. Report No. PH-29884. Study No. T9069211. Lab & Sponsor: Institute for Toxicology, Department of Short-Term Rodent Studies and Neurotoxicology, Bayer AG, 42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany. Study dates: 9th November 1999 to 11th January 2000. Report date: 15th May 2000.

Test Compound: SRA 3886 techn. (c.n.Fenamiphos); purity unspecified; sourced from Bayer AG, Wuppertal, Germany;
Batch: NCH-031*T
Test Species: Wistar rats (strain HsdCpd:Wu); 5 rats/dose; 243-298 g bw and approximately 9-weeks old (males); 201-236 g bw and approximately 12-weeks old (females); sourced from Harlan Winkelmann GmbH Borchen, Kreis Paderborn, Germany
Study Duration: 14 days
Laboratory: Institute for Toxicology, Department of Short-Term Rodent Studies and Neurotoxicology, Bayer AG, 42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany.
GLP & QA: GLP compliant [OECD Principles of GLP (as revised in 1997); Annex 1 German Chemical Act (Bundesgesetzblatt Part 1 of the 29th July 1994); FIFRA GLP Standards (40 CFR Part 160); Japanese Ministry of Agriculture. Forestry and Fisheries (JMAFF, 59 NohSan No. 3850)]
QA statement
Guidelines: OECD Test Guideline No. 402 "Acute Dermal Toxicity " (adopted February 24, 1987); Annex V Part B.3 (Acute toxicity [dermal] to Directive 67/548/EEC of the Council of the European Communities of June 27, 1967 and its subsequent amendments; Health Effects Test Guidelines (OPPTS 870.1200), Acute Dermal Toxicity (US EPA, 712-C-98-192, August 1988)

Dosing method	Vehicle	Observation Period	Doses tested (mg/kg bw)	Dermal LD_{50} (mg/kg bw)
Dermal application to clipped intact occluded skin for 24 hours	None	14 days	400, 1000 & 2000 (males); 400 & 2000 (females)	>2000

Clinical signs: There were no deaths in males, while a single high-dose female died two days after dosing. Clinical signs were observed in both sexes and their severity tended to increase with dose. Palmospasms occurred in all males at 1000 ("weak") and 2000 mg/kg bw ("moderate to strong"). In addition, "weak" laboured breathing occurred in one high-dose male and all clinical signs lasted for two days. In females, "moderate to severe" palmospasms, "weak" laboured breathing and "weak" to "moderate to severe" salivation occurred at 400 and 2000 mg/kg bw (affecting 2 or 3 and all rats, respectively) and lasted from 2-3 days at the low dose and 4 hours to 2 days at the high dose. Single females also exhibited "weak" decreased mobility and reactivity (400 mg/kg bw) or "weak" dyspnoea and chromodacryorrhea (2000 mg/kg bw). It was reported that there were no signs of skin irritation at the application site. In males, average bodyweight gains during the 14 day recovery period were consistent across all doses, suggesting no effect of treatment. At 2000 mg/kg bw, females gained relatively little bodyweight during the 14 day recovery period, with modest gains also noted at 400 mg/kg bw.

Necropsy findings: The single high-dose female that died had dark red discolouration of the liver. There were no macroscopic abnormalities detected in any survivors.

3.1.3 Inhalational Toxicity

Thyssen J (1979a) SRA 3886 (Nemacur active ingredient) Acute inhalational studies. Report No. 8210 Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal, Germany. Study dates: Report date: 23rd February 1979.

Test Compound:	SRA 3886 (c.n. fenamiphos); 89.8% purity, unspecified source
Batch:	Not specified
Test Species:	Albino rats (strain TNO/W 74); 10/sex/dose; females ~180 g bw, males ~200 g bw; age unspecified; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany
Study Duration:	14 days
Laboratory:	Institute of Toxicology, Bayer AG, Wuppertal, Germany.
GLP & QA:	None
Guidelines:	None

Dosing method	Vehicle	Observation period	Concentrations tested (mg/m^3)*	Inhalation LC_{50} (mg/m^3)
Head-only exposure to aerosols (particle size unspecified) for 1 or 4 hours	Ethanol:Lutrol (1:1)	14 days	83, 119, 145, 148 & 250 (1-hour exposure); 57, 62, 100, 155 (both sexes) & 191 (females) (4-hour exposure)	1-hour male 131 (range 114-151) 1-hour female 130 4-hour both sexes 100 (range 85-118 females)

* No vehicle control appears to have been tested

Clinical signs: Within one hour of a one hour exposure, mortalities occurred at and above 119 mg/m^3 in males and 145 mg/m^3 in females (2, 6, 9 and 10/10 at 119, 145, 148 and 250 mg/m^3, respectively, and 7, 9 and 10/10 at 145, 148 and 250 mg/m^3 in females, respectively) Following a 4 hour exposure, mortalities occurred at and above 100 mg/m^3 in both sexes within 2 or 4 hours of the exposure (6 and 10/10 at 100 and 155 mg/m^3, respectively, in males and 1, 5, 9 and 10/10 at 62, 100, 155 and 191 mg/m^3, respectively, in females). All rats in all groups exhibited muscle

twitching, with muscle cramps also noted at lethal concentrations. These signs lasted for up to 7 hours after exposure. In addition, more general clinical signs were exhibited by all rats, such as inactivity, stiff gait and rough hair coats for up to 5 days post-exposure. Higher exposure concentrations reportedly generated more severe clinical signs.

Necropsy: Not recorded.

Pauluhn J (2001) SRA 3886 (Common name fenamiphos) Study on acute inhalational toxicity in rats according to OECD No. 403 and OPPTS 870.1300 Report No. PH-30977. Study No. T4069685. Lab: Institute for Toxicology/Laboratory Inhalation Toxicology, Bayer AG, D-42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany. Sponsor: Bayer AG, Agriculture Division. Report date: 3rd May 2001.

Test Compound:	SRA 3886 (c.n.Fenamiphos); 95.45% purity, sourced from Bayer AG, Wuppertal, Germany
Batch:	NCH-031*T
Test Species:	Wistar rats (strain HsdCpd:Wu); 5 rats sex/dose; 163-209 g male bw range, 156-184 g female bw range; approximately 2-months old; sourced from Harlan Winkelmann GmbH Borchen, Kreis Paderborn, Germany
Study Duration:	14 days
Laboratory:	Institute for Toxicology/Laboratory Inhalation Toxicology, Bayer AG, D-42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany.
GLP& QA:	GLP compliant [OECD Principles of GLP (as revised in 1997); Annex 1 German Chemical Act (Bundesgesetzblatt Part 1 of the 29th July 1994); FIFRA GLP Standards (40 CFR Part 160); Japanese Ministry of Agriculture. Forestry and Fisheries (JMAFF, 59 NohSan No. 3850)] QA statement
Guidelines:	OECD Test Guideline No. 403 "Acute Inhalational Toxicity " (adopted 12 May 1981); EC guideline 92/69/EEC; FIFRA 81-3 (US EPA 1984) and OPPTS Guidelines (1998)

Dosing method	**Vehicle**	**Observation period**	**Concentrations tested (mg/m^3)**	**Inhalation LC_{50} (mg/m^3)**
Nose-only exposure to a liquid aerosol* for 4 hours	PEG 400:ethanol (1:1 w/v)	14 days	0, 64, 65, 92, 243 & 511	74 (range 67-81)

* average particle size was approximately 1.4 μm

Clinical signs: There was a concentration-related increase in mortalities starting from the lowest dose in males and 65 mg/m^3 in female. All males died at and above 92 mg/m^3 and all females at and above 243 mg/m^3. At these concentrations the onset of death was less than 4 hours, while at lower dose it was within one day of exposure. All rats exposed to fenamiphos exhibited clinical signs, with the onset and duration of signs ranging up to 3 and 9 days after exposure, respectively. The types of signs observed depended on the exposure concentration and included piloerection, rough hair-coats, bradypnoea, laboured breathing, dyspnoea, irregular breathing pattern, reduced mobility, limping, tremor, fasciculations, giddiness, high-legged gait, exophthalmos, miosis, corneal opacity, chromodacryorrhoea, red encrusted nostrils, salivation, pallor, emaciation and periorbicular red stains. Signs that were delayed in their onset included rough hair coats (1 day), irregular breathing (2 days), limp (1 day), tremor (1 or 3 days), red encrusted nostrils (1 day) and emaciation (2 or 3 days). Signs that persisted for 4 or more days after exposure included piloerection, rough hair coats, bradypnoea, laboured breathing, reduced motility and a limping.

In survivors, there was a significant ($p<0.01$) concentration-related decrease in rectal temperature at and above the lowest concentration within half an hour of the end of exposure (males: 0/63.6/64.7 mg/m^3 = 37.7/30.2/25.8°C; females: 0/63.6/64.7/92.1 mg/m^3 = 38.1/30.4/26.5/26.0°C).

Reflex measurements made one day after exposure revealed a number of abnormalities in some survivors of both sexes [i.e. at 64 and 65 mg/m^3 in males (1 or 2 rats); at and below 92 mg/m^3 in females (1, 2 or 3 rats)]. These abnormalities included reduced grip strength, reduced tonus, miosis and impaired or slightly uncoordinated righting response. Three days after exposure, the average bodyweights of male survivors at 64 and 65 mg/m^3 were 13 and 18% lower, respectively, compared to their pre-treatment weights. When compared to the control group, these losses were statistically significant ($p<0.05$), however, the bodyweights of these groups was already significantly lower than the control group prior to treatment (i.e. at day 0). There was a similar loss of bodyweight in females 3 days after exposure with a 10-20% loss occurring at 64, 65 and 92 mg/m^3. These losses were significantly different to the control ($p<0.01$) and it should be noted that the bodyweights of these groups were not significantly different to the control prior to exposure. The bodyweights of both sexes recovered over the remainder of the 14-day observation period.

Necropsy: All treated rats that died during the study had lungs that were less collapsed (than controls), with the majority also appearing pale and/or having a few dark red foci. In addition, bloated stomachs (and intestines in some rats) and pale organs (particularly the spleen) were observed. Most also had a discharge from the muzzle. Corneal opacity (uni/bilateral) was noted in a smaller proportion of dead rats (one male and 2 females at 92 mg/m^3; 2 males and 2 females at 511 mg/m^3). All survivors (including controls) sacrificed at the end of the 14-day observation period showed no macroscopic abnormalities, with the exception of one male exposed at 65 mg/m^3 that had less collapsed and pale lungs.

3.1.4 Skin and eye irritation studies

Skin irritation

Crawford CR & Anderson RH (1971) The skin and eye irritating properties of Bay 68138 technical to rabbits. Report No. 29988. Lab: Chemagro Corporation, Research Department, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 28th September 1971.

Test Compound:	Bay 68138 Technical (fenamiphos); unspecified purity & source
Batch:	9050536
Test Species:	6 NZW rabbits (mature); unspecified bw & source
Study Duration:	Not specified
Laboratory:	Chemagro Corporation, Research Department, unspecified location.
GLP & QA:	None
Guidelines:	None

Methods: Fenamiphos (50 mg in 0.5 mL acetone) was applied to abraded and non-abraded, clipped dorsal skin of rabbits via an occlusive dressing (abraded and non-abraded sites were used on each rabbit). No vehicle control group was included. The application site was one square inch (6.45 cm^2). The dressing was removed after 24 hours and the application site examined. The application site was examined again at 72 hours. Dermal irritation was scored using an unspecified scale.

Results: No signs of irritation occurred at the unabraded site on each of the 6 rabbits. Slight erythema occurred at the abraded site on 2/6 rabbits at 24 hours, while none was observed at 72 hours. On the basis of these findings fenamiphos is classifiable as a non-skin irritant

Kato M (1984a) Primary dermal irritation study of fenamiphos technical in rabbits. Study No. B-561. Lab: The Laboratory of Safety Evaluation, Kannami Laboratory, Bozo Research Center

Inc., 1308-125 Kuwabara Sanbonmatsu, Kannamicho, Tagata-gun, Japan. Sponsor: Nihon Tokushu Noyaku Seizo k.k., 4 Nihonbashi Honcho, 2-Chome, Chuo-ku, Tokyo 103, Japan. Study dates: 27th April to 29th September 1984. Report date: 29th September 1984.

Test Compound:	Fenamiphos technical; 90.7% purity; sourced from Nihon Tokushu Noyaku Seizo k.k Japan
Batch:	(lot number) pt.816396002
Test Species:	Japanese native-derived albino rabbits; 6 male rabbits; ~12 weeks old; 2.84-3.06 kg bw range; sourced from Ichikawaya Co. Ltd, Takenotsuka, Adachi, Tokyo, Japan
Study Duration:	27th April to 29th September 1984
Laboratory:	The Laboratory of Safety Evaluation, Kannami Laboratory, Bozo Research Center Inc., 1308-125 Kuwabara Sanbonmatsu, Kannamicho, Tagata-gun, Japan
GLP& QA:	None
Guidelines:	It was stated that the study protocol was similar to US EPA guidelines (unspecified)

*Method*s: 0.5 mL test compound was applied to the intact or abraded, clipped occluded dorsal skin of each rabbit. Twenty-four hours after application, the occlusive dressing was removed and the site decontaminated with distilled water. Skin reactions were subsequently scored according to the Draize Scale at 24, 48, 72 and 168 hours.

Results: There were no mortalities or any signs of systemic toxicity. At 24 hours, 4 rabbits exhibited very slight erythema and one had slight oedema, at both the abraded and unabraded sites. At 48 hours, no oedema was present, while 2 rabbits still had very slight erythema at both abraded and unabraded sites. At 72 hours, no rabbits exhibited any signs of skin irritation. On the basis of these findings, fenamiphos is classifiable as a slight skin irritant.

Eye irritation

Crawford CR & Anderson RH (1971) The skin and eye irritating properties of Bay 68138 technical to rabbits. Report No. 29988. Lab: Chemagro Corporation, Research Department, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 28th September 1971.

Test Compound:	Bay 68138 Technical (fenamiphos); unspecified purity and source
Batch:	9050536
Test Species:	6 NZW rabbits (mature); unspecified bw & source
Study Duration:	Not specified
Laboratory:	Chemagro Corporation, Research Department, unspecified location.
GLP & QA:	None
Guidelines:	None

Methods: 100 mg of fenamiphos (as a crystalline solid) was administered in to the left eye of 6 rabbits, with the right eye serving as the control. After 24 hours, eyes were scored for irritation according to the *Illustrated Guide for Grading Eye Irritation by Hazardous Substances* (US Department of Health, Education and Welfare, FDA). Corneal injury was examined under UV light following instillation of a drop of 2% fluorescein solution. Eye irritation was also scored at 48 and 72 hours.

Results: The table below summarises the mean eye irritation scores in rabbits. There was no corneal opacity, and no erythema or lachrymation of the conjunctivae. Signs of irritation occurred in 3 of the 6 rabbits. Iritis occurred in 3 rabbits (grade 1 or 2) at 24 hours, 2 of these same rabbits at 48 hours (grade <1) and had resolved by 72 hours. Chemosis occurred in 2 of the same 3 rabbits at 24 hours (grade 1), 48 hours (grade <1) and had resolved by 72 hours. One of these two rabbits had developed sclerotic congestion at 72 hours. On the basis of these findings, fenamiphos was

classifiable as a slight eye irritant in rabbits. The authors suggested that the observed reactions were due to mechanical rather than chemical irritation.

Table 17 Mean eye irritation[1] scores in rabbits (n=6)

Lesion	Time after administration					
	24 hours		48 hours		72 hours	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Iris	0	0.83	0	<0.2	0	0
Conjunctivae						
Chemosis	0	0.33	0	<0.33	0	0

1 = calculated by the evaluator based on individual animal scores

Kato M (1984b) Primary eye irritation study of fenamiphos technical in rabbits. Study No. B-562. Lab: The Laboratory of Safety Evaluation, Kannami Laboratory, Bozo Research Center Inc., 1308-125 Kuwabara Sanbonmatsu, Kannamicho, Tagata-gun, Japan. Sponsor: Nihon Tokushu Noyaku Seizo k.k., 4 Nihonbashi Honcho, 2-Chome, Chuo-ku, Tokyo 103, Japan. Study dates: 14th-21st May 1984. Report date: 29th September 1984.

Test Compound: Fenamiphos technical; 90.7% purity; Nihon Tokushu Noyaku Seizo k.k, Japan
Batch: (lot number) pt.816396002
Test Species: Japanese native-derived albino rabbits; 9 male rabbits; ~12 weeks old; 2.92-3.01 kg bw range; sourced from Ichikawaya Co. Ltd, Takenotsuka, Adachi, Tokyo, Japan
Study Duration: 14th to the 21st May 1984
Laboratory: The Laboratory of Safety Evaluation, Kannami Laboratory, Bozo Research Center Inc., 1308-125 Kuwabara Sanbonmatsu, Kannamicho, Tagata-gun, Japan
GLP & QA: None
Guidelines: It was stated that the study protocol was similar to US EPA guidelines (unspecified)

Methods: 0.1 mL of undiluted fenamiphos was instilled into the conjunctival sac of one eye of 6 rabbits, with the other eye serving as an untreated control. An additional group of 3 rabbits was similarly treated except that both eyes were washed with distilled water 20-30 seconds after sample application. Eye reactions were scored at 24, 48, 72, 96 hours and 10 days after application. Scoring was based on unspecified criteria.

Results: All rabbits in the unwashed group showed mydriasis in the treated eye approximately 10 minutes after sample application, which recovered after 2 or 3 days. In addition, the lid of the treated eye became closed 2 hours after sample application and recovered after 1 or 2 days. All rabbits in the unwashed group exhibited signs of systemic toxicity, which was reported to appear 3-4 hours after sample application, with all rabbits recovering by 6 hours. Clinical signs observed included salivation, rhinorrhoea, licks, increased respiration, cyanosis, slight convulsions and myasthenia. There were no signs of irritation or systemic toxicity in the 3 rabbits whose eyes were washed after sample application.

There were no signs of irritation in rabbits whose eyes were washed 20-30 seconds after the application of fenamiphos. In the unwashed group, all 6 rabbits exhibited signs of irritation in the cornea, iris and conjunctivae (see table below). These signs included: scattered/diffuse corneal opacity that affected the majority of the cornea at 24 hours, with the size of the lesion lessening over 7 days, hyperemia of the iris and slight erythema, swelling and discharge of/from the conjunctivae. Irritation was still evident, although improved, 7 days after sample application and had resolved completely at day 10. Based on these findings, fenamiphos is classified as a moderate eye irritant.

Table 18 Mean eye irritation scores in rabbits (n=6)

Lesions	Time after administration					
	24 hours	48 hours	72 hours	96 hours	168 hours	10 days
Cornea						
Opacity	1	1	1	1	0.67	0
Area	4	3.7	2.2	1.5	0.67	0
Iris	1	1	0.83	0.67	0.17	0
Conjunctivae						
Erythema	1	0.83	0.83	0.67	0.17	0
Chemosis	0.83	0	0.17	0.37	0.17	0
Lachrymation	1.3	0.17	0.17	0.17	0	0

3.1.5 Skin sensitisation

Watanabe M (1983) Fenamiphos dermal sensitization study in the guinea pigs. Report No. 252 Lab: Nihon Tokushu Noyaku Seizo k.k., Agricultural Chemical Institute Toxicological Research Laboratory, 1-1 Toyoda 3-chome, Hino-shi, Tokyo 191, Japan. Sponsor: Nihon Tokushu Noyaku Seizo k.k., 4 Nihonbashi Honcho, 2-Chome, Chuo-ku, Tokyo 103, Japan. Study Duration: Unspecified. Report Date: April 1983

Test Compound: Fenamiphos technical; 90.7% purity; sourced from Nihon Tokushu Noyaku Seizo k.k, Japan;
Batch: (lot number) pt.233190327
Test Species: Hartley-Strain albino guinea pigs; 20 females/group; ~300 g bw; sourced from SAITAMA Experimental Animals Supply Co., Japan
Study Duration: Unspecified
Laboratory: Nihon Tokushu Noyaku Seizo K.K., Agricultural Chemical Institute Toxicological Research Laboratory, 1-1 Toyoda 3-chome, Hino-shi, Tokyo, Japan
GLP & QA: None
Guidelines: None

Methods: The skin sensitisation potential of fenamiphos was tested in guinea pigs based on a modified Freund's Complete Adjuvant Test (Professor Yoshio Takase, Dept Dermatology, Shinshu University School of Medicine, Japan).

During the induction phase, 20 guinea pigs/group, were given 3 injections (totalling 0.3 mL; 0.1 mL/day) of a 0.5 or 5.0% solution of fenamiphos and Freund's complete adjuvant (1:1) in physiological saline. Of the 3 injections, one was in the subcutaneous tissue on the back of the shoulder, while the other 2 were in the right and left femoral muscles. The injection sites had previously been cleared of hair with electric clippers. The concentrations of fenamiphos were reportedly chosen based on preliminary results showing that 5.0% fenamiphos caused definite inflammation and 0.5% caused slight inflammation. A control group of 20 guinea pigs remained untreated. Two weeks after the final induction injection, 10 guinea pigs from each group of 20 (including the control) were challenged with a 0.05 mL intradermal injection of fenamiphos in physiological saline at 0.005, 0.01, 0.05, 0.1, 0.5 or 1.0%. The remaining 10 guinea pigs were challenged by patch-testing for 24 hours with 5.0, 10, 50 and 90.2% fenamiphos at sites that had previously been cleared of hair.

All guinea pigs were observed for mortalities and clinical signs. Control guinea pigs were sacrificed 24 hours after challenge, while those challenged via the intradermal route were sacrificed after 48 hours. Skin samples were excised and examined for the severity and area of erythema. Guinea pigs challenged via topical application (i.e. patch-tested) were sacrificed 24 and 48 hours after the patch was removed. The skin of these guinea pigs was macroscopically examined and evaluated

according to the scoring system of Tanioku Kihei et al ("Skin and Immunity, Allergy" Kanehara Shuppan Co. Ltd.; date unspecified). Results were statistically analysed using a χ^2-test, Fisher's exact test or Wilcoxon's rank sum test.

Results: Seven of the 20 guinea pigs induced with 5.0% fenamiphos died. One animal died 24 hours after the first induction injection, while the remaining 6 died within 24 hours of the third injection. Clinical signs noted in these animals were diarrhoea, salivation and tremors. The majority of animals (i.e. $\geq$8/10) that were challenged topically showed no signs of inflammation. Minimal erythema was observed in a few animals from each group (including the controls) and there were no significant differences between the groups. In guinea pigs that were challenged intradermally, a positive inflammatory response (erythema >2.5 mm in diameter) occurred in 0-8 animals per group of 10 (including the non-induced controls). However, there was no significant difference between the control and induced groups. Based on these findings, fenamiphos was classifiable as a non-skin sensitiser.

Stropp G (1995) SRA 3886: Study for the skin sensitization effect in guinea pigs (Guinea Pig Maximisation Test according to Magnusson and Kligman). Report No. 24341. Study No. T9058358. Lab: Department of Industrial Toxicology, Bayer AG, Fachbereich Toxikologie, Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany. Sponsor: Bayer AG, Agriculture Division. Study dates: 4-28th July 1995. Report date: 4th October 1995.

Test Compound: SRA 3886 (c.n.Fenamiphos); 93.10% purity; sourced from Bayer AG, Wuppertal, Germany
Batch: 809235173
Test Species: Male guinea pigs (Hsd Win:DH strain); 10 or 20/dose; 331-335 g bw range; approximately 5-6-weeks old; sourced from Harlan Winkelmann GmbH Borchen, Kreis Paderborn, Germany
Study Duration: 4-28th July 1995
Laboratory: Department of Industrial Toxicology, Bayer AG, Fachbereich Toxikologie, Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany
GLP& QA: GLP compliant [OECD Principles of GLP (Bundesanzeiger No. 42a, 2nd March 1983, German version); Annex 1 ChemG (Bundesgesetzblatt Part 1 of the 29th July 1994); FIFRA GLP Standards (40 CFR Part 160)]; QA statement
Guidelines: OECD Test Guideline No. 406 "Skin Sensitisation " (adopted 17th July 1995); EC guideline 92/69 (17th Adaption of Guideline 67/548/EEC): Classification, Packaging and Labeling of Hazardous Materials, "Skin Sensitisation" Method B.6; Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals (revised), guideline 81-6, US EPA (1984)

Methods: The skin sensitisation potential of fenamiphos was tested using the guinea pig maximisation test of Magnusson and Kligman. The sensitivity and reliability of the test method in the particular guinea pig strain had previously been confirmed by the performing laboratory using 2-mercaptobenzothiazole (Stropp 1995; Vohr 1995).

Induction and challenge concentrations were determined in a number of range-finding experiments. For the induction phase, a group of 20 guinea pigs was given 3 pairs of 0.1 mL intradermal injections in the shorn dorsal skin on the scapular region: (1) a 1:1 mixture of Freund's complete adjuvant in physiological saline; (2) 1% (w/v) fenamiphos in physiological saline containing 2% (v/v) Cremophor EL®; and (3) a 1:1 mixture of 1% (w/v) fenamiphos in physiological saline containing 2% (v/v) Cremophor EL®, and Freund's complete adjuvant. Two control groups of 10 guinea pigs were treated in the same manner except that injections 2 and 3 did not contain fenamiphos. The test formulation of fenamiphos was determined to be stable and homogenous.

A topical induction was performed one week after the intradermal induction. Test areas were shorn one day prior to treatment and then skin patches containing 0.5 mL test material were securely fixed

on and between the injection sites. Patches on the test group contained 25% (w/v) fenamiphos in physiological saline with 2% (v/v) Cremophor EL®, while patches on the control groups contained 0.5 mL physiological saline with 2% (v/v) Cremophor EL®. Pre-treatment with 10% (w/v) sodium lauryl sulphate in Vaseline was not performed reportedly due to the high toxicity occurring after intradermal induction. After 48 hours, patches were removed and the skin sites washed with physiological saline to remove the test material.

Three weeks after intradermal induction, guinea pigs were topically challenged via two skin patches containing 12 or 15% fenamiphos (0.5 mL total volume) for 24 hours. Skin sites were then washed with physiological saline to remove any test material and 21 hours later the sites were shorn. Skin reactions were scored according to the Draize Scale at 48 and 72 hours after the commencement of topical challenge.

Animals were observed daily for clinical signs. Bodyweights were recorded prior to commencing the study and at study termination (day 24). The criteria for concluding that the test material was a skin sensitiser was a $\geq$30% increase in animals showing a positive reaction compared to the control.

Results: In the test group, one guinea pig died one day after intradermal induction, which was preceded by lateral positioning and shortness of breath. Necropsy revealed reddening of the intestinal wall. A second animal exhibited decreased mobility, a ruffled coat, sunken flanks and pallor, and was sacrificed one day after intradermal induction. Necropsy of this animal revealed paleness of the liver, lungs and kidneys, a fluid-filled stomach and red to black intestinal contents. It was reported that there was a loss of bodyweight in one animal from days 8 to 12, however, only bodyweight data at day 0 (the day of application) and 24 were provided. These data showed no apparent difference in bodyweight between the control and treatment groups. Following topical induction, 3 animals developed open wounds at the treatment site, with these same animals showing encrustations on day 10, which healed by day 13. There were no abnormalities in the control group.

Forty-eight hours after topical challenge with 12 and 25% fenamiphos, 0/18 and 4/18 animals, respectively, exhibited slight erythema. After 72 hours, only 1/18 animals challenged with 25% fenamiphos still had slight erythema. There was no erythema observed in the control groups. Based on these findings, fenamiphos was classifiable as a non-skin sensitiser.

3.2 Metabolites

Toxicologically-significant fenamiphos metabolites include fenamiphos sulfoxide, fenamiphos sulfone, desisopropyl fenamiphos sulfoxide, desisopropyl fenamiphos sulfone and desisopropyl fenamiphos. A summary of the acute toxicity of these metabolites is provided in the following table.

Table 19 Summary of acute toxicity of fenamiphos metabolites

Study	Species	Results	Reference
Fenamiphos-sulfone			
Oral	Rat	LD_{50} 3 mg/kg bw (males) LD_{50} 2 mg/kg bw (females)	Crawford & Anderson (1974b)
Oral	Rat	LD_{50} 10-25 mg/kg bw	Thyssen (1974a)
Fenamiphos-sulfoxide			
Oral	Rat	LD_{50} 2 mg/kg bw (both sexes)	Crawford & Anderson (1974b)
Oral	Rat	LD_{50} 10-25 mg/kg bw	Thyssen (1974b)
Desisopropyl fenamiphos sulfoxide			
Oral	Rat	LD_{50} 4 mg/kg bw	Lamb & Matzkanin (1975)
Desisopropyl fenamiphos			
Oral	Rat	LD_{50} 1 mg/kg bw males LD_{50} 2 mg/kg bw females	Lamb & Matzkanin (1977)

3.2.1 Oral toxicity

Crawford CR & Anderson RH (1974b) The acute oral toxicity of ®Nemacur-sulfone and ®Nemacur-sulfoxide to rats. Report No. 40215. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 3rd April 1974.

Test Compound: ®Nemacur-sulfone, ®Nemacur-sulfoxide; unspecified purity & source
Batch: Not specified
Test Species: Male and female rats (4/sex/group); 230-300 g bw for males and 190-250 g bw for females; unspecified strain, age & source;
Study Duration: 14 days
Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Oral (gavage) Fasted for 20 hours	20% ethanol-80% propylene glycol	14 days	2.0, 2.5, 3.1& 4.1 (males) & 1.0, 2.0, 4.0& 8.0 (females) for ®Nemacur-sulfone 1.5, 2.0, 2.5 & 3.3 (males) & 1.6, 2.0, 2.5 & 3.1 (females) for ®Nemacur-sulfoxide	®Nemacur-sulfone: 2.6 (2.2-3.1) (males) 2.4 (1.7-3.4) (females) ®Nemacur-sulfoxide 2.4 (2.0-2.9) (males) 2.4 (2.1-2.6) (females)

* 95% confidence interval shown in parentheses

Clinical signs: There were no deaths at the lowest doses of either compound. Dosing with ®Nemacur-sulfone resulted in 2, 3 and 4/4 deaths at 2.5, 3.1 and 4.1 mg/kg bw, respectively, in males and 1, 4 and 4/4 deaths at 2.0, 4.0 and 8.0 mg/kg bw, respectively, in females. The time of death was up to 34 minutes in males and 22 minutes in females. Dosing with ®Nemacur-sulfoxide resulted in 1, 2 and 4/4 deaths at 2.0, 2.5 and 3.3 mg/kg bw, respectively, in males and 0, 3 and 4/4 deaths at 2.0, 2. and 3.1 mg/kg bw/day, respectively, in females. The time of death in both sexes was approximately 15 minutes. Clinical signs (unspecified) occurred at every dose of ®Nemacur-sulfone and started at 6-12 minutes after dosing in males and at 9-30 minutes in females. Clinical signs persisted for 3-6 hours in males and for 1 hour in females. Clinical signs (unspecified) also occurred at every dose of ®Nemacur-sulfoxide, starting at 4-10 minutes after dosing in males and

5-40 minutes in females. These clinical signs persisted for 3 hours in males and one hour in females.

Necropsy findings: Not recorded.

Thyssen J (1974a) ®Nemacur-sulfoxide acute toxicity in rats. Report No. unspecified. Lab & Sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study dates: unspecified. Report date: 11th February 1974.

Test Compound: ®Nemacur-sulfone; unspecified purity & source
Batch: Not specified
Test Species: Female Wistar II albino rats (3/group); 160-180 g bw; unspecified age & source
Study Duration: 7 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	Water and Emulsifier W233*	7 days	5, 10, 25 & 100	10-25

* unspecified concentration

Clinical signs: All rats died within 17-45 minutes of dosing at 25.0 and 100.0 mg/kg bw. Cholinergic signs (muscle tremor, spasms, salivation and "respiratory disturbances") were observed at these same doses and reportedly developed 6-10 minutes after dosing. There were no mortalities or clinical signs observed at 5 or 10 mg/kg bw/day.

Necropsy: Not recorded.

Thyssen J (1974b) ®Nemacur-sulfoxide acute toxicity in rats. Report No. unspecified. Lab & Sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study dates: unspecified. Report date: 11th February 1974.

Test Compound: ®Nemacur-sulfoxide; unspecified purity & source
Batch: Not specified
Test Species: Female Wistar II albino rats (3/group); 160-180 g bw; unspecified age & source
Study Duration: 7 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	Water and Emulsifier W233	7 days	2.5, 5.0, 10.0, 25.0 & 100.0	10-25

Clinical signs: All rats died within 13-30 minutes of dosing at 25.0 and 100.0 mg/kg bw. There were no mortalities at any of the lower doses. Cholinergic signs (muscle tremor, spasms, salivation and "respiratory disturbances") were observed at and above 5.0 mg/kg bw/day. These developed 30-60 minutes after dosing at 5 and 10 mg/kg bw, and at 5-16 minutes after dosing at 25 and 100 mg/kg bw.

Necropsy: Not recorded.

Lamb DW & Matzkanin CS (1975) The acute oral toxicity of Nemacur technical, desisopropyl Nemacur sulfoxide and desethyl Nemacur. Report No. 44531. Lab: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location. Sponsor: unspecified. Study duration: unspecified. Report date: 4th April 1975.

Test Compound: Desisopropyl nemacur sulfoxide; >95% purity; unspecified source
Batches: 74-173-16
Test Species: Male and female SD rats (5/sex/group); 200-245 g bw for females & 268-326 g bw for males; unspecified age & source
Study Duration: 14 days
Laboratory: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location
GLP& QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Oral (gavage) Fasted for 22-24 hours	20% ethanol:80% propylene glycol	14 days	3.2, 3.7, 4.2 & 4.9 males 2.8, 3.2, 3.7 & 4.2 females	4.1 (2.7-3.8) males 3.7 (3.3-4.0) females

* 95% confidence interval shown in parentheses

Clinical signs: Deaths occurred at every dose in males (1, 2, 2 and 4/5 at 3.2, 3.7, 4.2 and 4.9 mg/kg bw, respectively) and at and above 3.2 mg/kg bw in females (1, 2 and 5/5 at 3.2, 3.7 and 4.2 mg/kg bw, respectively). The time to death was within 90 minutes in males and 40 minutes in females. Clinical signs (tremors, salivation, lacrimation, diarrhoea and convulsions) occurred in all rats of both sexes within 30 minutes of dosing and lasted for 2 hours.

Necropsy findings: Not recorded.

Lamb DW & Matzkanin CS (1977) The acute oral toxicity of ®Nemacur desisopropyl Report No. 51597. Lab: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 9th February 1977.

Test Compound: Nemacur desisopropyl; unspecified purity & source
Batches: 76-207-1
Test Species: Male and female SD rats (10/sex/group); 160-188 g bw for females & 170-236 g bw for males; unspecified age & source
Study Duration: 14 days
Laboratory: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral (gavage) Fasted for 18-20 hours	20% ethanol:80% propylene glycol	14 days	1.0, 1.2, 1.4, 1.6, 1.8, 2.1 & 2.4 (males) 1.4, 1.6, 1.8, 2.1, 2.4 & 2.8 (females)	1.4 (1.2-1.6) males 2.1 (1.9-2.3) females

* 95% confidence interval shown in parentheses

Clinical signs: Deaths occurred at and above 1.2 mg/kg bw in males and 1.6 mg/kg bw in females (3, 4, 7, 8, 8 and 10/10 at 1.2, 1.4, 1.6, 1.8, 2.1, and 2.4 mg/kg bw, respectively, in males and 3, 1, 6, 9 and 10/10 at 1.6, 1.8, 2.1, 2.4 and 2.8 mg/kg bw, respectively, in females). The time to death was

30 minutes after dosing. Clinical signs (lacrimation, salivation, muscular fasciculations and convulsions) occurred in all rats at and above 1.2 mg/kg bw. Clinical signs began 10 minutes after dosing and persisted for 24 hours.

Necropsy findings: Not recorded.

3.3 Impurities

Toxicologically-significant fenamiphos impurities include aryldiamide, diarylamide, diaryl ethyl ester, diethyl ester, diethylmonamide, di-SCH_3 compound, ethyl aryl ester, ethyldiamide, 4-(methylthio)-meta-cresol (MTMC). A summary of the toxicity of these metabolites is provided in the following table.

Table 20 Summary of acute toxicity of fenamiphos impurities

Study	Species	Results	Reference
Diethylmonamide, ethyldiamide, diethylester, aryldiamide, di-SCH_3 compound, diarylethylester, diarylamide, ethylarylester acid			
Oral	Rat	LD_{50} >5 mg/kg bw(males) LD_{50} > 4 mg/kg bw (females)	Crawford & Anderson (1973)
4-(methylthio)-meta-cresol (MTMC)			
Oral	Rat	LD_{50} >5 mg/kg bw(males) LD_{50} > 4 mg/kg bw (females)	Crawford & Anderson (1973)
Oral	Rat	LD_{50} 1418 mg/kg bw males LD_{50} 1333 mg/kg bw females	Crawford & Anderson (1974a)
4-(methylthio)-meta-cresol sulfone (MTMC-sulfone)			
Oral	Rat	LD_{50} 1250 mg/kg bw males LD_{50} 1854 mg/kg bw females	Crawford & Anderson (1974a)
4-(methylthio)-meta-cresol sulfoxide (MTMC-sulfoxide)			
Oral	Rat	LD_{50} 1418 mg/kg bw males LD_{50} 1175 mg/kg bw females	Crawford & Anderson (1974a)
4-methylmercapto-m-cresol			
Oral	Rat	LD_{50} >2500 mg/kg bw	Thyssen (1974c)
3-methyl-4-methylmercaptophenol			
Oral	Rat	LD_{50} 500-1000 mg/kg bw	Thyssen (1974d)
3-methyl-4-methanesulfonylphenol			
Oral	Rat	>1000 mg/kg bw was non-toxic	Thyssen (1974e)
Desmethyl fenamiphos			
Oral	Rat	LD_{50} 1000-4000 mg/kg bw males LD_{50} ~1000 mg/kg bw females	Lamb & Matzkanin (1975)

3.3.1 Oral toxicity

Crawford CR & Anderson RH (1973) Comparative oral toxicity in rats of several impurities and a technical compound of ®Nemacur with analytical grade Nemacur. Report No. 3446. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 7th February 1973.

Test Compound: Diethylmonamide, ethyldiamide, diethylester, 4-methylthio-3-cresol, aryldiamide, di-SCH_3 compound, diarylethylester, diarylamide, ethylarylester acid; purity and source unspecified

Batch: Not specified

Test Species: SD rats (4/sex/dose); 200-250 g bw; unspecified age & source

Study Duration: 14 days

Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.

GLP & QA: None

Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage Fasted for 20 hours	20% ethanol-80% propylene glycol	14 days	3.2 & 4.7 (males) 2.4 & 3.6 (females)	>4.7 (males) >3.6 (females

Clinical signs: There were no mortalities or clinical signs.

Necropsy findings: Not recorded.

Crawford CR & Anderson RH (1974a) The acute oral toxicity of two ®Nemacur phenolic metabolites and MTMC to male and female rats. Report No. 39700. Lab: Chemagro Division of Baychem Corporation, Research & Development, unspecified location. Sponsor unspecified. Study dates: unspecified. Report date: 27th February 1974.

Test Compound: MTMC, MTMC-sulfone, MTMC-sulfoxide; 96.4, 95 & 99% purities, respectively; unspecified source
Batches: 73-87-151, 73-87-150 & 73-87-149, respectively (lab reference No.s)
Test Species: Male and female rats (4/sex/group); unspecified strain, age & source; 225-286 g bw for males and 190-225 g bw for females
Study Duration: 14 days
Laboratory: Chemagro Division of Baychem Corporation, Research & Development, unspecified location.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral (gavage) Fasted for 20 hours	20% ethanol-80% propylene glycol	14 days	910, 1183, 1538 & 2000 [MTMC, MTMC-sulfoxide, MTMC-sulfone (males)] 1280, 1600, 2000 & 2500 [MTMC-sulfone (females)]	MTMC: 1418 (1250-1610) (males); 1333 (1083-1638) (females) MTMC-sulfone: 1250 (1100-1418) (males); 1854 (1259-2733) (females) MTMC-sulfoxide: 1418 (1250-1610) (males); 1175 (1013-1356) (females)

* 95% confidence interval shown in parentheses

Clinical signs: MTMC caused mortalities at and above 1538 mg/kg bw in males and 1183 mg/kg bw in females, with the time of death up to 48 hours and 2 hours, respectively (0, 0, 3 & 4/4 deaths in males and 0, 2, 2 & 4/4 deaths in females at 910, 1183, 1538 and 2000 mg/kg bw, respectively). MTMC-sulfone caused mortalities at and above 1183 mg/kg bw in males and at every dose in females, with the time of death up to 4 days and 12 hours, respectively (0, 1, 4 & 4/4 deaths in males at 910, 1183, 1538 and 2000 mg/kg bw, respectively; 1, 1, 2 & 4/4 deaths in females at 1280, 1600, 2000 & 2500, respectively). MTMC-sulfoxide caused mortalities at and above 1538 mg/kg bw in males and 1183 mg/kg bw in females, with the time of death up to 4 and 24 hours, respectively (0, 0, 3 & 4/4 deaths in males and 0, 2, 4 & 4/4 deaths in females at 910, 1183, 1538 and 2000 mg/kg bw, respectively). Ataxia and decreased activity were observed in all rats treated with each of the 3 test compounds, which started from 5 minutes after dosing and lasted for 1 or 2 hours.

Necropsy findings: Not recorded.

Thyssen J (1974c) 4-methylmercapto-m-cresol acute toxicity in rats. Report No. unspecified. Lab & sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study dates: unspecified. Report date: 29th April 1974.

Test Compound: 4-methylmercapto-m-cresol; unspecified purity & source
Batch: Not specified
Test Species: Female Wistar II albino rats (10 or 15/group); 160-180 g bw; unspecified age & source
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	Lutrol® (PEG)	14 days	500, 1000 & 2500	>2500

Clinical signs: Four rats died at the highest dose, 5 hours to 10 days after dosing. There were no deaths at the two lower doses. Clinical signs (poor general condition, sedation and respiratory disturbances) reportedly occurred in all rats at every dose during the first 2 days after dosing.

Necropsy: Not recorded.

Thyssen J (1974d) 3-methyl-4-methylmercaptophenol acute toxicity in rats. Report No. unspecified. Lab & sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study date: unspecified. Report date: 25th February 1974.

Test Compound: 3-methyl-4-methylmercaptophenol; unspecified purity & source
Batch: Not specified
Test Species: Female Wistar II albino rats (2/group); 160-180 g bw; unspecified age & source
Study Duration: 7 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	Water and Emulsifier W233*	7 days	250, 500 & 1000	500-1000

* unspecified concentration

Clinical signs: Deaths occurred only at the highest dose (time of death unspecified). Clinical signs (described as a depression in general condition) occurred in all rats at 500 and 1000 mg/kg bw, but were absent at the lowest dose. The duration of clinical signs was unreported.

Necropsy: Not recorded.

Thyssen J (1974e) 3-methyl-4-methanesulfonylphenol acute toxicity in rats. Report No. unspecified. Lab & sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study date: unspecified. Report date: 25th February 1974.

Test Compound: 3-methyl-4-methanesulfonylphenol; unspecified purity & source
Batch: Not specified
Test Species: 3 Female Wistar II albino rats; 160-180 g bw; unspecified age & source
Study Duration: 7 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)
Oral gavage	Water and Emulsifier W233*	7 days	1000	>1000

* unspecified concentration

Clinical signs: There were no deaths or clinical signs.

Necropsy: Not recorded.

Lamb DW & Matzkanin CS (1975) The acute oral toxicity of Nemacur technical, desisopropyl Nemacur sulfoxide and desethyl Nemacur. Report No. 44531. Lab: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location. Sponsor: unspecified. Study dates: unspecified. Report date: 4th April 1975.

Test Compound: Desethyl nemacur; 75-80% purity; unspecified source
Batch: 74-173-28
Test Species: Male and female SD rats (5/sex/group); 200-220 g bw for females & 294-297 g bw for males; unspecified age & source
Study Duration: 14 days
Laboratory: Chemagro Agricultural Division, Mobay Chemical Corporation, Research and Development, unspecified location
GLP & QA: None
Guidelines: None

Dosing method	**Vehicle**	**Observation Period**	**Dose tested (mg/kg bw)**	**Oral LD_{50} (mg/kg bw)**
Oral (gavage) Fasted for 22-24 hours	20% ethanol:80% propylene glycol	14 days	1000 & 4000	1000-4000 males ~1000 females

Clinical signs: All males died at the high dose, while there were no deaths at the low dose. Deaths occurred at both doses in females (3 and 5/5 at 1000 and 4000 mg/kg bw, respectively). The time to death was 24 or 48 hours post-dose. Clinical signs (tremors, salivation, lacrimation, diarrhoea and convulsions) occurred in all rats of both sexes within 30 minutes of dosing and lasting for 24 or 74 hours.

Necropsy findings: Not recorded.

3.4 Products/formulations

The results of acute toxicity studies conducted on fenamiphos products similar to 3 Australian products are evaluated below (see Appendix IV for the formulation details of these products). These products were the same types of formulation (granular or emulsifiable concentrate), contained equivalent concentrations of fenamiphos and had the same types of non-active constituents. The results of these studies are summarised in the table below.

Table 21 Summary of acute toxicity of fenamiphos products

Study	Species	Results	Reference
Nemacur 5 GR			
Oral	Rat	LD_{50} 47 mg/kg bw (males) LD_{50} 68 mg/kg bw (females)	Mihail & Thyssen (1980a)
Dermal 24 hours exposure	Rat	LD_{50} 3650 mg/kg bw (males) LD_{50} 3690 mg/kg bw (females)	
Inhalation 1 hour head-only exposure	Rat	LC_{50} >125 mg/m^3	
Inhalation 4 hour head-only exposure	Rat	LC_{50} >106 mg/m^3 (males) LC_{50} ~106 mg/m^3 (females)	
Nemacur GR 10			
Oral	Rat	LD_{50} 26 mg/kg bw (fasted males) LD_{50} 34 mg/kg bw (fasted females) LD_{50} 72 mg/kg bw (non-fasted males) LD_{50} 77 mg/kg bw (non-fasted females)	Heimann & Thyssen (1981)
Dermal	Rabbit	>5000 mg/kg bw	
Inhalation 1 hour head-only exposure	Rat	>118 mg/m^3	
Inhalation 4 hour head-only exposure	Rat	>44 mg/m^3	
Skin irritation	Rabbit	Non skin irritant	
Eye irritation	Rabbit	Slight eye irritant	
Nemacur 400 EC			
Oral	Rat	LD_{50} 10 mg/kg bw	Mihail & Thyssen (1980b)
Dermal 24 hours exposure	Rat	LD_{50} 208 µL/kg bw (males) (83 mg/kg bw fenamiphos) LD_{50} 161 µL/kg bw (females) (64 mg/kg bw fenamiphos)	
Inhalation 1 hour head only exposure	Rat	LC_{50} 330-400 mg/m^3 (females)	
Inhalation 4 hour head-only exposure	Rat	LC_{50} 132-198 mg/m^3	
Skin irritation	Rabbit	Severe skin irritant	
Eye irritation	Rabbit	Severe eye irritation	
Nemacur 400 EC (0.7% aqueous dilution)			
Oral	Rat	LD_{50} 1895 µL/kg bw (13 mg/kg bw fenamiphos)	Mihail & Thyssen (1980b)
Inhalation 4 hour head-only exposure	Rat	LC_{50} >91 mg/m^3 (males) LC_{50} 90 mg/m^3 (females)	
Skin irritation	Rabbit	Non skin irritant	
Eye irritation	Rabbit	Non eye irritant	

3.4.1 Oral toxicity

Mihail F & Thyssen J (1980a) Nemacur 5 GR studies on formulation toxicology. Report No. 8950. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: June to September 1979. Report date: 12th February 1980

Test Compound: Nemacur 5 GR (5% granular formulation of fenamiphos; see appendix IV for composition; Ivory Coast Manufacture
Batch: Unspecified
Test Species: Male and female Wistar albino rats (TNO/W 74); 15/dose; age unspecified; 160-210 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Oral gavage Fasted for 16 hours	Distilled water & Cremophor EL (unspecified conc.)	14 days	0.1, 1, 30, 40, 50, 60 & 75 (males); 0.1, 1, 25, 40, 50, 75, 100 and 150 (females)	47.2 (43-52) males 67.5 (56-81) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred at and above 40 mg/kg bw in both sexes (3, 10, 13 and 15/15 deaths in males at 40, 50, 60 and 75 mg/kg bw, respectively; 1, 4, 8, 13 and 15/15 deaths in females at 40, 50, 75, 100 and 150 mg/kg bw, respectively). The time to death was 10-28 minutes after dosing in males and 8-60 minutes in females. Clinical signs occurred in all rats at and above 1 mg/kg bw. These clinical signs included laboured breathing, muscle cramps, salivation, bloody tears and dyspnoea (at the highest dose). The onset of clinical signs was within minutes of dosing and lasted for up to 24 hours. Survivors reportedly exhibited apathy for up to two days after dosing.

Necropsy: No treatment-related abnormalities were detected.

Heimann KG & Thyssen J (1981) Nemacur GR 10 study for formulation toxicity. Report No. 10125. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: February to March 1981. Report date: 14th August 1981

Test Compound: Nemacur GR 10 (10% granular formulation of fenamiphos; see appendix IV for composition); source unspecified
Batch: Unspecified
Test Species: Male and female Wistar albino rats (strain WISW, SPF-CPB); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Oral gavage Fasted (16 hours) and non-fasted	Distilled water & Cremophor EL (unspecified conc.)	14 days	Fasted for 16 hours: 1, 5, 15, 20, 25, 35 & 50 (males); 5, 10, 25, 35, 50 & 100 (females) Non-fasted: 5, 10, 50, 55, 60, 75, 100 & 250 (males); 5, 10, 50, 60, 75, 100 & 250 (females)	Fasted: 26 (21-32) males 34 (26-44) females Non-fasted: 72 (58-96) males 77 (68-89) females

* The 95% confidence interval is shown in parentheses

Clinical signs: In rats fasted for 16 hours prior to dosing, mortalities occurred at and above 20 mg/kg bw in males and 25 mg/kg bw in females (1, 5, 9 and 10/10 at 20, 25, 35 and 50 mg/kg bw, respectively, in males; 1, 6, 9 and 10/10 at 25, 35, 50 and 100 mg/kg bw, respectively, in females). The time to death was 10-15 minutes after dosing in males and 7-40 minutes after dosing in females. In non-fasted rats, deaths occurred at and above 55 mg/kg bw in males and 60 mg/kg bw in females (2, 4, 5, 8 and 10/10 at 55, 60, 75, 100 and 250 mg/kg bw, respectively in males and 2, 4, 9 and 10/10 at 60, 75, 100 and 250 mg/kg bw, respectively, in females). The time of death ranged from 7-20 minutes after dosing in males and 10-30 minutes in females.

Clinical signs (dyspnoea, reduced mobility, muscular spasms, salivation and exophthalmos) occurred in all fasted rats at and above 5 and 25 mg/kg bw in males and females, respectively, while in non-fasted rats, they occurred in all rats at and above 10 mg/kg bw. The onset of clinical signs ranged from 3 minutes at lethal doses to one hour at lower doses, with signs lasting for up to 4 days. In survivors, apathy lasted for one to three days.

Necropsy: The following macroscopic findings were described in an unspecified number of decedents: slightly distended lungs, slightly pale spleen and slightly reddened renal pelvis. In an unspecified number of survivors, the following macroscopic findings were described: lung patch with small dots and rough spleen surface

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound: Nemacur 400 EC (40% fenamiphos; see appendix IV for composition); source unspecified
Batch: 233 821 208
Test Species: Male and female Wistar albino rats (strain TNO/W74); 15/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Oral gavage Fasted for 16 hours	Distilled water	14 days	0.1, 6.0, 7.5, 10.0, 12.5, 15.0 & 20.0 (males); 0.1, 5.0, 7.5, 10.0, 15.0 & 20.0 (females)	10 (8.6-11.6) males 10.25 (8.6-12.2) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Deaths occurred at and above 7.5 mg/kg bw (3, 6, 12, 13 and 15/15 at 7.5, 10.0, 12.5, 15.0 and 20.0 mg/kg bw, respectively, in males, and 2, 8, 13 and 15/15 at 7.5, 10.0, 15.0 and 20.0 mg/kg bw, respectively, in females). The time to death was 7-27 minutes after dosing in males and 9-46 minutes after dosing in females. Clinical signs occurred in all rats except at the lowest dose of 0.1 mg/kg bw (reduced mobility, muscular cramps, involuntary jumping, salivation and bloody tears, with laboured breathing and transient lateral recumbency occurring at lethal doses). Survivors exhibited apathy for 1-3 days after dosing.

Necropsy: No treatment-related abnormalities were observed.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound: 0.7% aqueous dilution of Nemacur 400 EC (40% fenamiphos; see appendix IV for composition); source unspecified
Batch: 233 821 208
Test Species: Male Wistar albino rats (strain TNO/W74); 15/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested	Oral LD_{50}*
Oral gavage Fasted for 16 hours	Distilled water	14 days	1000, 1500, 2000 & 2500 µL/kg bw: equivalent to 7, 10.5, 14 and 17.5 mg/kg bw fenamiphos	1895 µL/kg bw (1656-2168): equivalent to 13.2 mg/kg bw (11.6-15.2)

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred at and above 1500 µL/kg bw (3, 9, and 12/15 at 1500, 2000 and 2500 µL/kg bw, respectively). Cholinergic signs consistent with the undiluted formulation occurred within minutes of dosing and lasted for up to 24 hours. Apathy occurred in survivors for 1-3 days.

Necropsy: There were no treatment-related gross abnormalities reported.

3.4.2 Dermal toxicity

Mihail F & Thyssen J (1980a) Nemacur 5 GR studies on formulation toxicology. Report No. 8950. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: June to September 1979. Report date: 12th February 1980

Test Compound: Nemacur 5 GR (5% granular formulation of fenamiphos; see appendix IV for composition); Ivory Coast Manufacture
Batch: Unspecified
Test Species: Male and female Wistar albino rats (TNO/W 74); 10/dose; age unspecified; 160-210 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Dermal LD_{50}*
Moistened granules applied to intact, clipped dorsal skin under an occlusive dressing for 24 hours	None	14 days	250, 1000, 1500, 2500 & 5000 (males): 250, 1000, 2500, 3500 & 5000 (females)	3650 males 3690 (3005-4531) females

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred at and above 1500 mg/kg bw in males and 2500 mg/kg bw in females (2, 4 and 1/10 at 1500, 2500 and 5000 mg/kg bw, respectively, in males; 1, 3 and 9/10 at

2500, 3500 and 5000 mg/kg bw, respectively in females). The time to death decreased with dose and ranged from 40 minutes to 6 days in males and 1 hour to 3 days in females. Cholinergic signs similar to those observed during the oral dosing component of this study (see above) occurred from 1-2 hours after dosing. In survivors, apathy was reported to last for up to 9 days after dosing.

Necropsy: No treatment-related abnormalities were detected.

Heimann KG & Thyssen J (1981) Nemacur GR 10 study for formulation toxicity. Report No. 10125. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: February to March 1981. Report date: 14th August 1981

Test Compound: Nemacur GR 10 (10% granular formulation of fenamiphos; see appendix IV for composition); source unspecified
Batch: Unspecified
Test Species: Male and female Wistar albino rats (strain WISW, SPF-CPB); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested (mg/kg bw)	Oral LD_{50} (mg/kg bw)*
Moistened granules applied to intact, clipped dorsal skin under occlusive dressing for 24 hours	None	14 days	5000	>5000

Clinical signs: There were no deaths. Transient apathy occurred in an unspecified number of rats.

Necropsy: One rat had a rough spleen surface.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound: Nemacur 400 EC (40% fenamiphos; see Appendix IV for composition; source unspecified)
Batch: 233 821 208
Test Species: Male and female Wistar albino rats (strain TNO/W74); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation Period	Dose tested	Oral LD_{50} (µL/kg bw)
Dermal application to intact, clipped dorsal skin under an occlusive dressing for 24 hours	None	14 days	25, 100, 150, 250, 350 and 500 µL/kg bw: equivalent to 10, 40, 60, 100, 140 & 200 µg/kg bw, respectively	208 (164-265) males 161 (92-282) females [Equivalent to 83.2 (65.6-106) and 64.4 (36.8-112.8) mg/kg bw fenamiphos, respectively]

* The 95% confidence interval is shown in parentheses

Clinical signs: Mortalities occurred at and above 150 µL/kg bw in males and 100 µL/kg bw in females (2, 7, 9 and 10/10 at 150, 250, 350 and 500 µL/kg bw, respectively, in males and 2, 5, 8, 9 and 10/10 at 100, 150, 250, 350 and 500 µL/kg bw, respectively, in females). The time to death ranged from 1 hour to 4 days after application, depending on the dose. Cholinergic signs consistent with those seen during the oral dosing component of this study occurred from 30 minutes to 2.5 hours after application

Necropsy: Slight pulmonary emphysema and dark spots on lungs were reported in an unspecified number of rats that died during the study and those that were sacrificed at the end of the study.

3.4.3 Inhalational toxicity

Mihail F & Thyssen J (1980a) Nemacur 5 GR studies on formulation toxicology. Report No. 8950. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: June to September 1979. Report date: 12th February 1980

Test Compound: Nemacur 5 GR (5% granular formulation of fenamiphos; see Appendix IV for composition); Ivory Coast Manufacture
Batch: Unspecified
Test Species: Male and female Wistar albino rats (TNO/W 74); 10/dose; age unspecified; 160-210 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation period	Concentrations tested (mg/m^3)*	Inhalation LC_{50} (mg/m^3)
Inhalation of dust 1 or 4 hour head-only exposure**	None	14 days	23, 60, & 106 (4 hour exposure)	>125 (1-hour exposure) >106 (4 hour exposure) males ~106 (4 hour exposure) females

* concentrations tested for the 1 hour exposure period not specified; ** aerosol size unspecified

Clinical signs: No deaths or clinical signs occurred at the maximum concentration of 125 mg/m^3 following the 1 hour exposure period. Deaths occurred in males at 60 and 106 mg/m^3 (1 and 3/10, respectively) and only at 106 mg/m^3 in females (5/10) following 4 hours of exposure. The time to death was less than 4 hours. Clinical signs occurred in all rats at every concentration and included sluggish movement, piloerection, sedation (at lethal concentrations), staggering and slightly laboured breathing (unspecified number of rats). In survivors, these signs persisted for up to 8 days after exposure.

Necropsy: All rats that died had pulmonary emphysema. In survivors, no macroscopic abnormalities occurred at 23 mg/m^3, while slight pulmonary emphysema occurred at higher concentrations in an unspecified number of rats.

Comment: In the absence of a control group, it is unclear whether the effects seen in this study were due to fenamiphos *per se* or to the inhaled dust. In particular, the clinical signs were not consistent with typical ChE inhibition.

Heimann KG & Thyssen J (1981) Nemacur GR 10 study for formulation toxicity. Report No. 10125. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: February to March 1981. Report date: 14th August 1981

Test Compound: Nemacur GR 10 (10% granular formulation of fenamiphos; see Appendix IV for composition); source unspecified
Batch: Unspecified
Test Species: Male and female Wistar albino rats (strain WISW, SPF-CPB); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation period	Concentrations tested (mg/m^3)	Inhalation LC_{50} (mg/m^3)
Inhalation of dust 1 and 4 hour head-only exposures*	None	14 days	118 (1-hour exposure) 44 (4-hour exposure)	>118 (1 hour exposure) >44 (4 hour exposure)

* aerosol size unspecified

Clinical signs: There were no deaths or clinical signs. Bodyweights were unaffected by treatment.

Necropsy: There were no macroscopic abnormalities reported.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound: Nemacur 400 EC (40% fenamiphos; see Appendix IV for composition; source unspecified)
Batch: 233 821 208
Test Species: Male and female Wistar albino rats (strain TNO/W74); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation period	Concentrations tested (mg/m^3)*	Inhalation LC_{50} (mg/m^3)
Head-only exposure to aerosols for 1 or 4 hour*	ethanol:Lutrol (1:1)	14 days	17, 90, 206, 254, 330, 400, 485 & 593 (1-hour exposure); 24, 94, 98, 132, 198, 201 and 323 (4-hour exposure)	330-400 (1-hour exposure) 132-198 (4-hour exposure)

* aerosol size unspecified

Clinical signs: Following a one hour exposure, mortalities occurred at and above 254 mg/m^3 in males and 330 mg/m^3 in females (1, 2, 10, 9 and 10/10 at 254, 330, 400, 485 and 593 mg/m^3, respectively, in males and 1, 10, 9 and 10/10 at 330, 400, 485 and 593 mg/m^3, respectively, in females). The time to death was one day. Clinical signs occurred at and above 206 mg/m^3 in males and 330 mg/m^3 in females and included restlessness and ruffled coat (few minutes to 8 days after exposure) in addition to muscle twitching, cramps and salivation (for up to one day after exposure). At lethal concentrations, rats appeared drowsy, prostrate or laterally recumbent for up to 2 hours prior to death.

Following the 4 hour exposure, mortalities occurred at and above132 mg/m^3 (1, 10, 6 and 10/10 at 132, 198, 201 and 323 mg/m^3, respectively, in males and 3, 8, 10 and 9/10 at 132, 198, 201 and 323 mg/m^3, respectively, in females). The time to death was one day. Clinical signs were observed in all rats at and above 94 mg/m^3 (as after 1 hour exposure).

Necropsy: Gross abnormalities detected in decedents included pink-coloured lungs and slight to marked emphysema, with an unspecified number also having a pale spleen and a lobular pattern of the liver. An unspecified number of rats sacrificed at the end of the study exhibited slightly dilated lungs.

Supplementary study (vapour exposure): Groups of 5rats/sex were exposed to vapours of Nemacur 400 EC (whole body exposure) for 3 or 7 hours then observed for 14 days. One male rat died at the end of the 7 hour exposure period. Central nervous system disturbances (behavioural abnormalities, breathing disorders, drowsiness, unconsciousness, prostration, lateral recumbency and uncoordinated movements) occurred in all rats at approximately 15 minutes after exposure and lasted for a day. No cholinergic signs were reported. All survivors subsequently made a full recovery. Mucosae of the eyes and nose were reportedly irritated but only during exposure. Post-mortem examination revealed "old pink-coloured" lungs and mild emphysemas in an unspecified number of rats. The authors attributed the findings to the xylene content of this product.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound: 0.7% aqueous dilution of Nemacur 400 EC (40% fenamiphos; see Appendix IV for Composition); source unspecified
Batch: 233 821 208
Test Species: Male Wistar albino rats (strain TNO/W74); 10/dose; age unspecified; ~160-200 g bw; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany.
Study Duration: 14 days
Laboratory: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA: None
Guidelines: None

Dosing method	Vehicle	Observation period	Concentrations tested (mg/m^3)	Inhalation LC_{50} (mg/m^3)
Head-only exposure to aerosols for 4 hour*	ethanol:Lutrol (1:1)	14 days	91	>91 (males) 90 (females)

* aerosol size unspecified

Clinical signs: Two males and 6 females (of 10) died within 24 hours of exposure. An unspecified number of rats exhibited cholinergic signs.

Necropsy: Not reported.

3.4.4 Skin and eye irritation studies

Skin irritation

Heimann KG & Thyssen J (1981) Nemacur GR 10 study for formulation toxicity. Report No. 10125. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: February to March 1981. Report date: 14th August 1981

Test Compound:	Nemacur GR 10 (10% granular formulation of fenamiphos; see Appendix IV for composition); source unspecified
Batch:	Unspecified
Test Species:	Male and female NZW rabbits; unspecified number/group; age unspecified; ~3-4 kg bw; sourced from Hacking & Churchill Ltd., Huntingdon, England.
Study Duration:	24 hour
Laboratory:	Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA:	None
Guidelines:	None

Methods: 0.5 g of the ground formulation was applied to cellulose squares (2.5 x 2.5 cm) and affixed to the clipped skin of rabbits for 24 hours (the location of the application site was unspecified). No other methodological details were given.

Results: No signs of skin irritation were observed after removal of the dressing. On this basis, Nemacur GR 10 was classified by the authors as a non-skin irritant.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: May to July 1979. Report date: 7th January 1980.

Test Compound:	Nemacur 400 EC (40% fenamiphos; see Appendix IV for composition); source unspecified
Batch:	233 821 208
Test Species:	Male and female NZW rabbits (3/sex); unspecified number/group; age unspecified; ~3-4 kg bw; sourced from Hacking & Churchill Ltd., Huntingdon, England.
Study Duration:	14 days
Laboratory:	Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA:	None
Guidelines:	None

Methods: 0.5 mL of the formulation was applied to small gauze pads (2.5 x 2.5 cm) and affixed to the hairless auricle of 6 rabbits (presumably 3/sex). At 2, 4 and 8 hours after application, pads were removed from 1 rabbit/sex and any signs if irritation recorded. All rabbits were observed for a further 7 days. No details of any scoring criteria were provided.

Results: No signs of irritation were reported following 2 hours of dermal exposure. Four hours of exposure reportedly caused moderate erythema at the application site. Eight hours of exposure caused "very severe" erythema that persisted for the 7 day observation period. In addition, mild

oedema occurred for up to 3 days after exposure. On the basis of these findings, Nemacur 400 EC was classified by the authors as a severe skin irritant.

Supplementary study: A 0.7% aqueous dilution of Nemacur 400 EC caused no irritation when applied to rabbit skin for 24 hours under similar conditions.

Eye irritation

Heimann KG & Thyssen J (1981) Nemacur GR 10 study for formulation toxicity. Report No. 10125. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study dates: February to March 1981. Report date: 14th August 1981

Test Compound:	Nemacur GR 10 (10% granular formulation of fenamiphos; see Appendix IV for Composition); source unspecified
Batch:	Unspecified
Test Species:	Male and female NZW rabbits; unspecified number/group; age unspecified; ~3-4 kg bw; sourced from Hacking & Churchill Ltd., Huntingdon, England.
Study Duration:	7 days
Laboratory:	Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA:	None
Guidelines:	None

Methods: Approximately 50 mg of the ground formulation was applied to the conjunctival sac of one eye/rabbit and observed for 7 days. No further details were provided.

Results: Moderate erythema (unspecified Draize score) and slight oedema of the conjunctivae were observed in the treated eyes of an unspecified number of rabbits 24 hours after application. No signs of irritation were reported after this time. On this basis, Nemacur GR 10 is classifiable as a slight eye irritant.

Mihail F & Thyssen J (1980b) Nemacur 400 EC acute toxicity studies (occupational toxicity studies) Study No. SRA 3886 400 EC/003-006. Report No. 8799. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study duration: May to July 1979. Report date: 7th January 1980.

Test Compound:	Nemacur 400 EC (40% fenamiphos; see Appendix IV for composition); source unspecified
Batch:	233 821 208
Test Species:	2 NZW rabbits; sex and age unspecified; ~3-4 kg bw; sourced from Hacking & Churchill Ltd., Huntingdon, England.
Study Duration:	14 days
Laboratory:	Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany.
GLP & QA:	None
Guidelines:	None

Methods: 100 µL of the test material was applied to the conjunctival sac of one eye of two rabbits. Rabbits were then observed for 7 days. Details of the scoring criteria used to assess irritation were not reported.

Results: The following signs of ocular irritation occurred and did not resolve during the observation period: moderate redness and mild chemosis of the conjunctivae; slight reddening and swelling of the iris; and mild and diffuse corneal opacity. On the basis of these findings Nemacur 400 EC was classifiable as a severe eye irritant.

Supplementary study: A 0.7% aqueous dilution of Nemacur 400 EC caused no irritation when applied to rabbit eyes for 24 hours under similar conditions.

3.5 Antidote studies

Kimmerle G (1972) Nemacur active ingredient antidotal experiments on rats. Report No. 3560. Lab & Sponsor: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld. Study duration: unspecified. Report date: 19th July 1972.

Technical grade fenamiphos (Batch No. 8016/71; unspecified purity & source) was dissolved in an unspecified concentration of polyethylene glycol 400 and administered by oral gavage to 15 male wistar II rats/group (unspecified age, bw & source) at doses of 10, 12.5, 14, 16, 17 or 18 mg/kg bw. The dose volume was 0.5 mL/100 g bw. Separate groups of 15 rats were given a single oral gavage dose of the same test material at 20, 30, 40 or 50 mg/kg bw. At 2-10 minutes after dosing ("just prior to the appearance" of acute cholinergic signs), these rats were given a single intravenous injection of atropine sulphate (50 mg/kg bw), pyridine-2-aldoxime (2-PAM) (50 mg/kg bw), toxogonin (20 mg/kg bw) and atropine sulphate plus 2-PAM or atropine sulphate plus toxigonin. The author calculated that the acute oral LD_{50} for fenamiphos was 15.6 mg/kg bw. Treatment with the various antidotes resulted in an approximate doubling of the LD_{50} for fenamiphos (26.6, 26.7, 27.6, 30.2 and 30.5 mg/kg bw for atropine sulphate, 2-PAM, toxogonin, atropine sulphate plus 2-PAM and atropine sulphate plus toxigonin, respectively).

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Oral administration

There were no short-term repeat-dose oral dosing studies submitted for evaluation.

4.2 Dermal administration

4.2.1 Rats

Krotlinger F (2000c) SRA 3886 (c.n. Fenamiphos) Study for subacute dermal toxicity in rats (4-week treatment period). Report No. PH-30472. Study No. T6068110. Lab & Sponsor: Institute for Toxicology, Department of Short-Term Rodent Studies and Neurotoxicology, Bayer AG, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Study duration: 2nd March – 10th August 2000. Report date: 27th November 2000.

GLP & QA: GLP compliant [OECD Principles of GLP (as revised in 1997); Annex 1 German Chemical Act (Bundesgesetzblatt Part 1 of the 29th July 1994); FIFRA GLP Standards (40 CFR Part 160); Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 59 NohSan No. 3850)] except that the documentation for the weekly formulation of fenamiphos was incomplete (the author stated that this did not limit the assessment of the results); QA statement

Guidelines: OECD Test Guideline No. 410 "Repeated dose dermal toxicity: 21/28-day study" (adopted May 12, 1991); EEC Annex V Part B.9 (Repeated dose [28 days] toxicity [dermal]) to Directive 67/548/EEC of the Council of the European Communities of June 27, 1967 and its subsequent amendments; US EPA FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Series 82-2, Repeated dose dermal toxicity: 21 day study (November 1984)

Materials and Methods

Fenamiphos (Batch No. 809435148; 95.2% purity; sourced from Bayer AG, Germany), formulated in polyethylene glycol 400, was applied to the clipped dorsal skin of 5 wistar rats/sex/dose [strain HsdCpd:Wu; ~9-weeks old and 246-279 g bw (males), ~11-weeks old and 178-204 g bw (females); sourced from Harlan Winkelmann GmbH Borchen, Kreis Paderborn, Germany] at 0, 2.5, 10 or 40 mg/kg bw/day for 6 hours/day over 22 (males) or 23 days (females). Treatment was for 5 days/week over the first 3 weeks and for the full 7 days of the fourth week. [The dose selection was based on a previous range-finding study (Lab Study No. T3067280), where dermal application of 0, 10 or 120 mg/kg bw/day fenamiphos for 4 days failed to cause erythema or oedema.] The application volume was 1 mL/kg bw and the application area was approximately 20.3-30.25 cm^2. Application sites were occluded with a gauze dressing and secured with adhesive tape. Rats were also immobilised during the treatment period using a "Lomir Biomedical Inc" rat jacket. Following each exposure, the jacket, gauze and tape were removed and the application sites cleaned with soap and water.

Rats had been acclimatised for at least 5 days prior to experimentation and were housed individually under standard conditions. Food and water were available *ad libitum*. Rats were randomly assigned to control and treatment groups on the basis of plasma and RBC ChE activities. The back and flanks of rats were shorn one day prior to treatment and were maintained free of hair by clipping twice/week.

Rats were observed twice daily for clinical signs and any other abnormalities. Bodyweights were recorded prior to experimentation and then weekly. Food consumption was determined weekly.

Application sites were examined for any signs of irritation (i.e. erythema or oedema) prior to experimentation and before each treatment; erythema was scored according to the Draize Scale and oedema was assessed by measuring skinfold thickness in the centre of the application area (skinfold thickness was measured on days 0, 3, 7, 10, 14, 17, 21, 24 and 28). Blood samples were collected from non-fasted rats prior to experimentation and at the end of the 4-week treatment period. For glucose analysis, blood was collected from fasted rats. The standard range of haematology and clinical chemistry parameters were analysed (see Appendix V). Plasma, RBC and brain ChE activities were analysed. No urinalysis was performed. Following the treatment period, rats were sacrificed by exsanguination under ether anaesthesia and macroscopically examined. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, thymus, ovaries and uterus. These organs along with the lungs, vagina and treated and untreated skin were histopathologically examined.

Bodyweight, bodyweight gain, food consumption and organ weight data were statistically analysed using the Dunnett's test. It was unclear from the study report how the remaining data were statistically analysed. It was stated that some of the individual bodyweight and food consumption data were missing due to a "technical defect which occurred during on-line data collection" and "weighing errors".

Results

Chemistry: Analytical data indicated that fenamiphos was stable in the test formulation at 0.25-4.0% (w/v) for at least 7 days at room temperature. It was stated that an analysis of homogeneity was unnecessary because fenamiphos was " clearly soluble in the vehicle".

Mortalities, clinical signs, bodyweights and food consumption: There were no mortalities and no treatment-related clinical signs (diarrhoea occurred in 2-4 males in each group but showed no relationship to treatment). There was a slight (<3%) decrease in average bodyweight in all groups and in both sexes from day 21 to 28, which was unrelated to treatment. Food consumption was unaffected by treatment. There was no treatment-related erythema or skin thickening at the application site.

Haematology and clinical chemistry: There was a significant increase in monocytes in males at 40 mg/kg bw/day ($p<0.01$) and non-significant increases in leucocytes, neutrophils and lymphocytes (see table below). In the absence of historical control data, the toxicological significance of these findings was unclear. However, in the absence of an effect in females, these findings are unlikely to be treatment-related. All other haematological findings were unremarkable in both sexes. There was no treatment-related effect on any clinical chemistry parameter.

Table 22 Selected haematology findings

Parameter	Dose (mg/kg bw/day)							
	0		**2.5**		**10**		**40**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Monocytes	0.22	0.25	0.21	0.19	0.29	0.26	0.38*	0.25
Leucocytes	8.64	8.45	9.97	6.82	9.87	8.39	11.70	7.90
Neutrophils	0.55	0.81	0.78	0.54	0.60	0.80	0.95	0.86
Lymphocytes	7.60	7.19	8.69	5.89	8.68	7.11	10.03	6.61

Results expressed as the mean 10^9/L; * $p<0.05$

ChE activity: There was a significant decrease in plasma ChE activity at 40 mg/kg bw/day in males (30%; $p<0.01$) and a non-significant decrease in females (~20%), both of which were considered

treatment-related and toxicologically significant. There was no effect on plasma ChE activity at lower doses. There was no treatment-related inhibition of RBC or brain ChE activity.

Pathology: There were no treatment-related macro- or microscopic abnormalities and no effect on absolute organ weights. At 40 mg/kg bw/day, relative female liver weights were significantly lower than the control group ($p<0.05$; 3733 *versus* 4025 mg), but in the absence of any pathology, abnormal clinical chemistry or similar effect in males, this finding was considered incidental and unrelated to treatment.

Conclusions: The NOEL following 4 weeks of dermal administration to rats was 10 mg/kg bw/day, based on the inhibition of plasma ChE activity at 40 mg/kg bw/day. There was no inhibition of RBC or brain ChE activity at the highest dose.

4.2.2 Rabbits

Mihail F & Schilde B (1980) SRA 3886 (active ingredient of nemacur) subacute dermal toxicity study on rabbits. Report No. 9297. Study No's. SRA 3886/008 & 7847/002. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: 21st November – 13th December 1978. Report date: 8th July 1980.

Materials and Methods

Fenamiphos (Batch No. unspecified; 89.8% purity; sourced presumably from Bayer AG, Germany), formulated in deionised water and Cremophor EL (5 drops/10 mL formulation), was applied to the clipped dorsal skin of 6 NZW rabbits/sex/dose (2.0-3.0 kg bw; age unspecified; sourced from Hacking & Churchill, Huntingdon, England) at 0, 2.5 or 10.0 mg/kg bw/day for 6 hours/day on 15 consecutive working days. The application volume and site were 0.5 mL/kg bw and approximately 7 x 9 cm, respectively. For 3 rabbits/group, the application site had been abraded 12 hours prior to sample application. Rabbits were immobilised during treatment and prevented from accessing food and water. Following each exposure period, application sites were washed with soap and water, and maintained free of hair by clipping twice/week. Outside of the treatment period, rabbits were housed individually under standard conditions, with food and water available *ad libitum*. The stability and concentration of the test formulations under the experimental conditions were not analysed.

Rabbits were observed daily for clinical signs. Bodyweights were recorded prior to experimentation and then on a weekly basis. The application sites were examined for any signs of irritation (i.e. erythema or oedema) prior to experimentation and then after every 6-hour exposure; skin irritation was scored according to the Draize Scale. Blood and overnight urine samples were collected from all rabbits prior to experimentation and after the 3-week treatment period. The following haematology parameters were analysed: RBC, white blood cell, Hb, Hct, thrombocyte count and differential blood count. The following clinical chemistry parameters were analysed: aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen and RBC, plasma and brain ChE activities. The following urinalysis parameters were analysed: pH, albumin, glucose, Hb, reducing substances, urobilinogen and microscopic sediment.

Rabbits were sacrificed 24-48 hours after the final treatment with sodium hexobarbital (0.5 g) then exsanguinated. All rabbits were subjected to a gross pathological examination and the following organs weighed: heart, lung, liver, spleen, kidneys, adrenals, testes or ovaries and thyroid. These

organs were histopathologically examined along with the following: epididymides, uterus and treated and untreated skin.

No statistical analysis was performed.

Due to inhibition of plasma ChE inhibition at both doses, a supplementary experiment was performed using 0 or 0.5 mg/kg bw/day, and following the same procedures as outlined above.

Results

There were no mortalities and no treatment-related clinical signs. Average bodyweight gain over the 3-week treatment period was marginally lower (~3-5%) at 10 mg/kg bw/day compared to the control and 2.5 mg/kg bw/day groups. Macro- and microscopic examination revealed no evidence of any irritation or abnormalities of the skin.

There was no treatment-related effect on any haematology, clinical chemistry or urinalysis parameters. Results of ChE activity measurements are summarised in the table below, noting that no statistical analysis was performed on these data. In males, plasma ChE activity was inhibited at 10 mg/kg bw/day on days 10 and 15 by approximately 30 and 60%, respectively (when corrected for decreased plasma ChE activity in controls at day 10). The apparent inhibition of plasma ChE activity in males at 2.5 mg/kg bw/day (measured at day 10) was not considered biologically significant because the control group showed a similar decrease in activity. Further, the inhibition that occurred at 2.5 mg/kg bw/day on day 15 was not considered toxicologically significant as it was <20% (relative to pre-treatment activity). In females, there was a dose-related inhibition of plasma ChE activity at days 10 and 15; 14-30% inhibition at 2.5 mg/kg bw/day and 35-53% inhibition at 10 mg/kg bw/day (relative to pre-treatment activity). Like the male control group, female controls also showed decreased plasma ChE activity at day 10 (up to 11%). Inhibition of RBC ChE activity occurred in both sexes only at 10 mg/kg bw (up to 38% in males and 25% in females). There was no treatment-related inhibition of brain ChE activity. There was no difference in the level of inhibition of plasma or RBC ChE activity in abraded and non-abraded rabbits. In the supplementary experiment, there was no effect on plasma, RBC or brain ChE activities at 0.5 mg/kg bw/day.

Table 23 ChE activity in rabbits following dermal application of fenamiphos

Parameter	Dose (mg/kg bw/day)					
	0		2.5		10	
	Intact	Abraded	Intact	Abraded	Intact	Abraded
Male						
Plasma ChE – Day 0	0.59	0.72	0.66	0.58	0.64	0.72
Plasma ChE – Day 10	0.39 (34%)	0.48 (34%)	0.46 (30%)	0.36 (38%)	0.23 (64%)	0.26 (64%)
Plasma ChE – Day 15	0.67	0.74	0.59 (11%)	0.49 (16%)	0.24 (62%)	0.40 (48%)
RBC ChE – Day 0	1.56	1.65	1.46	1.72	1.63	1.82
RBC ChE – Day 10	1.67	1.83	1.80	1.87	1.30 (20%)	1.42 (22%)
RBC ChE – Day 15	1.62	1.78	1.86	1.83	1.17 (28%)	1.13 (38%)
Brain ChE – Day 15	2.03	1.79	1.95	1.94	1.81	2.14
Female						
Plasma ChE – Day 0	0.56	0.79	0.49	0.61	0.71	0.68
Plasma ChE – Day 10	0.50 (11%)	0.76 (4%)	0.35 (29%)	0.40 (34%)	0.30 (58%)	0.32 (53%)
Plasma ChE – Day 15	0.59	0.82	0.42 (14%)	0.44 (28%)	0.46 (35%)	0.32 (53%)
RBC ChE – Day 0	1.76	1.31	1.90	1.63	1.35	1.34
RBC ChE – Day 10	2.11	1.76	2.11	1.89	1.25 (7%)	1.26 (3%)
RBC ChE – Day 15	1.83	1.54	1.81 (5%)	1.66	1.09 (19%)	1.0 (25%)

Brain ChE – Day 15	2.88	2.81	2.56	2.07	2.25	2.22

Results expressed as the mean activity in U/mL, with the % inhibition relative to Day 0 activity shown in parentheses

Pathology and histopathology revealed no treatment-related abnormalities and no effect on organ weights.

Conclusion: The NOEL following dermal application to rabbits for 15 days was 0.5 mg/kg bw in females and 2.5 mg/kg bw/day in males, based on the inhibition of plasma ChE activity at and above 2.5 and 10 mg/kg bw/day, respectively. Inhibition of RBC ChE activity occurred in both sexes only at 10 mg/kg bw. There was no difference in the level of inhibition of plasma or RBC ChE activity in abraded and non-abraded rabbits. There was no inhibition of brain ChE activity.

4.3 Inhalational administration

Thyssen J (1979a) SRA 3886 (Nemacur active ingredient) Acute inhalational studies. Report No. 8210 Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal, Germany. Study duration: unspecified. Report date: 23rd February 1979.

This acute toxicity study, which has been evaluated in Section 3.1.3 of this report, also contained a repeated exposure component.

Materials and Methods

Albino rats (strain TNO/W 74; 10/sex/dose; females ~180 g bw, males ~200 g bw; age unspecified; sourced from Winkelmann, Borchen, Kreis Paderborn, Germany) were exposed to aerosols of fenamiphos (unspecified Batch No. & source; 89.8% purity) over five, 4-hour periods. In the first experiment, rats were exposed to concentrations of 0, 4, 9 or 28 mg/m^3, while a second experiment tested concentrations of 0, 0.3, 0.6 or 3.3 mg/m^3. Fenamiphos was dissolved in ethanol:polyethylene glycol 400 (i.e. Lutrol) and then aerosolised in a dynamic flow inhalation apparatus (Kimmerle & Eben 1973), which reportedly allows head- and nose-only exposure. Following the exposure period, rats were observed for 14 days. The chamber concentration of fenamiphos was analysed but there was no analysis of the aerosol size. Food and water were available *ad libitum*.

Rats were observed daily for clinical signs, physical and behavioural abnormalities. Bodyweights were recorded prior to exposure, at the end of the 5 exposures and then weekly over the observation period. Blood was collected prior to the first exposure, following the 1st, 3rd and 5th exposures, and 72 hours after the 5th exposure, for analysis of plasma and RBC ChE activities. Rats were sacrificed by exsanguination under ether anaesthesia and macroscopically examined. No statistical analysis was performed.

Results

There were no mortalities in either experiment, with the exception of a single control male in the second experiment, which the author attributed to bacterial septicaemia. During the exposure period, clinical signs occurred in all males at and above 9 mg/m^3 and in all females at and above 4 mg/m^3. These included "mild behavioural reactions" (inactivity, stiff gait and rough hair coats) and muscle twitching. No clinical signs, behavioural or physical abnormalities were observed during the post-exposure observation period. In both experiments, all groups (including controls) lost bodyweight during the 5 day exposure period, which subsequently recovered during the 14-day observation period; there was no treatment-related effect on bodyweight. There were no treatment-related macroscopic abnormalities.

Results of ChE activity measurements from both experiments are summarised in the Tables below. In the first experiment, there was a concentration-related increase in plasma ChE inhibition, which was toxicologically-significant (i.e. >20%) at every concentration and every timepoint in both sexes. Three days after the cessation of exposure, plasma ChE activity had recovered to toxicologically-insignificant levels, with the exception of 28 mg/m^3 females, whose plasma ChE activity was still depressed by 45% relative to pre-exposure activity. Toxicologically-significant inhibition of RBC ChE activity occurred in females at 9 and 28 mg/m^3 following the 3rd and 5th exposures. There was marginal inhibition of RBC ChE activity at these same concentrations in males, which only just became toxicologically significant following 5 exposures to 28 mg/m^3. RBC ChE activity had recovered at 3 days after the 5th exposure.

Table 24 ChE activity in rats exposed to aerosols of fenamiphos (Experiment 1)

Parameter	Concentration (mg/m^3)							
	0		4		9		28	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE – pre-exposure	0.50	1.37	0.44	1.37	0.43	1.46	0.48	1.44
Plasma ChE – 1st exposure	0.50	1.37	0.24 (45%)	0.30 (78%)	0.24 (44%)	0.31 (79%)	0.10 (79%)	0.11 (92%)
Plasma ChE – 3rd exposure	0.46 (8%)	1.22 (11%)	0.21 (52%)	0.25 (82%)	0.08 (81%)	0.11 (92%)	0.05 (90%)	0.07 (95%)
Plasma ChE – 5th exposure	0.44 (12%)	1.16 (15%)	0.20 (55%)	0.24 (82%)	0.07 (84%)	0.12 (92%)	0.05 (90%)	0.07 (95%)
Plasma ChE – 72 hours after 5th exposure	0.47 (6%)	1.42	0.44	1.13 (18%)	0.44	1.18 (19%)	0.43 (10%)	0.79 (45%)
RBC ChE – pre-exposure	2.53	2.51	2.27	2.55	2.38	2.70	2.38	2.68
RBC ChE – 1st exposure	2.71	2.81	2.68	2.45 (4%)	2.69	2.62 (3%)	1.99 (16%)	2.21 (18%)
RBC ChE – 3rd exposure	2.76	2.89	2.61	2.55	2.01 (15%)	2.17 (20%)	1.95 (18%)	2.04 (24%)
RBC ChE – 5th exposure	2.80	2.92	2.61	2.32 (9%)	1.96 (18%)	1.95 (28%)	1.89 (21%)	1.78 (34%)
RBC ChE – 72 hour after 5th exposure	2.76	3.09	2.78	2.54	2.57	2.47 (9%)	2.55	2.57 (4%)

Results expressed as the mean activity in U/mL with the percent inhibition relative to pre-exposure activity given in parentheses (note: standard deviations/error were unreported).

In the second experiment, when data were corrected for any depression in plasma ChE activity in the control group, toxicologically-significant inhibition of plasma ChE occurred at 0.6 and 3.3 mg/m^3 in females from the 1st exposure activity subsequently recovered when exposure ceased. In males, toxicologically-significant inhibition of plasma ChE activity occurred only at the highest concentration. There was no toxicologically-significant inhibition of RBC ChE in either sex.

Table 25 ChE activity in rats exposed to aerosols of fenamiphos (Experiment 2)

Parameter	Concentration (mg/m^3)							
	0		0.3		0.6		3.3	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE – pre-exposure	0.45	1.30	0.41	1.58	0.39	1.52	0.41	1.68
Plasma ChE – 1st exposure	0.49	1.33	0.42	1.48 (6%)	0.35 (10%)	1.22 (20%)	0.28 (32%)	0.74 (56%)
Plasma ChE – 3rd exposure	0.50	1.12 (14%)	0.44	1.10 (30%)	0.34 (13%)	0.83 (45%)	0.24 (41%)	0.40 (76%)
Plasma ChE – 5th exposure	0.53	1.06 (18%)	0.46	0.96 (39%)	0.32 (18%)	0.77 (49%)	0.24 (41%)	0.40 (76%)
Plasma ChE – 72 hours after 5th exposure	0.53	1.21 (7%)	0.47	1.38 (13%)	0.39	1.29 (15%)	0.45	1.35 (20%)
RBC ChE – pre-exposure	2.93	2.97	2.95	2.98	2.92	2.89	2.99	2.99
RBC ChE – 1st exposure	2.77 (5%)	2.83 (5%)	2.71 (8%)	2.86 (4%)	2.77 (5%)	2.98	2.77 (7%)	2.91 (3%)
RBC ChE – 3rd exposure	2.47 (16%)	2.66 (10%)	2.62 (11%)	2.57 (14%)	2.71 (7%)	2.98	2.59 (13%)	2.74 (8%)
RBC ChE – 5th exposure	3.02	3.21	3.02	3.32	2.77 (5%)	3.21	2.71 (9%)	2.85 (5%)
RBC ChE – 72 hours after 5th exposure	2.53 (14%)	3.03	2.75 (7%)	2.95	2.68 (8%)	3.20	2.89 (3%)	3.05

Results expressed as the mean activity in U/mL with the percent inhibition relative to pre-exposure activity given in parentheses (note: standard deviations/error were unreported).

Conclusions: The NOECs in rats following five 4-hourly exposures to aerosols of fenamiphos were 0.3 mg/m^3 in females and 0.6 mg/m^3 in males, based on toxicologically-significant inhibition of plasma ChE inhibition at the next highest concentrations (0.6 mg/m^3 in females and 3.3 mg/m^3 in males). RBC ChE activity was inhibited at 9 mg/m^3 in females and 28 mg/m^3 in males.

Thyssen J (1979b) Nemacur active ingredient (SRA 3886) subacute inhalational toxicity study on rats. Report No. 8669. Study No. unspecified. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfield, Germany. Study duration: unspecified. Report date: 10th October 1979.

Materials and Methods

Wistar Rats (strain TNO/W 74 albino; 10 sex/group; 200-244 g bw (males), 1680-200 g bw (females); age unspecified; sourced from Winkelmann, Borchen, Germany) were exposed to aerosols of fenamiphos (Batch No. 808817123; 92.2% purity; source unspecified) at 0, 0.1, 1.0 or 10 mg/m^3 for 6 hours/day on 5 consecutive days for 3 weeks. The test concentrations were based on a previous acute inhalational study (see Thyssen 1979a). Chamber air analysis revealed that these concentrations equated to 0, 0.03, 0.25 and 3.5 mg/m^3, respectively. Fenamiphos had been diluted in ethanol: polyethylene glycol 400 (1:1) to generate a 0.5% stock solution from which the aerosol mixtures were made daily. The stock solution also contained 0.05% oil red to assist with the measurement of fenamiphos in the inhalation chamber. The stock solution was reportedly stable for the duration of the experiment. Exposure was performed in a dynamic flow inhalation apparatus (Kimmerle & Eben 1973), which reportedly allowed only inhalational exposure (it was not specified whether this meant head- or nose-only exposure). Aerosol droplet size was measured once

during the study in the control and low-concentration groups; >95% of aerosols were <3.0 μm in diameter and therefore respirable. Rats were housed 5/cage, with food and water available *ad libitum*.

Rats were observed daily for clinical signs and physical and behavioural abnormalities. Bodyweights were recorded prior to experimentation and then at the end of each week. Blood samples were collected from 5 rats/sex/dose 24 hours after the final exposure. The following haematology parameters were analysed: Hct, Hb and erythrocyte, leukocyte, differential blood and thrombocyte counts. Plasma and RBC ChE activities were measured prior to experimentation and then after the 5^{th}, 10^{th} and 15^{th} exposures. Brain ChE activity was measured at the end of the study. The following clinical chemistry parameters were analysed: SGOT, SGPT, ALP, urea and glucose. Urine was collected from 5 rats/sex/dose over 16 hours during the final week. The following urinary parameters were analysed: glucose, Hb, protein, pH, urobilinogen and spun deposits (i.e. microscopic sediment).

Rats were sacrificed 24 hours after the final exposure by exsanguination under ether anaesthesia and macroscopically examined. The following organs were weighed: thyroid, heart, lung, liver, spleen, kidneys, adrenals, testes and ovaries. These organs, along with the following, were histopathologically examined in 5 rats/sex/group: oesophagus, stomach, duodenum, brain, ovaries, eyes, bronchial lymph glands, acetyl ChE a, larynx, the nasopharyngeal area of the head and bone marrow.

Group means were statistically analysed by the ranking test of Wilcoxon.

Results

Mortalities, clinical signs and bodyweight: There were no mortalities or clinical signs. There was no treatment-related effect on bodyweight.
Haematology, clinical chemistry and urinalysis: Haematology, clinical chemistry and urinary parameters were unaffected by treatment.

ChE activity: Results of ChE activity measurements are summarised in the table below. At 3.5 mg/m^3, significant inhibition ($p<0.01$) of plasma ChE activity occurred in males at every sampling interval and was approximately 45% relative to pre-exposure activity or the activity in the concurrent control group. This result was clearly toxicologically significant. There was no inhibition of plasma ChE activity at 0.03 or 0.25 mg/m^3 in males.

Taking into account the depression in plasma ChE activity in the control group (of up to 22% relative to pre-exposure activity), no inhibition of plasma ChE activity was considered to occur in females at 0.03 mg/m^3. At 0.25 mg/m^3, plasma ChE inhibition was up to 21% relative to pre-exposure activity, following the 15^{th} exposure (when corrected for the control). While this value is above the 20% cut-off normally considered as a toxicologically significant finding, it was not statistically significant when compared to the concurrent control; on balance it is considered marginal and not toxicologically significant due to the variability in the data. At 3.5 mg/m^3, plasma ChE activity was significantly inhibited (0.01%) by up to 35% relative to pre-treatment activity and when corrected for the control group, with this finding considered toxicologically significant.

Table 26 ChE activity in rats following inhalational exposure to fenamiphos

Parameter	Concentration of fenamiphos (mg/m^3)							
	0		0.03		0.25		3.5	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE – pre-exposure	0.54 ± 0.06	1.44 + 0.17	0.47 ± 0.08	1.34 ± 0.43	0.57 ± 0.06	1.79 ± 0.41	0.49 ± 0.07	1.60 ± 0.45
Plasma ChE – 5th exposure	0.49 ± 0.06	1.13 ± 0.28 (22%)	0.46 ± 0.15	1.11 ± 0.40 (17%)	0.51 ± 0.05	1.16 ± 0.25 (35%)	0.26 ± 0.03** (47%)	0.32 ± 0.06** (80%)
Plasma ChE – 10th exposure	0.50 ± 0.05	1.26 ± 0.29 (12%)	0.50 ± 0.12	1.28 ± 0.47 (4%)	0.51 ± 0.06	1.21 ± 0.25 (32%)	0.29 ± 0.04** (41%)	0.35 ± 0.04** (78%)
Plasma ChE – 15th exposure	0.51 ± 0.07	1.29 ± 0.29 (10%)	0.49 ± 0.13	1.21 ± 0.35 (10%)	0.50 ± 0.06	1.24 ± 0.22 (31%)	0.27 ± 0.05** (45%)	0.28 ± 0.07** (82%)
RBC ChE – pre-exposure	2.72 ± 0.16	2.94 ± 0.29	2.69 ± 0.11	2.92 ± 0.17	2.85 ± 0.14	2.94 ± 0.12	2.99 ± 0.14	2.88 ± 0.14
RBC ChE – 5th exposure	3.17 ± 0.23	3.41 ± 0.13	3.00 ± 0.20	3.17 ± 0.13*	3.14 ± 0.46	3.18 ± 0.14*	2.92 ± 0.45	2.90 ± 0.30**
RBC ChE – 10th exposure	2.79 ± 0.12	2.75 ± 0.20 (6%)	2.50 ± 0.16* (7%)	2.52 ± 0.26 (14%)	2.70 ± 0.18 (5%)	2.51 ± 0.17 (15%)	2.65 ± 0.09 (11%)	2.24 ± 0.13** (22%)
RBC ChE – 15th exposure	2.86 ± 0.19	3.03 ± 0.17	2.95 ± 0.28	3.01 ± 0.29	2.90 ± 0.26	2.89 ± 0.08	2.63 ± 0.26 (12%)	2.50 ± 0.19** (13%)
Brain – day 15	1.46	1.82	1.56	1.71	1.75	1.80	2.08	1.98

Plasma and RBC ChE results expressed as the mean U/mL ± SD, with the % inhibition relative to pre-exposure activity shown in parentheses; brain ChE activity is expressed as the mean U/g; * $p<0.05$ compared to the control; ** $p<0.01$ compared to the control

A slight depression in RBC ChE activity occurred across all treatment groups in both sexes following the 10th exposure relative to their pre-exposure activity. However, when the results were corrected for the inhibition in the concurrent control groups, all were <20% and therefore not considered toxicologically significant. Statistical comparisons between the control and treated groups revealed a significance depression in RBC ChE activity in females at the highest concentration at each sampling interval. However, these findings were not considered toxicologically significant because inhibition was <20% of pre-exposure activity.

There was no treatment-related effect on brain ChE activity.

Pathology: There were no treatment-related macro- or microscopic abnormalities and no effects on absolute or relative organ weights.

Conclusions: The NOEC following inhalational exposure of rats to aerosols of fenamiphos on 15 occasions was 0.25 mg/m^3, based on the inhibition of plasma ChE activity in both sexes at 3.5 mg/m^3. Inhibition of RBC ChE activity only occurred in females at 3.5 mg/m^3 after the 10th exposure. There was no inhibition of brain ChE activity.

5. SUBCHRONIC TOXICITY STUDIES

5.1 Oral administration

5.1.1 Rats

Löser E (1968a) BAY 68138 Subchronic toxicological study in rats. Report No. 745. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 29th March 1969.

This did not appear to be the original report but rather a re-typed or re-written version and did not contain individual animal data. It was deemed unsuitable for regulatory purposes due to the lack of reporting detail and therefore transparency. However, it was included in the current fenamiphos review because there were few short-term repeat-dose or subchronic oral dosing studies available for evaluation.

Mawdesley-Thomas LE & Urwin C (1970a) Pathology report of Bay 68138 sub-chronic toxicological studies in rats (Addendum to report No. 745.) Report No. 3353/70/165. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 23rd April 1970.

Materials and Methods

Fenamiphos (unspecified Batch No. & source; 82% purity) in 40% Silkasil® S was admixed in the diet and fed to 15 Wistar rats/sex/dose (~28-32 days old; ~58 and 59 g bw for males and females, respectively; sourced from Winkelmann Breeders, Paderborn, Germany) at 0, 4, 8, 16 or 32 ppm (equivalent to 0, 0.4, 0.8, 1.6 and 3.2 mg/kg bw/day, respectively, calculated by dividing by a dietary conversion factor of 10) for 3 months. No rationale was given for the dose selection. The control group consisted of 30 rats/sex. Rats were housed under standard conditions, with food and water available *ad libitum*. There was no analysis of the stability, homogeneity or concentration of fenamiphos in the diet.

There was no specific mention of observations for mortalities or clinical signs. Bodyweights and food consumption were recorded weekly. Plasma and RBC ChE activities were measured in 5 rats/sex/dose prior to the initiation of treatment and then after 1, 4, 8 and 12 weeks. There was no analysis of brain ChE activity. No other clinical chemistry parameters were analysed. The following haematology parameters were analysed in 5 rats/sex/dose at 4 and 12 weeks after the initiation of treatment: Hb, Hct and erythrocyte, leucocyte, reticulocyte and differential blood counts. The following urinary parameters were analysed in 5 rats/sex/dose at 4 and 12 weeks after the initiation of treatment: glucose, protein, reducing substances, urobilinogen and microscopic sediment.

All survivors were sacrificed at the end of the 3-month treatment period by exsanguination under ether anaesthesia. These, along with any rats that died during the study, were macroscopically examined. The following organs were weighed: thyroid, heart, lungs, liver, spleen, kidneys, adrenals and testes. The following tissues were histopathologically examined: heart, kidneys, gonads, liver, spleen, brain, thyroids, adrenals, lungs and duodenum.

Results were statistically analysed using the Wilcoxon rank sum test.
Results

A single control male died, while there were no deaths in any other groups. Typical signs of cholinesterase inhibition were reportedly confined to males and females in the high-dose group

during the first 2 months of treatment. It was unclear form the report whether all rats were affected, however it was stated that "these signs did not occur simultaneously in all animals". Graphically-presented data illustrated that there was no treatment-related effect on bodyweight gain. Average food intake was unaffected by treatment.

Results of ChE activity measurements are provided in the table below. While >20% inhibition occurred at and above 0.8 mg/kg bw/day for plasma ChE activity and at and above 1.6 mg/kg bw/day for RBC ChE activity, there was no indication as to whether any findings were statistically significant. In addition, the absence of the complete results for ChE activity measurements (means, standard deviations and individual animal data) made the interpretation of these findings difficult.

There was a dose-related increase in plasma ChE inhibition in both sexes. In males, inhibition of >20% occurred at and above 0.8 mg/kg bw/day at week 1, then at and above 1.6 mg/kg bw/day at weeks 4 and 8. At week 12, plasma ChE activity recovered somewhat such that it was <20% across all doses and therefore not considered toxicologically significant. In females, inhibition of >20% occurred at and above 0.8 mg/kg bw/day across all sampling times and did not appear to recover by the end of the study as was the case in males. Toxicologically significant inhibition of RBC ChE activity (i.e. >20% relative to the controls) occurred at 1.6 mg/kg bw/day (week 8 only) and 3.2 mg/kg bw/day (all sampling times) in both sexes. There appeared to be some recovery of RBC ChE activity as measured at week 12.

Table 27 Results of ChE activity measurements

Parameter	Dose (mg/kg bw/day)									
	0		0.4		0.8		1.6		3.2	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE – week 1	0	0	18.3	6.8	27.1	20.0	35.8	24.4	38.5	40.0
Plasma ChE – week 4	0	0	7.1	16.7	15.4	30.1	20.8	35.2	23.3	47.2
Plasma ChE – week 8	0	0	14.0	-	11.2	39.3	29.4	50.8	37.8	51.3
Plasma ChE – week 12	0	0	13.3	11.0	4.1	33.6	11.9	41.8	19.7	44.3
RBC ChE - week 1	0	0	0	0	0	7.1	3.0	5.7	20.0	36.2
RBC ChE - week 4	0	0	0	0	1.8	0	10.6	7.8	27.7	35.3
RBC ChE - week 8	0	0	0	2.4	7.0	11.8	40.3	55.7	37.0	46.2
RBC ChE - week 12	0	0	0	0	0	1.0	7.6	12.9	25.7	22.0

Results expressed as the % inhibition relative to the control group (note: no raw data were provided in the report); - no data

There were no treatment-related effects on haematology or urinalysis parameters. It was reported that there were no macroscopic abnormalities that were attributable to treatment. The absolute liver weights of males at 1.6 and 3.2 mg/kg bw/day (12.33 and 12.38 g, respectively) were significantly greater (no p value given) than the control (10.86 g), which was attributed to the increased bodyweights of these groups (i.e. there was no effect on relative liver weights). There were no other possible effects on absolute or relative organ weights. There were no treatment-related histopathological abnormalities.

Conclusions: The NOEL following 12 weeks of dietary administration of fenamiphos to rats was 0.4 mg/kg bw/day, based on the toxicologically-significant inhibition of plasma ChE at and above 0.8 mg/kg bw/day. Inhibition of RBC ChE activity occurred at and above 1.6 mg/kg bw.

Hayes RH (1986a) Ninety-day cholinesterase study on rats with technical fenamiphos (®Nemacur) in diet. Toxicology Report No. 717. Study No. 83-171-01. Lab: Mobay Chemical Corporation, Environmental Health Research, Corporate Toxicology Department, Metcalf, Stilwell Kansas, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 18th April to 28th July 1983. Report Date: 26th February 1986.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice (US EPA, 40 CFR Part 160); QA statement

Materials and Methods

Technical fenamiphos [Reference No. 77-297-55; 89% purity (see Appendix II for composition); sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) was admixed in the diet and fed to 20 Fischer 344 rats/sex/dose at 0, 0.36, 0.6 or 1.0 ppm for 14 weeks. There was no rationale for the dose selection.

Diets were prepared weekly using 1% w/w corn oil as the vehicle and an unspecified concentration of acetone as the solvent. Diets were stored frozen until use. The concentration, homogeneity and stability of fenamiphos in the test diets were analysed. Rats were sourced from Charles River Breeding laboratories (Wilmington, Massachusetts, USA) and acclimatised for 7 days prior to experimentation. Rats were 5 weeks old at the commencement of dosing. Males and females weighed between 67-112 and 66-100 g, respectively, at the start of the study. Rats were housed individually under standard conditions, with food and water available *ad libitum*.

Observations for mortality and clinical signs were made at least once daily. An examination for palpable masses and other abnormalities was made weekly. Bodyweights and food consumption were recorded weekly. Blood was collected from 10 rats/sex/dose at weeks 5, 9, 12 and 14 for analysis of plasma and RBC ChE activities. There was no analysis of haematology, clinical chemistry or urinalysis parameters. Rats were sacrificed after 14 weeks by carbon dioxide asphyxiation. Only rats utilised for ChE activity measurements were necropsied and macroscopically examined. Brains were removed for analysis of ChE activity. No organ weights were recorded and there was no histopathological analysis.

Results were statistically analysed by ANOVA followed by a Duncan's Multiple Range test if the F value showed a significant difference at $p<0.05$.

Results

Dietary analysis: Analysis of the diets at weeks 1, 6, 11 and 14 revealed mean analytical levels of 0.52 (range: 0.30-0.93), 0.62 (range: 0.52-0.68) and 1.0 (range: 0.76-1.3) ppm, which corresponded to nominal concentrations of 0.36, 0.6 and 1.0 ppm, respectively. The high variability in the low concentration diet prompted the author to analyse the weekly diet samples for this dose level. This analysis revealed a mean analytical concentration of 0.41 ppm, which is still 12% higher than the nominal level of 0.36 ppm. The author reportedly calculated the mean effective concentration based on the degradation rate over a week and found that the analytical levels were within 91-104% of the nominal levels. However, no data were provided to illustrate these findings.

It was reported that an analysis of the homogeneity of the 0.36 ppm diet revealed significant differences between the middle and the top and bottom layers of the diet. However, the author concluded that the diet was homogenous as the analytical differences between the layers was small and the ranges of each layer overlapped. No data was provided to substantiate this finding. It was reported that stability analysis revealed a half-life of 24 days at room temperature and 105 days when stored in a freezer (unspecified temperature). On this basis, the author concluded that fenamiphos was stable in the diet for the 7 day feeding period. But again, no data were provided to substantiate this finding.

Mortalities and clinical signs: There were no deaths. A number of clinical signs were observed only in the treated groups including corneal opacity (a single 0.36 ppm females at week 13), lachrymation [two females each at 0.36 (weeks 1 & 10) and 1.0 ppm (week 10)], blindness (two 0.60 ppm females at weeks 11, 12 & 13), loose stools (a single 1.0 ppm male at week 4), an ocular lesion (a single 1.0 ppm female at week 13) and inflammation of the abdomen (a single 1.0 ppm female at week 10). However, the occurrence of these signs showed no relationship with dose and therefore was not considered treatment-related. The author reported that the eye effects were due to the collection of blood from the orbital plexus.

Bodyweights and food consumption: Bodyweights and food consumption were unaffected by treatment, noting that there were a number of significant differences ($p<0.05$) with the control that were incidental in nature. Based on the food consumption and bodyweight data, the author calculated that the dose of fenamiphos ingested by rats were 0.030, 0.045 and 0.072 mg/kg bw/day for males and 0.035, 0.053 and 0.084 mg/kg bw/day for females at nominal levels of 0.36, 0.60 and 1.0 ppm, respectively.

ChE activity: Statistically significant inhibition of plasma ChE activity occurred in both sexes at various times and doses, however, all but one value was less than 20% (relative to the control) and therefore not considered toxicologically significant. The inhibition of plasma ChE activity by 24% in 1.0 ppm females at week 9 was viewed as equivocal as there was no similar or greater effect at any other time. Further, the absence of pre-treatment ChE activity measurements for each group made the interpretation of this finding difficult. There was no effect on RBC or brain ChE activities.

Pathology: There were no treatment-related macroscopic abnormalities.

Conclusions: The NOEL in rats following 14 weeks of dietary exposure to fenamiphos was 1 ppm (equal to 0.072 and 0.084 mg/kg bw/day in males and females, respectively), the highest dose tested, based on the absence of plasma, RBC and brain ChE inhibition at this dose.

5.1.2 Dogs

Löser E (1968b) BAY 68138 Subchronic toxicological study on dogs. Report No. 837. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 28th May 1968.

Materials and Methods: Fenamiphos (unspecified Batch No. & source; 82% purity) in 40% Silkasil® S was admixed in the diet and fed to 2 beagle dogs/sex/group at 0, 2, 6 or 18 ppm for 3 months (estimated to be equal 0, 0.05, 0.15 and 0.45 mg/kg bw, respectively, by dividing by a dietary conversion factor of 40). There was no rationale for the dose selection. Dogs were sourced from Foster Dog Farm (Birmingham, England) and quarantined for 3 weeks prior to experimentation. Dogs were 4-5 months old and weighed approximately 8.5-9.5 kg. Dogs were

housed individually under standard conditions; with 200 g feed offered each day. Water was available *ad libitum*. There was no analysis of the concentration, stability or homogeneity of fenamiphos in the diet.

There was no mention of observations for mortalities or clinical signs. Dogs were weighed weekly. Any uneaten food was weighed. Ophthalmoscopy was performed at an unspecified time. The following haematology, clinical chemistry and urinalysis parameters were measured prior to treatment and then at one and 3 months: haematology - Hb, erythrocyte, thrombocyte and leucocyte counts, Hct, MCHC and MCV; clinical chemistry - ALP, SGOT, SGPT, sorbit dehydrogenase, leucine aminopeptidase (LAP) urea, creatinine and glucose; urinalysis – glucose, protein, bile pigments, LAP and microscopic sediment. Plasma and RBC ChE activities were measured prior to treatment and at weeks 1, 4 and 12. Dogs were sacrificed by "opening their thoracic cavities" under sodium evipan anaesthesia. Internal organs were macroscopically examined and the following organ weights recorded: thyroid gland, heart, lungs, pancreas, liver, spleen, kidneys, adrenals and testes. There was no measurement of brain ChE activity. No histopathology was performed. There was no statistical analysis of results.

Results: There were no mortalities. Clinical signs (mild muscle tremors and vomiting) occurred only at 18 ppm in an unspecified number of dogs mainly during week 3-6. There was no treatment-related effect on bodyweight or food consumption. No ophthalmoscopic abnormalities were detected. There was no treatment-related effect on any haematology, clinical chemistry or urinalysis parameter. Dose-related inhibition of plasma ChE activity occurred in both sexes at every sampling interval, which was toxicologically-significant (i.e. >20% relative to the control) at every dose [week 1 (M/F): 32/37, 56/56 and 69/52% at 2, 6 and 18 ppm, respectively; week 4 (M/F): 15/30, 46/51 and 63/60% at 2, 6 and 18 ppm, respectively; week 12 (M/F): 31/28, 61/42 and 61/60% at 2, 6 and 18 ppm, respectively]. Toxicologically-significant inhibition of RBC ChE activity occurred at and above 6 ppm [week 1 (M/F): 0/11, 24/36 and 26/33% at 2, 6 and 18 ppm, respectively; week 4 (M/F): 0/8, 31/52 and 32/56% at 2, 6 and 18 ppm, respectively; week 12 (M/F): 0/11, 38/45 and 58/64% at 2, 6 and 18 ppm, respectively]. There were no treatment-related macroscopic abnormalities or organ weight effects.

Conclusion: The LOEL following 3 months of dietary exposure to fenamiphos in dogs was 2 ppm (estimated to be 0.05 mg/kg bw/day), based on the occurrence of toxicologically-significant plasma ChE inhibition. Toxicologically-significant inhibition of RBC ChE activity occurred at and above 6 ppm (estimated to be 0.15 mg/kg bw/day). Clinical signs (mild muscle tremors and vomiting) occurred only at the highest dietary level of 18 ppm (estimated to be 0.45 mg/kg bw/day). No individual animal data were provided.

Löser E (1969) BAY 68138 Subchronic toxicological study on dogs (3 months feeding test). Report No. 1655. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 22nd August 1969.

Mawdesley-Thomas LE & Urwin C (1970b) Pathology report of Bay 68138: Sub-chronic toxicological studies in dogs (feeding experiment of 3 months) (Addendum to report No. 1655) Report No. 3354/70/166. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 23rd April 1970.

Materials and Methods: Fenamiphos (unspecified Batch No. & source; 99.4% purity) was admixed in the diet and fed to 2 beagle dogs/sex/group at 1, 2 or 5 ppm for 3 months (estimated to be equal to 0.025, 0.05 and 0.125 mg/kg bw, respectively, by dividing by a dietary conversion factor of 40).

The control group consisted of 3 dogs/sex. There was no rationale for the dose selection. Dogs were sourced from The Dog Kennel (Appleton, England) and quarantined for 3 weeks prior to experimentation. Dogs were 5-6 months old and weighed approximately 8-10 kg. Dogs were housed individually under standard conditions, with 300 g feed offered each day. Water was available *ad libitum*. There was no analysis of the concentration, stability or homogeneity of fenamiphos in the diet. Examinations were performed essentially as described above in Löser (1968b), with the following additional parameters measured: prothrombin time, thrombocyte counts and differential blood count; serum lactic dehydrogenase, malic dehydrogenase, bilirubin and cholesterol; measurement of plasma and RBC ChE activities at week 8 (in addition to weeks 1, 4 and 12); and weight of the suprarenal capsule. The following tissues were histopathologically examined: heart, lungs, thymus, lymph nodes, liver, gall bladder, spleen, pancreas, kidneys, bladder, uterus/prostate, gonads, thyroid, adrenals, salivary gland, oesophagus, skeletal muscle, brain, GIT, rib and pituitary gland.

Results: There were no mortalities or clinical signs. There was no treatment-related effect on food consumption or bodyweight. No ophthalmoscopic abnormalities were detected. There was no treatment-related effect on any haematology, clinical chemistry or urinalysis parameter. Toxicologically-significant inhibition of plasma ChE activity (i.e. >20% relative to the control) occurred in both sexes at and above 2 ppm [week 1 (M/F): 8/0, 0/<10 and 43/43% at 1, 2 and 5 ppm, respectively; week 4: (M/F): 17/12, 23/24 and 52/49% at 1, 2 and 5 ppm, respectively; week 8: (M/F): 11/16, 22/22 and 53/59% at 1, 2 and 5 ppm, respectively; week 12 (M/F): 13/18, 0/13 and 41/44% at 1, 2 and 5 ppm, respectively]. Toxicologically-significant inhibition of RBC ChE activity occurred only at the highest dose [Males: 10, 21, 45 and 22% at weeks 1, 4, 8 and 12, respectively; females: 0, 27, 18 and 26% at weeks 1, 4, 8 and 12, respectively). There were no treatment-related macroscopic abnormalities or organ weight effects. There were no treatment-related histopathological abnormalities.

Conclusion: The NOEL following 3 months of dietary exposure to fenamiphos in dogs was 1 ppm (estimated to be 0.025 mg/kg bw/day), based on the occurrence of toxicologically-significant plasma ChE inhibition at and above 2 ppm (estimated to be 0.05 mg/kg bw/day). Toxicologically-significant inhibition of RBC ChE activity occurred only at the highest dietary level of 5 ppm (estimated to be 0.125 mg/kg bw/day). Deficiencies to this study were the lack of statistical analysis and the provision of individual animal data.

Hayes RH (1983) Ninety-day cholinesterase study on dogs with fenamiphos in diet. Toxicology Report No. 444. Study No. 83-174-01. Lab: Mobay Chemical Corporation, Environmental Health Research, Corporate Toxicology Department, Metcalf, Stilwell Kansas, USA. Sponsor: Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 11th April to 22nd July 1983. Report Date: 24th October 1986.

GLP & QA: It was stated that the "study was conducted in accordance with applicable Good Laboratory Practice regulations"; QA statement

Materials and Methods

Fenamiphos (Reference No. 77-297-55; 89% purity; sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) was admixed in the diet and fed to 4 purebred beagle dogs/sex/dose at 0, 0.6, 1.0 or 1.7 ppm for 100 days. There was no rationale for the dose selection. Diets were prepared weekly using 1% w/w corn oil as the vehicle and an unspecified concentration of acetone as the solvent. Diets were stored frozen until use. The concentration of fenamiphos in the diets was analysed in duplicate monthly samples, while random samples taken

from the top, middle and bottom thirds of the 0.6 and 1.7 ppm batches, were tested for homogeneity.

Dogs were sourced from Laboratory Research Enterprises Inc. (Kalamazoo, Michigan, USA) and were approximately 4-months old. They were maintained for approximately 3 months prior to the commencement of the study. Males and females weighed between 7.82-12.30 and 7.03-9.89 kg, respectively, at the start of the study. Dogs were housed individually under standard conditions. Water was available *ad libitum* while the amount of food offered was not specified.

Observations for mortality and clinical signs were made at least once daily. An examination for external abnormalities was made weekly. Bodyweights and food consumption were recorded weekly. Blood was collected from all dogs at weeks 0, 4, 6, 8, 10 and 12 for analysis of plasma and RBC ChE activities. There was no analysis of haematology, clinical chemistry or urinalysis parameters. Dogs were sacrificed after 100 days (unspecified method), necropsied and macroscopically examined. Brains were removed for analysis of ChE activity. No organ weights were recorded and there was no histopathological analysis.

Bodyweight and food consumption data were statistically analysed by ANOVA followed by Duncan's New Multiple Range Test.

Results

Dietary analysis: A 20% variation in the concentration of fenamiphos in the top, middle and bottom thirds of the 0.6 ppm diet was reported, while a 30% variation was determined for the 1.7 ppm diet. On this basis, the author concluded that these batches of diet were homogenous. In the majority of cases, analytical levels of fenamiphos were within $\pm$10% of nominal levels. Exceptions were the 0.6 ppm diet, which had 26% less fenamiphos at 3 months compared to the start of the study, and the 1.7 ppm diet, which had 16 and 21% more fenamiphos after 1 and 2 months, respectively. The interpretation of these findings is made difficult by the small sample size (n=2) and the incomplete and unclear reporting of these results in the study report.

Mortalities, clinical signs, effects on bodyweight and food consumption: There were no mortalities and no treatment-related clinical signs. There was no effect on bodyweight or food consumption. Taking into consideration food consumption and bodyweight data, the author calculated that the actual doses received at dietary levels of 0, 0.6, 1.0 and 1.7 ppm were equal to 0, 0.134, 0.231 and 0.439 mg/kg bw/day, respectively, in males, and 0, 0.143, 0.233 and 0.358 mg/kg bw/day, respectively, in females.

ChE activity: There was no treatment-related effect on RBC or brain ChE activities. Results of plasma ChE activity measurements are summarised in the table below. Toxicologically significant inhibition of plasma ChE activity (i.e. >20% relative to pre-treatment activity) occurred only at the highest dietary level of 1.7 ppm at every sampling interval. While inhibition reached 35% in males and 29% in females (when corrected for the loss of activity in the control), there was no indication in the study report that these findings were statistically significant.

Table 28 Plasma ChE activity in dogs following dietary exposure to fenamiphos

Sample Time	Dietary Level (ppm)							
	0		0.6		1.0		1.7	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 0	1.62 ± 0.31	1.45 ± 0.17	1.45 ± 0.18	1.67 ± 0.22	1.69 ± 0.29	1.58 ± 0.21	1.58 ± 0.22	1.74 ± 0.36
Week 4	1.41 ± 0.21 (13%)	1.25 ± 0.19 (14%)	1.12 ± 0.11 (23%)	1.28 ± 0.17 (23%)	1.25 ± 0.19 (26%)	1.13 ± 0.14 (28%)	0.93 ± 0.09 (41%)	1.03 ± 0.22 (41%)
Week 6	1.45 ± 0.28 (10%)	1.31 ± 0.20 (10%)	1.15 ± 0.14 (21%)	1.35 ± 0.16 (19%)	1.34 + 0.14 (21%)	1.18 ± 0.21 (25%)	1.03 ± 0.12 (35%)	1.08 ± 0.21 (38%)
Week 8	1.40 ± 0.26 (14%)	1.28 ± 0.23 (12%)	1.19 ± 0.15 (18%)	1.34 ± 0.14 (20%)	1.26 ± 0.10 (25%)	1.14 ± 0.15 (28%)	0.98 ± 0.06 (38%)	1.06 ± 0.27 (39%)
Week 10	1.37 ± 0.23 (15%)	1.21 ± 0.17 (17%)	1.12 ± 0.12 (23%)	1.32 ± 0.12 (21%)	1.20 ± 0.08 (29%)	1.13 ± 0.22 (28%)	0.93 ± 0.11 (41%	1.06 ± 0.21 (39%)
Week 12	1.44 ± 0.26 (12%)	1.42 ± 0.18 (2%)	1.20 ± 0.17 (17%)	1.45 ± 0.24 (13%)	1.36 ± 0.20 (23%)	1.29 ± 0.20 (18%)	1.04 ± 0.14 (34%)	1.20 ± 0.22 (31%)

Results expressed as the mean IU/mL ± 1 standard deviation, with the % inhibition relative to week 0 activity contained in parentheses.

Conclusions: The NOEL in dogs following 100 days of dietary exposure to fenamiphos was 1.0 ppm (equal to 0.23 mg/kg bw/day), based on toxicologically significant inhibition of plasma ChE activity at the next highest level of 1.7 ppm (equal to 0.44 mg/kg bw/day in males and 0.36 mg/kg bw/day in females). There was no inhibition of RBC or brain ChE activities.

5.2 Dermal or inhalational administration

There were no subchronic dermal or inhalational toxicity studies submitted for evaluation.

6. CHRONIC TOXICITY STUDIES

6.1 Oral administration

6.1.1 Mice

Hayes RH (1982) Technical fenamiphos (®Nemacur) oncogenicity study in mice. Report No. 241. Lab: Mobay Chemical Corporation, Environmental Health Research Institute, Corporate Toxicology Department, South Metcalf, Stilwell, Kansas, USA. Sponsor: Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 12th June 1978 to 14th February 1980. Report date: 12th February 1982

GLP and QA: QA statement

Materials and Methods

Technical fenamiphos (Batch No. 77-297-55; 90% purity; see Appendix II for composition; sourced from Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) was admixed in the diet and fed to 50 CD1 mice/sex/group at 0, 2, 10 or 50 ppm for 20 months. Ten

rats/sex/group were also assigned as replacements during the first month of the study if required. No rationale was given for the dose selection.

Diets were prepared weekly using 1% w/w corn oil as the vehicle and an unspecified concentration of acetone as the solvent. Diets were offered to mice on a weekly basis, with all uneaten feed destroyed at the end of each week. The homogeneity of the diet was determined by analysing 13 x 50 g random samples of an 8 kg batch of the 50 ppm diet. The stability of ^{14}C-labelled fenamiphos in the diet was determined at room and freezer temperatures (30 and –15°C, respectively) at days 0, 3, 4, 7, 14, 32 and 60. The concentration of fenamiphos in each diet was analysed every 3 months.

Mice were sourced from Charles River Breeding Laboratories (Wilmington, Massachusetts, USA) and acclimatised for one week prior to the commencement of dosing. At the commencement of dosing, mice were 6 weeks of age. Males had an average bodyweight of 26 g, while females weighed approximately 22 g. Mice were housed individually under standard conditions, with food and water available *ad libitum* throughout all phases of the study.

Observations for mortality and clinical signs were made at least once daily. Mice were palpated for masses weekly. Bodyweights and food consumption were recorded weekly. Blood was collected from 10 mice/sex/group at 6, 12, 18 and 30 months for the analysis of the following haematology parameters: Hct, Hb, MCV, erythrocyte counts, leukocyte counts and differential leukocyte counts. No urinalysis was performed and there was no analysis of ChE activity or any other clinical chemistry parameter.

All survivors were sacrificed by carbon dioxide asphyxiation. These along with any rats found dead or sacrificed in a moribund condition during the study, were necropsied; this included an examination of all external surfaces, all orifices, cranial cavity, carcass, external and cut surfaces of the brain and spinal cord, the thoracic abdominal and pelvic cavities and their viscera, and the cervical tissues and organs. The following organs were weighed: adrenals, brain, gonads, heart, kidneys, liver, lungs and spleen. Histopathology was performed on the standard set of organs and tissues (see Appendix V) from all rats.

Bodyweight, food consumption, haematology parameters and relative and absolute organ weights were statistically analysed by ANOVA followed by Duncan's Multiple Range test if significance was attained at $p<0.05$

Results

Dietary analysis: Homogeneity analysis of a batch of the 50 ppm diet found an average fenamiphos concentration of 44.7 ppm, with a variance of 9.6 ppm. However, the actual results of this experiment were not provided in the current report, with a "Mobay Report Number 66680" cited to support the conclusion that this particular batch was homogenous. The half-life of C^{14}-fenamiphos in the diet was reportedly 24 days at room temperature and 105 days when stored in the freezer, with a "Mobay Report No. 66678" cited as the original data source. Further, it was reported that there was an 18% loss of fenamiphos over a week; a sulfoxide metabolite was apparently formed "gradually" over 60 days. The analytical concentration of fenamiphos ranged from 80-126, 75-105 and 73-98% at nominal levels of 2, 10 and 50 ppm, respectively, but again no raw data were provided only reference to a "Mobay Chemists Notebook Reference Numbers B-89 and C-51".

Mortalities and clinical signs: At 12 months, mortality was elevated in 50 ppm females compared to the control (2, 4, 4 and 18% at 0, 2, 10 and 50 ppm, respectively). Over the remainder of the study, mortality in 50 ppm females remained slightly higher than the control (18 months: 34, 44, 48 and

56% at 0, 2, 10 and 50 ppm, respectively; 20 months: 56, 62, 60 and 68 at 0, 2, 10 and 50 ppm, respectively). While statistical analysis using a χ^2 test found no significant difference between any groups, the increased mortality at 12 months is considered a treatment-related finding. In males, there was only a marginal increase in mortality at the highest concentration (4, 2, 4 and 6% at 12 months; 44, 48, 46 and 52% at 18 months; 56, 66, 64 and 60% at 20 months at 0, 2, 10 and 50 ppm, respectively), which is not considered treatment-related.

While it was stated that none of the various clinical signs observed during the study were treatment-related, this was not supported by any observational data (i.e. the incidences of clinical signs were unreported).

Bodyweights and food consumption: All groups showed a similar pattern of bodyweight gain over the course of the study, however, the average weekly bodyweight of 50 ppm mice was significantly lower ($p<0.05$) than the control (up to approximately 10% lower) over the majority of the study. There were a few statistically significant differences at the two lower doses that were considered incidental.

While there were significant differences in food consumption between treated and control mice at certain times, graphically-presented data illustrated that the pattern of food consumption over time was similar across all groups. On this basis, there was no treatment-related effect on food consumption.

Based on food consumption and bodyweight data, the author calculated that the average dose of fenamiphos ingested by mice were 0.3, 1.4 and 7.4 mg/kg bw/day for males and 0.3, 1.8 and 8.8 mg/kg bw/day for females at nominal levels of 2, 10 and 50 ppm, respectively.
Haematology: There was no treatment-related effect on any haematology parameter.

Pathology: There were no tissue masses or lesions in mice sacrificed or dying during the study, or surviving to termination, which were treatment-related.

Average absolute heart and kidney weights were significantly lower ($p<0.05$) than the control in 50 ppm females (heart: 0.233 *versus* 0.267 g, respectively; kidney: 0.561 *versus* 0.658 g, respectively). In females, average absolute brain weight was significantly reduced ($p<0.05$) across all treated groups (0.469, 0.450, 0.452 and 0.443 g at 0, 2, 10 and 50 ppm, respectively). In males, absolute lung and liver weights were significantly lower ($p<0.05$) than the control at 50 ppm (lungs: 0.328 *versus* 0.376 g, respectively; liver: 2.220 *versus* 2.482 g, respectively). Analysis of relative organ weights indicated that only relative brain weight of 50 ppm females was significantly higher ($p<0.05$) than the control (1.609 *versus* 1.476 g, respectively), a finding that was attributable to the significantly lower terminal bodyweight of 50 ppm females (27.4 *versus* 32.4 g in the control). On this basis and in the absence of any histopathological abnormalities, none of these findings were considered treatment-related.

Histopathology: Neoplastic lesions occurred with similar frequency and severity in both treated and control mice. On this basis, fenamiphos was not considered carcinogenic.

There were a number of non-neoplastic lesions that were elevated in treated mice: acute rhinitis in females (2/50, 0/50, 0/49, and 6/49 at 0, 2, 10 and 50 ppm, respectively); amyloid in the submaxillary salivary gland of males (23/49, 34/48, 31/50 and 32/49 at 0, 2, 10 and 50 ppm, respectively); cyst in the thymus in males (0/44, 2/44, 0/46 and 2/48 at 0, 2, 10 and 50 ppm, respectively); chronic myocarditis in females (10, 15, 11 and 17/50 at 0, 2, 10 and 50 ppm, respectively); amyloid in the spleen in males (12, 20, 15 and 21/50 at 0, 2, 10 and 50 ppm,

respectively); acute bronchopneumonia (0, 0, 3 and 3/50 in males and 1, 0, 3 and 4/50 in females at 0, 2, 10 and 50 ppm, respectively); amyloid in the liver of males (7, 27, 19 and 15/50 at 0, 2, 10 and 50 ppm, respectively); multiple granuloma in the liver of males (0, 6, 7 and 0/50 at 0, 2, 10 and 50 ppm, respectively); chronic cystitis of the urinary bladder in males (1/41, 0/45, 4/38 and 3/43 at 0, 2, 10 and 50 ppm, respectively); and amyloid in the ovaries (16, 25, 23 and 18/50 at 0, 2, 10 and 50 ppm, respectively. The occurrence of amyloid deposition in a number of organs/tissues suggested generalised inflammation, which may have been related to the age of the rats. However, in the absence of any dose-response relationships, including any increase in severity, these non-neoplastic findings are not considered treatment-related.

Conclusions: The NOEL in mice following 2 years of dietary exposure to fenamiphos was 10 ppm (equal to 1.4 mg/kg bw/day in males and 1.8 mg/kg bw/day in females), based on the significant reduction in bodyweight at the next highest level of 50 ppm (equal to 7.4 and 8.8 mg/kg bw/day in males and females, respectively). Increased mortality at 12 months occurred in females at 50 ppm (8.8 mg/kg bw). There was no evidence that fenamiphos was carcinogenic. Deficiencies noted in this study were the absence of urinalysis, ChE activity measurements and clinical chemistry. No pre-treatment bodyweight or haematology parameters were recorded.

6.1.2 Rats

Löser E (1972a) Bay 68138 chronic toxicological studies on rats (2-year feeding experiment). Report No. 3539. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 20th June 1972.

Cherry CP & Newman AJ (1973) Pathology report of Bayer 68138 chronic toxicological studies in rats (2 year's feeding experiment. Addendum to report No. 3539. Report No. 5464/72/860. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Friedrich-Ebertstrasse, Germany. Report date: 1st January 1973.

Materials and Methods

A 50% premix of fenamiphos (Sample No. FL 1384/20; unspecified purity & source) in Silkasil S was admixed in the diet and fed to 40 SPF wistar rats/sex/dose for 2 years at levels of 0, 3, 10 or 30 ppm. There was no rationale for the choice of these dietary levels. Control groups consisted of 80 rats/sex. Rats were sourced from Winkelmann (Kirchborchen, Kreis Paderborn, Germany) and housed individually under standard conditions. Rats were approximately 28-32 days old and weighed 45-55 g at the start of the experiment. Food and water were available *ad libitum*. There was no analysis of the concentration, stability or homogeneity of the diet although it was stated that "checks on the LD_{50} levels during and at the end of the experiment showed that they were always normal".

There was no specific mention of observations for mortalities or clinical signs. Bodyweights were recorded weekly during the first 6 months and thereafter on a fortnightly basis. Food consumption was recorded weekly. Blood was taken from 5 rats/sex/dose at 6 and 12 months, and from 10 rats/sex/dose at 24 months. The following haematology parameters were analysed: Hb, Hct, MCH, MCV, prothrombin time and erythrocyte, leucocyte, reticulocyte and differential blood counts. The following clinical chemistry parameters were analysed: SGOT, SGPT, ALP, ornithine-carbamyl transferase, bilirubin, urea, creatinine and glucose. Plasma and RBC ChE activities were analysed in 5 rats/sex/dose at weeks 4, 6, 17, 26, 52, 78 and 105. There was no analysis of brain ChE activity. Urine was collected from an unspecified number of rats at unspecified times and analysed for the following: protein, glucose, blood, bile pigment and microscopic sediment.

All survivors were sacrificed at the end of the 2-year treatment period by exsanguination under ether anaesthesia. These, along with any rats that died during the study, were macroscopically examined. The following organs were weighed: thyroid, heart, lungs, liver, spleen, kidneys, adrenals and gonads. Internal organs (not specified) were macroscopically examined for pathological abnormalities. Histopathology was performed on the following organs: adrenals, brain, duodenum, epididymis, heart, kidneys, liver, lung, ovaries, pituitary, spleen, stomach, testes, thyroid, urinary bladder and uterus.

Group means were statistically analysed using the Wilcoxon rank sum test

Results

Mortalities and clinical signs: After one year, mortality rates were ≤5.0% across all groups and showed no relationship with treatment. After 2 years, male mortality at 0, 3, 10 and 30 ppm was 29, 25, 13 and 25%, respectively. Female mortality rates after 2 years were marginally elevated at 30 ppm (38%) compared to the control, 3 and 10 ppm groups (29, 25 and 23%, respectively). However, this finding is considered unlikely to be toxicologically significant due to the absence of a dose-response effect, the small magnitude of the 'increase' and the absence of any other toxicological effects. At the highest dietary level, rats were reported to exhibit "mild muscle twitching" during the first 6 weeks, which was not evident over the remainder of the study.

Bodyweights and food consumption: Graphically-presented data illustrated that there was no treatment-related effect on bodyweight. Average food consumption was unaffected by treatment. Taking into consideration food consumption and bodyweight data, the author calculated that the actual doses received at dietary levels of 0, 3, 10 or 30 ppm were 0, 0.17, 0.56 and 1.72 mg/kg bw/day, respectively, in males, and 0, 0.23, 0.76 and 2.20 mg/kg bw/day, respectively, in females.

Haematology: While there were no significant effects on any haematology parameter at 6, 12 or 24 months, there were a number of possible treatment-related effects based on apparent dose-response relationships or increases in the high-dose group. However, the toxicological significance of these findings is difficult to interpret due to the absence of pre-treatment analysis of these parameters, the absence of individual animal data and the lack of suitable historical control data. At 6 months, high-dose males had marginally increased Hb (17.1 *versus* 16.4 g/mL in the control), MCH (26.5 *versus* 22.3 γγ in the control) and MCV (71.3 *versus* 64.0 μm^3 in the control). There were no similar increases in females. The table below summarises possible treatment-related effects on selected haematology parameters at 12 months. There was a dose-related decrease in Hb, Hct and erythrocytes in males, which suggested anaemia, but no such effect in females. There was a dose-related increase in reticulocytes in both sexes, while thrombocytes were decreased in males and leucocytes increased in females. At the end of the study, leucocytes were increased in males (7.8, 7.7, 9.8 and 10.8 x 10^3 at 0, 3, 10 and 30 ppm, respectively), while prothrombin spin time was decreased (15.0, 14.9, 13.9 and 13.9 sec at 0, 3, 10 and 30 ppm, respectively). There were no possible effects in females at the end of the study. On balance, these findings are unlikely to be toxicologically significant as there were no consistent effects between males and females or at the 3 sampling times.

Table 29 Selected haematology findings at 12 months

Parameter	Dietary level (ppm)			
	0	**3**	**10**	**30**
Males				
Hb (g/100 mL)	17.3	16.9	16.5	16.6
Hct (% vol)	48	48	47	45
Erythrocytes (10^6)	9.37	9.07	8.96	8.46
Reticulocytes (o/oo)	12	16	16	21
Thrombocytes (10^3)	846	808	776	684
Females				
Reticulocytes (o/oo)	12	16	18	20
Leucocytes (10^3)	6.8	7.0	7.6	9.0

Results expressed as means

Clinical chemistry: The majority of clinical chemistry parameters were unaffected by treatment. There was a dose-related increase in SGPT activity, which only occurred at 6 months: in males, activities of 12.3, 13.1, 19.8 and 20.9 mU/mL occurred at 0, 3, 10 and 30 ppm, respectively; in females, activities of 14.7, 16.3, 19.4 and 19.8 mU/mL occurred at 0, 3, 10 and 30 ppm, respectively. While this finding is suggestive of minor liver damage, its clinical significance is unclear due to the absence of any other indicators of liver damage [e.g. elevated ALP, SGOT, lactate dehydrogenase and bilirubin; liver pathology], the lack of statistical differences with the control group and the lack of suitable historical control data. Cholesterol was elevated in males at 30 ppm (216.1 *versus* 158.0 mg/mL in the control) but only at the end of the study. This finding is unlikely to be toxicologically significant as all groups of males (and females) showed increased cholesterol levels relative to those measured at 12 months.

Urinalysis: There was no treatment-related effect on any urinary parameter.

ChE activity: Dose-related inhibition of plasma and RBC ChE activities occurred in both sexes relative to their respective controls; however, there was no indication in the study report that any results were statistically significant. In males, plasma ChE inhibition of 4-11, 13-19% and 24-45% occurred at 3, 10 and 30 ppm, respectively; with the level of inhibition reasonably consistent across the entire 2 years (i.e. there was no obvious "peak" of inhibition). In males, RBC ChE inhibition of 0-9, 0-21 and 22-54% occurred at 3, 10 and 30 ppm, respectively, with the highest level of inhibition occurring at 26 weeks. In females, plasma ChE inhibition of 0-8, 41-55 and 49-59% occurred at 3, 10 and 30 ppm, respectively, with the level of inhibition consistent across the 2 year study period. In females, RBC ChE inhibition of 0-9, 6-20 and 42-65% occurred at 3, 10 and 30 ppm, respectively, and was consistent across the study. Collectively, the toxicological significance of these findings is somewhat difficult to interpret due to the absence of pre-treatment ChE measurements for each group, a measure of group variability (e.g. standard deviations or errors) and individual animal data. In addition, mean control activities fluctuated over the study. On balance it is considered that toxicologically-significant inhibition of plasma and RBC ChE activities occurred in males only at 30 ppm. In females, plasma ChE activity was inhibited at and above 10 ppm, while RBC ChE activity was only inhibited at 30 ppm.

Pathology: Deaths occurring during the study were reportedly attributable to pneumonia or hypotonicity or atony of the small intestine. It was reported that these deaths showed no relationship with treatment. In rats that were sacrificed at the end of the study, mild to severe pneumonia, chronic nephritis and testicular atrophy were observed but showed no relationship with treatment (incidences not specified). No increased tumour rates were seen in treated rats and the types of tumours seen did not differ to those seen in the controls. There were no treatment-related histopathological abnormalities.

Some absolute organ weights were significantly higher or lower than the controls but were incidental in nature and followed no dose-response relationship nor were consistent in males and females. Some relative organ weights were also significantly different to the control but again the findings were incidental in nature. Overall there was no evidence of any treatment-related effect on absolute or relative organ weights.

Conclusion: The NOEL following dietary exposure to fenamiphos for 2 years was 10 ppm in males (equal to 0.56 mg/kg bw/day) and 3 ppm in females (equal to 0.23 mg/kg bw) based on the inhibition of plasma ChE activity at 30 and 10 ppm, respectively (equal to 1.72 and 0.76 mg/kg bw/day, respectively). RBC ChE activity was inhibited only at 30 ppm (equal to 1.72 and 2.20 mg/kg bw/day in males and females, respectively). There was no evidence that fenamiphos was carcinogenic in rats. The absence individual animal data was a deficiency to the study report.

Hayes RH (1986b) Combined chronic toxicity/oncogenicity study of technical fenamiphos (®Nemacur) in rats. Toxicology Report No. 721. Study No. 83-271-01. Lab: Mobay Corporation, Environmental Health Research, Corporate Toxicology Department, South Metcalf, Stilwell Kansas, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 28th February 1983 to 20th March 1985. Report Date: 28th February 1986.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice (US EPA, 40 CFR Part 160); QA statement

Guidelines: FIFRA Pesticide Assessment Guidelines Subdivision F, Subsection 83-5 (Combined Chronic Toxicity/Oncogenicity Study)

Materials and Methods

Technical fenamiphos (Reference No. 77-297-55; 89% purity, see Appendix II for composition; sourced from Mobay Corporation, Agricultural Chemicals Division) was admixed in the diet and fed to 50 Fischer 344 rats/sex/dose for 2 years at 0, 2, 10 or 50 ppm. Satellite groups of 10 and 20 rats/sex for the control and high-dose groups, respectively, were assigned for sacrifice after one year. Ten rats/sex/group were also assigned as replacements during the first month of the study if required. No rationale was given for the dose selection.

Diets were prepared weekly using 1% w/w corn oil as the vehicle and an unspecified concentration of acetone as the solvent. Diets were stored frozen until use. The concentration, homogeneity and stability of fenamiphos in the test diets were analysed. Rats were sourced from Charles River Breeding laboratories (Wilmington, Massachusetts, USA) and were acclimatised for 10 days prior to experimentation. At the start of the study, rats were 6-weeks old and had average bodyweights of approximately 110 g in males and 95 g in females. Rats were housed individually under standard conditions, with food and water available *ad libitum*.

Observations for mortality and clinical signs were made at least once daily. An examination for palpable masses and other abnormalities was made weekly. Bodyweights and food consumption were recorded weekly. Ophthalmoscopic examinations were performed on 10 rats/sex/group prior to the commencement of dosing and then at 24 months. Blood was collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. Standard haematology parameters were analysed (see Appendix V), with the exception of clotting parameters. Standard clinical chemistry parameters were analysed (see Appendix V), but there was no analysis of triglycerides. Plasma and RBC ChE activities were analysed in 10 rats/sex/group at 1, 2, 3, 5, 12, 18 and 24 months. Brain ChE activity was analysed in 10 rats/sex/group at the end of the study and in all rats from the satellite group designated for

sacrifice after one year. Urine was collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months and analysed for the standard range of parameters (see Appendix V), except for reducing substances.

All survivors were sacrificed by carbon dioxide asphyxiation. These along with any rats found dead or sacrificed in a moribund condition during the study, were necropsied; this included an examination of all external surfaces, all orifices, cranial cavity, brain, body cavities and their viscera and cervical tissues and organs. The following organs were weighed: adrenals, brain (and brain stem), gonads, heart, kidneys, liver, lungs and spleen. Histopathology was performed on the standard set of organs and tissues from all rats (see Appendix VI).

Results were statistically analysed by ANOVA followed by Duncan's Multiple Range test if significance was attained at $p<0.05$

Results

Dietary analysis: Average analytical levels of fenamiphos, as determined from weekly dietary analysis over the entire study period, were 1.9, 8.6 and 41.0 ppm [coefficients of variation (CV) of 10, 10 and 13%, respectively] at nominal levels of 2, 10 and 50 ppm, respectively. The author reportedly calculated the mean effective concentration based on the degradation rate over a week and found that the analytical levels were within 74-85% of the nominal levels.

It was reported that an analysis of the homogeneity of the high- and low-dose diets revealed no significant differences between the top, middle and bottom layers of the mixing bowel used to prepare the diets. The concentration of fenamiphos in the 2 ppm diet reportedly had a 40% range and a 13% CV, while the 50 ppm diet had a 28% range and 10% CV. On this basis the author concluded that the diets were homogenous. While no data were provided to substantiate these findings, the author cited a previous memo regarding the homogeneity of fenamiphos in rodent ration (Moore 1983).

Previous analysis (Lenz & Gronberg 1978) reportedly found that fenamiphos was stable when frozen and had a relative decline of approximately 5% after 60 days. This study also found that fenamiphos was not stable at room temperature, with fenamiphos sulfoxide and fenamiphos sulfone the reported degradants. The half-life of fenamiphos in rodent ration was approximately 25 days.

Mortalities and clinical signs: There was no treatment-related effect on mortality at 52, 78 or 105 weeks. A range of clinical signs were observed across all groups and generally showed no relationship with treatment. Alopecia was elevated in high-dose females (12/50 *versus* 3, 2, 1/50 at 0, 2 and 10 ppm, respectively) in addition to rough coat (34/50 *versus* 13, 13 and 10/50 at 0, 2 and 10 ppm, respectively). The incidence of blindness was somewhat elevated at 50 ppm (0, 2, 1 and 4/50 in males and 2, 1, 1 and 5/50 in females at 0, 2, 10 and 50 ppm, respectively). Females in the 50 ppm satellite group had rough coat (5/20), alopecia (4/20) and eye opacity (4/20) in contrast to the controls, which did not show these signs. The incidence of palpable masses showed no relationship with treatment.

Bodyweights and food consumption: Average bodyweights of males and females at 50 ppm were significantly lower than the controls ($p<0.05$) over most of the 2-year study period (up to approximately 10% and 15% lower in males and females, respectively). In the 50 ppm satellite group sacrificed after one year, average male bodyweights were also lower relative to the control, however, statistical significance ($p<0.05$) was attained only over the first 8 weeks of treatment. In the female satellite group, average bodyweights were significantly lower ($p<0.05$) than the control

over the entire one-year treatment period. No treatment-related effect on bodyweight occurred at 2 or 10 ppm. Food consumption was unaffected by treatment. Based on food consumption and bodyweight data, the author calculated that the dose of fenamiphos ingested by rats was equal to 0.098, 0.464 and 2.454 mg/kg bw/day for males and 0.121, 0.603 and 3.361 mg/kg bw/day for females at nominal levels of 2, 10 and 50 ppm, respectively.

Ophthalmology: Lens opacity was increased in females at 50 ppm (9/50) compared to the other groups (4, 0 and 3 at 0, 2 and 10 ppm, respectively). Ocular abnormalities occurring only in treated groups included corneal opacity (one male each at 10 and 50 ppm; 2 females at 10 ppm), exophthalmia (one male each at 10 and 50 ppm; 4 females at 10 ppm) and closed eyes (one and two males at 2 and 50 ppm, respectively; one female in each dose group).

Haematology: Selected haematology findings are summarised in the table below. At 3 months, RBC and Hct were significantly lower ($p<0.05$) at 50 ppm relative to the control in both males and females, with Hb also significantly reduced in females. There were also significant increases ($p<0.05$) in MCV and MCHC in both sexes. Collectively these findings suggest treatment-related anaemia, however, as all findings were within historical control ranges [RBC: 7.0-10 $10^6/mm^3$; Hct: 42.0-50.0%; Hb: 13.1-16.5 g/dL; MCV 48.3-56.1 fl; MCHC: 35.3-39.2 g/dL; 18-20 week old F344 rats (Derelanko 2000) they were not considered toxicologically significant.

At 6 months, Hct and MCV were significantly higher ($p<0.05$) across all groups of females relative to the control, while MCHC was significantly lower ($p<0.05$) at 10 and 50 ppm; these findings tended to be dose-related. Similar effects on these parameters were not seen in males. Of these findings, only the effect on MCV was slightly above the historical control range for 32-34 week old F344 rats of 48.0-56.0 μ^3 (Derelanko 2000) and could suggest macrocytic anaemia. At 6 months, platelets were significantly increased ($p<0.05$) at 10 and 50 ppm in both sexes relative to the controls but fell within the historical control range of 800-1200 $10^3/mm$ (Derelanko 2000) and was therefore not considered toxicologically significant.

At 12 months, RBC was significantly lower across all male groups ($p<0.05$) compared to the control, with Hb also significantly lower at 50 ppm and Hct at 10 and 50 ppm. No such effects were seen in females. These findings were not toxicologically significant as they fell with the historical control range for 58-60 week old F344 rats [RBC: 7.2-9.5 $10^6/mm^3$; Hb: 15.7-17.5 g/dL; Hct: 40-46.6%; 58-60 week old F344 rats (Derelanko 2000)]. At 18 and 24 months there were no significant effects in males or females that were treatment-related.

Table 30 Selected haematology findings

Parameter	Dietary Level (ppm)							
	0		2		10		50	
	♂	♀	♂	♀	♂	♀	♂	♀
3 months								
RBC ($10^6/m^3$)	9.58 ± 0.42	8.59 ± 0.22	9.43 ± 0.27	8.59 ± 0.24	9.80 ± 0.40	8.86* ± 0.24	9.17* ± 0.25	8.22* ± 0.31
Hb (g/dL)	17.6 ± 0.5	17.2 ± 0.4	17.4 ± 0.5	17.1 ± 0.4	18.1* ± 0.6	17.7* ± 0.6	17.3 ± 0.3	16.8* ± 0.6
Hct (%)	46.4 ± 1.7	45.1 ± 1.2	45.7 ± 1.2	45.3 ± 1.3	47.2 ± 1.7	46.4* ± 1.2	45.1* ± 1.1	43.9* ± 1.7
MCV (μ^3)	48.4 ± 0.8	52.4 ± 0.4	48.4 ± 0.4	52.7 ± 0.4	48.4 ± 0.6	52.3 ± 0.5	49.5* ± 1.9	53.4* ± 0.4
MCH (g/dL)	18.4 ± 0.5	20.0 ± 0.3	18.5 ± 0.3	19.9 ± 0.4	18.4 ± 0.3	19.9 ± 0.3	18.8* + 0.4	20.4* ± 0.5
6 months								
Hct (%)	45.7 ± 2.4	42.0 ± 4.8	44.7 ± 2.2	45.1* ± 1.7	46.1 ± 1.9	45.5* ± 2.4	44.7 ± 2.8	44.4* ± 2.0
MCV (μ^3)	49.8 ± 0.4	53.9 ± 0.4	49.7 ± 0.5	54.7* ± 0.5	49.7 ± 0.7	54.9* + 1.3	50.3* ± 0.8	57.1* ± 1.6
MCHC (g/dL)	37.1 ± 0.7	37.6 ± 0.7	37.8 ± 0.9	37.3 ± 0.7	36.6 ± 0.5	36.8* ± 1.2	37.3 ± 2.2	35.5* ± 0.2
Platelets ($10^3/m^3$)	827 ± 42	799 ± 54	861 ± 110	830 ± 47	939* ± 82	896* ± 111	926* ± 64	948* ± 90
12 months								
RBC ($10^6/m^3$)	9.08 ± 0.32	7.81 ± 0.64	8.75* ± 0.23	7.75 ± 0.38	8.66* ± 0.26	7.89 ± 0.39	8.43* ± 0.78	7.66 ± 0.32
Hb (g/dL)	17.1 ± 0.4	16.7 ± 1.0	16.9 ± 0.5	16.8 ± 0.5	16.7 ± 0.4	16.9 ± 0.4	16.5* ± 1.1	16.7 ± 0.6
Hct (%)	44.2 ± 1.3	42.4 ± 2.6	43.3 ± 1.8	42.1 ± 2.1	42.3* ± 1.2	42.8 ± 2.2	41.6* ± 3.5	42.4 ± 1.7

Results expressed as group averages ± standard deviation; * $p<0.05$

Clinical chemistry: At week 27, blood urea nitrogen was significantly increased ($p<0.05$) in males and females across all groups relative to the controls (13, 23, 24 and 25 mg/dL in males and 20, 25, 27 and 25 mg/dL in females at 0, 2, 10 and 50 ppm, respectively). An examination of individual animal data revealed that these significant differences were attributable to low blood urea nitrogen in 4 or 5 control rats/sex [range of 7-24 mg/dL in males and 9-31 mg/dL in females compared to the historical control range for 32-34 week old F344 rats of 12-24 mg/dL (Derelanko 2000)]. The absence of a dose-response relationship also indicated that these findings were not treatment-related. Cholesterol was significantly elevated in both sexes at 50 ppm (males: 82 *versus* 69 mg/dL in the control; females: 109 *versus* 95 mg/dL in the control) but fell within the historical control range of 50-80 mg/dL in males and 85-130 mg/dL in females (Derelanko 2000) and was therefore not considered toxicologically-significant. Creatinine was significantly lowered across all groups (0.8, 0.5, 0.5 and 0.5 mg/dL in males and 0.9, 0.5, 0.5 and 0.4 mg/dL in females at 0, 2, 10 and 50 ppm, respectively). While the absence of a dose-response relationship for these findings indicates that they were unrelated to treatment, no historical control data were available for 33-week old Fischer 344 rats to confirm the normality of these results; however, they did fall within the range for 18-20 week old F344 rats [0.4-0.8 mg/dL (Derelanko 2000)].

At week 53, glucose was significantly reduced ($p<0.05$) in males (147, 134, 134 and 128 mg/dL at 0, 2, 10 and 50 ppm, respectively). Glucose was also significantly lower ($p<0.05$) at 2 and 50 ppm in females (146, 124, 136 and 125 mg/dL at 0, 2, 10 and 50 ppm, respectively). As the findings in the treated groups fell within the historical control range of 90-140 mg/L for 58-60 week old F344 rats (Derelanko 2000) and did not follow a dose-response relationship, they were not considered

due to fenamiphos. Chloride was significantly elevated ($p<0.05$) in males at 10 and 50 ppm (112 and 114 mEq/L, respectively, *versus* 107 in the control and 103 in the 2 ppm groups) but was only slightly above the historical control range of 100-112 mEq/L at 50 ppm and therefore not considered treatment-related. In females, cholesterol was significantly lower ($p<0.05$) than the control across all groups (132, 114, 108 and 100 mg/dL at 0, 2, 10 and 50 ppm, respectively), with the findings at 10 and 50 ppm below the historical control range for 58-60 week-old F344 females rats of 110-150 mg/dL (Derelanko 2000). An examination of individual rat data revealed a definite dose-related decrease in the range of cholesterol (96-162, 102-145, 84-123 and 65-111 mg/dL at 0, 2, 10 and 50 ppm, respectively). While these findings suggest a treatment-related effect at 10 and 50 ppm, their toxicological significance is unclear. In females, globulin was significantly reduced ($p<0.05$) at every dose (3.2, 2.5, 1.7 and 1.7 g/dL at 0, 2, 10 and 50 ppm, respectively), with the effect at 10 and 50 ppm below the historical control range of 2.3-3.5 g/dL (Derelanko 2000). In the absence of any similar effect in males or at any other times, the toxicological significance of these findings is unclear

There were no possible treatment-related effects on clinical chemistry parameters in males and females at 79 or 109 weeks.

ChE activity: Results of ChE activity measurements are summarised in the table below. Significant ($p<0.05$) dose-related inhibition of plasma ChE activity occurred at every dietary level of fenamiphos: at 2 ppm, average inhibition was up to 37% in males (week 79) and 55% in females (week 15); at 10 ppm, average inhibition was up to 68% in males (week 109) and 83% in females (week 79); at 50 ppm, average inhibition was up to 87% in males and 95% in females (week 79 for both sexes). At the lowest dietary level, toxicologically-significant inhibition of plasma ChE activity did not occur until week 79, while in females, toxicologically-significant inhibition of plasma ChE activity occurred at every sampling period. On this basis, and given the difference in magnitude in inhibition at 10 and 50 ppm, females appeared more sensitive to the effect of fenamiphos based on plasma ChE inhibition. Toxicologically- and statistically-significant ($p<0.05$) inhibition of RBC ChE activity occurred at 10 and 50 ppm: at 10 ppm, average inhibition acetyl ChE d 25% in males (week 15) and 43% in females (week 79), while at 50 ppm, average inhibition acetyl ChE d 80% in males (week 27) and 82% in females (week 8). In the satellite groups treated with 50 ppm fenamiphos for one year, significant inhibition ($p<0.05$) of brain ChE activity occurred in both sexes. In the main study, significant inhibition ($p<0.05$) of brain ChE activity occurred only in males (14% relative to the control).

Table 31 Results of ChE activity measurements

Time	**Dietary Level (ppm)**							
	0		**2**		**10**		**20**	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE - week 8	0.78	2.06	0.64* (18%)	1.20* (42%)	0.44* (44%)	0.41* (80%)	0.23* (71%)	0.21* (90%)
Plasma ChE - week 10	0.62	2.41	0.58 (6%)	1.41* (41%)	0.50* (19%)	0.53* (78%)	0.25* (60%)	0.18* (93%)
Plasma ChE - week 15	0.63	2.53	0.59* (6%)	1.64* (55%)	0.47* (25%)	0.60* (76%)	0.28* (56%)	0.29* (89%)
Plasma ChE - week 27	0.63	2.73	0.58* (8%)	1.63* (40%)	0.48* (24%)	0.49* (82%)	0.17* (73%)	0.22* (92%)
Plasma ChE - week 53	0.81	3.15	0.70* (14%)	2.12* (33%)	0.58* (28%	0.63* (80%)	0.23* (72%)	0.18* (94%)
Plasma ChE - week 79	1.28	2.78	0.80* (37%)	1.64* (41%)	0.49* (62%)	0.47* (83%)	0.16* (87%)	0.15* (95%)
Plasma ChE - week 109	1.32	2.04	0.93* (30%)	1.60* (22%)	0.42* (68%)	0.54* (74%)	0.32* (76%)	0.22* (90%)

Time	Dietary Level (ppm)							
	0		2		10		20	
	♂	♀	♂	♀	♂	♀	♂	♀
RBC ChE - week 8	1.93	1.75	1.79* (7%)	1.65* (6%)	1.44* (25%)	1.02* (42%)	0.38* (80%)	0.35* (82%)
RBC ChE - week 10	1.84	1.79	1.83 (0%)	1.84	1.61* (12%)	1.25* (30%)	0.44* (76%)	0.39* (78%)
RBC ChE - week 15	1.87	1.73	1.77* (5%)	1.71	1.41* (25%)	1.14* (34%)	0.40* (79%)	0.43* (75%)
RBC ChE - week 27	1.84	1.63	1.80* (2%)	1.61	1.44* (22%)	1.01* (38%)	0.36* (80%)	0.31* (81%)
RBC ChE - week 53	1.86	1.76	1.90 (0%)	1.70* (9%)	1.63* (12%)	1.26* (28%)	0.59* (68%)	0.50* (72%)
RBC ChE - week 79	1.96	1.92	1.88* (4%)	1.70* (11%)	1.51* (23%)	1.10* (43%)	0.50* (74%)	0.44* (77%)
RBC ChE - week 109	1.63	1.71	1.66	1.56 (9%)	1.43* (12%)	1.35* (21%)	0.72* (56%)	0.64* (63%)
Brain ChE - week 63 (satellite)	17.0	15.6	-	-	-	-	12.7* (25%)	11.8* (24%)
Brain ChE - week 109	13.2	21.1	12.5 (5%)	12.4	12.8 (3%)	12.1	11.4* (14%)	11.1 (8%)

Results expressed as group averages (IU/mL) $\pm$ 1 standard deviation; % inhibition relative to the control is contained in parenthesis; * $p<0.05$

Urinalysis: At 3 months, average urinary protein[2] was elevated in treated rats, with the findings in males significantly different to the controls at every dose ($p<0.05$) (1.6, 2.1, 2.0 and 2.0 at 0, 2, 10 and 50 ppm, respectively; 0.9, 1.5, 1.6 and 1.6 at 0, 2, 10 and 50 ppm, respectively, in females). However, these findings were not considered treatment-related due to the lack of a dose-response relationship and the wide range in the control groups (1.0-2.0 in males and 0.5-2.0 in females). At 6 months, urinary protein was significantly lowered ($p<0.05$) at every dose and in both sexes relative to the controls (1.8, 0.6, 0.6 and 0.5 in males and 2.0, 1.5, 1.4 and 1.0 in females at 0, 2, 10 and 50 ppm, respectively) a finding which has no toxicological significance. There were no significant effects on urinary protein at later times.

At 18 months, significantly decreased ($p<0.05$) urobilinogen occurred in males (3.1, 3.1, 3.0 and 0.5 Ehrlich units/dL at 0, 2, 10 and 50 ppm, respectively). In females, urobilinogen was also significantly lowered ($p<0.05$) at 10 and 50 ppm, although it was significantly higher ($p<0.05$) at 2 ppm (2.6, 3.2, 0.6 and 0.5 Ehrlich units/dL at 0, 2, 10 and 50 ppm, respectively). An examination of individual animal data indicated that there were no outliers that could have contributed to the significance of these findings (ranges of 3.0-4.0, 3.0-4.0, 3.0-3.0 and 0.5-0.5 in males and 1.0-3.0, 3.0-4.0, 0.5-1.5 and 0.5-0.5 females at 0, 2 10 and 50 ppm, respectively). As there were no suitable historical control data to assess the normalcy of these results, their biological significance is unclear. At 24 months, significantly increased urobilinogen was detected in females across all groups (0.3, 0.5, 0.5 and 0.5 Ehrlich units/dL at 0, 2, 10 and 50 ppm, respectively) but was not considered treatment-related due to the absence of a dose-response effect. Further, the lack of any concomitant perturbation of serum bilirubin indicates that these findings were of no toxicological significance.

Gross pathology: In both sexes, absolute liver weights were significantly lower at 50 ppm compared to the controls (13.848 *versus* 15.534 g in males; 8.213 *versus* 10.291 g in females). Absolute lung weights were significantly higher at 50 ppm (2.389 *versus* 1.917 g in males; 1.428 *versus* 1.735 g in

[2] Relative scale of 0.5+ to 4+, with a trace designated as 0.5+

females). In the satellite groups sacrificed after one year, the absolute lung weights of females were significantly lower at 50 ppm (8.606 *versus* 9.698 g in the control).

In both sexes, relative brain, heart and lung weights were significantly higher at 50 ppm compared to the controls (see table below). Relative kidney weights were also significantly higher ($p<0.05$) in females at 50 ppm. In the satellite groups sacrificed after one year, significantly higher ($p<0.05$) relative brain, heart and lung weights also occurred in females but not males. In the absence of any histopathology in these organs or effects on clinical chemistry parameters, these findings are probably attributable to the decrease in terminal bodyweights in both sexes.

Table 32 Relative organ weights

Organ	Dietary Level (ppm)							
	0		2		10		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Main study								
Brain	0.578	0.742	0.546	0.737	0.550	0.706	0.645*	1.001*
Heart	0.338	0.384	0.328	0.377	0.334	0.358	0.366*	0.480*
Kidneys	1.004	0.964	0.957	1.059	1.351	0.946	1.009	1.155*
Lung	0.552	0.584	0.581	0.652	0.614	0.568	0.772*	1.028*
Satellite groups sacrificed after one year								
Brain	0.502	0.752	-	-	-	-	0.503	0.831*
Heart	0.294	0.329	-	-	-	-	0.299	0.367*
Kidneys	0.801	0.845	-	-	-	-	0.798	0.910*
Lung	0.449	0.518	-	-	-	-	0.453	0.597*

Results expressed as % of bodyweight; * $p<0.05$

Histopathology: There were a range of non-neoplastic lesions that showed some relationship with treatment (summarised in the table below). In females, acute inflammation of the larynx was elevated at 50 ppm (27% *versus* 6% in the control) concomitant with an increase in the severity of this finding; no such effect was seen in males. Granulomatous inflammation of the lungs was evident in both sexes at 50 ppm, with the severity of this finding increasing with dose (males: 16 *versus* 0% in the control; females: 50 *versus* 2% in the control). The author hypothesised that the increased incidence of inflammatory lesions in the nasal, laryngeal and lung tissue of 50 ppm male and female rats was related to the "marked ChE inhibition in these rats which produces hyperactivity, increased respiratory rates and secretions, inhalation of feed and dander debris with possible effects on swallowing and on epiglottal reflex closure. The increased secretions and exposure of the respiratory epithelium to debris may have elicited enhanced defence inflammatory process . . "

In males, there was an increased incidence of medial lobe anomalies (2, 4, 6 and 12% at 0, 2, 10 and 50 ppm, respectively), angiectasis (0, 12, 14 and 10% at 0, 2, 10 and 50 ppm, respectively) and clear cell cytoplasmic change (0, 0, 8 and 10% at 0, 2, 10 and 50 ppm, respectively) in the liver. In the absence of suitable historical control data, the toxicological significance of these findings is unclear. Focal atrophy of the pancreas was increased above the controls at every dose (38, 55, 55 and 58% at 0, 2, 10 and 50 ppm, respectively) but in the absence of a dose-response relationship and with no apparent increase in the severity of this finding, it was not considered treatment-related. There was a slight increase in atrophy of the seminal vesicles at every dose (64, 84, 70 and 88% at 0, 2 10 and 50 ppm, respectively) but the absence of a dose-response relationship excludes this finding as treatment-related. Hyperplasia of the pituitary gland was increased at 50 ppm (60 *versus* 40% in the control) but is a common age-related finding in this particular rat strain.

In females, fatty metamorphosis of the adrenals was increased at the highest dose (36, 42, 32 and 56% at 0, 2, 10 and 50 ppm, respectively) but in the absence of a dose-response relationship or with no increase in severity, this finding was not considered treatment-related. Osteosclerosis of the skull was increased in females (2, 4, 6 and 12% at 0, 2, 10 and 50 ppm, respectively) but the toxicological relevance of this finding is unclear.
In the eyes of both sexes, there was a slight increase in phthisis bulbi (2, 6, 6, and 8% in males and 2, 2, 6 and 8% in females at 0, 2, 10 and 50 ppm, respectively). In females, chronic inflammation of the eye was elevated at 50 ppm (12% *versus* 2% in the control) in addition to atrophy of the optic nerve (2, 4, 0 and 9% at 0, 2, 10 and 50 ppm, respectively), findings which are consistent with previous ophthalmoscopic abnormalities detected at 50 ppm.

Table 33 Selected histopathology findings in the main study

Finding	Dietary Level (ppm)							
	0		2		10		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Larynx								
Acute inflammation	7/50 (2.0)	3/50 (1.7)	3/4 (1.7)	5/50 (2.2)	2/50 (2.0)	1/50 (3.0)	5/49 (2.6)	13/49 (2.6)
Lungs								
Granulomatous inflammation	0/50	1/50 (2.0)	0/50	1/50 (3.0)	1/50 (2.0)	0/50	8/50 (3.4)	25/50 (3.2)
MCL	10/50	14/50	10/50	16/50	18/50	10/50	17/50	6/50
Liver								
Medial lobe anomaly	1/50 (2.0)	3/50 (2.0)	2/50 (2.0)	1/50 (2.0)	3/50 (2.7)	6/50 (2.0)	6/50 (1.8)	4/50 (2.0)
Angiectasis	0/50	6/50 (2.0)	6/50 (2.5)	1/50 (2.0)	7/50 (1.9)	3/50 (2.0)	5/50 (2.2)	2/50 (2.0)
Clear cell cytoplasmic change	0/50	4/50 (2.0)	0/50	0/50	4/50 (2.0)	4/50 (2.0)	5/50 (2.0)	0/50
Spleen								
MCL	14/50	14/50	14/50	21/50	20/50	13/50	21/50	7/50
Pancreas								
Focal atrophy	19/50 (2.1)	12/49 (1.7)	27/49 (1.8)	16/50 (1.8)	27/50 (1.9)	16/50 (2.3)	29/50 (2.3)	17/50 (2.2)
Adrenals								
Fatty metamorphosis	23/50 (1.6)	18/50 (1.6)	20/50 (1.8)	21/50 (1.9)	14/50 (1.9)	16/50 (1.8)	26/50 (1.6)	28/50 (1.8)
MCL	7/50	11/50	6/50	7/50	15/50	4/50	13/50	1/50
Testes								
MCL	2/50	-	2/50	-	3/50	-	5/50	-
Seminal vesicles								
Atrophy	32/50	-	43/50	-	35/50	-	44/49	-
Eyes								
Phthisis bulbi	1/50 (5.0)	1/50 (5.0)	3/50 (5.0)	1/50 (5.0)	3/50 (5.0)	3/50 (5.0)	4/50 (5.0)	4/50 (5.0)
Chronic inflammation	0/50	1/50 (2.0)	0/50	1/50 (5.0)	3/50 (2.3)	0/50	0/50	6/50 (2.0)
Optic nerve								
Atrophy	0/50	1/46 (3.0)	1/49 (5.0)	2/46 (3.0)	1/47 (5.0)	0/50	1/50 (5.0)	4/45 (4.0)
Pituitary gland								
Hyperplasia	20/49 (1.8)	13/50 (2.1)	21/50 (2.1)	12/50 (1.9)	16/50 (1.8)	13/50 (2.1)	30/50 (2.0)	16/50 (2.0)
Skull								
Osteosclerosis	0/50	1/50 (3.0)	0/50	2/50 (2.5)	0/50	3/50 (2.7)	0/50	6/50 (3.0)

Results expressed as the number of rats displaying the finding/total rats in the group; the average severity is given in parentheses; MCL = mononuclear cell leukaemia

In the satellite groups of rats that were sacrificed after one year, there were only a few findings that were elevated at the highest dose. There was a slight increase in cardiomyopathy of the heart in males but not females (75% *versus* 50% in the control), which is common age-related finding in male F344 rats. Pigmentation (haemosiderin) of the spleen was increased in males at 50 ppm (95 *versus* 60% in the control). In females at 50 ppm, calcification in the eye (10 *versus* 0% in the control), retinal degeneration (25 versus 0% in the control) and cataracts (15% versus 0% in the control) were elevated. Calculus of the penis occurred in all males at 50 ppm (9/9) and was absent in the control group.

In terms of neoplastic findings, there was a slight increase in the incidence of mononuclear cell leukaemia (MCL) in the lungs, spleen, adrenals and testes at 10 and 50 ppm in males (lungs: 34 and 36%, respectively, *versus* 20% in the control; spleen: 40 and 42%, respectively, *versus* 20% in the control; adrenals: 30% and 26%, respectively, *versus* 14% in the control; testes: 6 and 10%, respectively, *versus* 4% in the controls) (see table above). However, MCL is a common age-related neoplasm in this particular rat strain and was therefore not considered treatment-related.

The types and incidences of tumours detected in treated rats were similar to those occurring in the controls. In females there was a slight increase in granulosa cell tumour of the ovaries at 50 ppm (2, 2, 0 and 6% at 0, 2, 10 and 50 ppm, respectively). Adenomas of the uterus were also slightly elevated at 10 and 50 ppm (22, 26, 40 and 32% at 0, 2 10 and 50 ppm, respectively). In the absence of a dose-response effect and statistical significance, neither of these findings were considered treatment-related. Overall, there were no differences between groups in the number of rats with tumours (98% in all groups), the number of rats with benign tumours and the number of rats with malignant tumours (including rats with single or multiple benign/malignant tumours). On this basis, fenamiphos was found to have no carcinogenic potential in this particular rat strain.

Conclusions: The LOEL following 2 years of dietary exposure to fenamiphos was 2 ppm (equal to 0.098 mg/kg bw/day in males and 0.121 mg/kg bw/day females) due to toxicologically- and statistically-significant inhibition of plasma ChE activity at and above this dose. At and above 10 ppm (equal to 0.464 mg/kg bw/day in males and 0.603 mg/kg bw/day in females), toxicologically- and statistically-significant inhibition of RBC ChE activity occurred. At the highest level of 50 ppm (equal to 2.454 mg/kg bw/day in males and 3.361 mg/kg bw/day in females), toxicologically- and statistically-significant inhibition of brain ChE activity occurred at one year, with statistically significant inhibition occurring in males at 2 years. A range of other treatment-related effects were also evident at 50 ppm and included significantly lower bodyweights, clinical signs in females (alopecia and rough coats), increased blindness, lens opacity (females), increased incidence of inflammatory lesions in the nasal, laryngeal and lung tissue (due possibly to chronic ChE inhibition), increased absolute lung and liver weights and increased relative brain, heart and lung weights. Females appeared more sensitive to the effects of fenamiphos on ChE activity than males. Fenamiphos was not carcinogenic in Fischer 344 rats.

6.1.3 Dogs

Löser E (1972b) Bay 68138 chronic toxicological studies in dogs (2-year feeding experiment). Report No. 3561. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 3rd July 1972.

Thomson C, Newman AJ & Urwin C (1972) Pathology report of Bayer 68138 chronic toxicity study in dogs (administration in the diet for 2 years) (addendum to report No. 3561). Report No. 5380/72/776. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 22nd September 1972.

Materials and Methods

A 50% premix of fenamiphos (Sample No. FL 1384/20; unspecified purity & source) in Silkasil S was admixed in the diet and fed to 4 Beagle dogs /sex/dose for 2 years at levels of 0, 0.5, 1, 2, 5 and 10 ppm. There was no rationale given for the choice of these dietary levels. Dogs were sourced from Appleton dog farm (England) and housed individually under standard conditions. Dogs were approximately 6-8 months old, with males and females weighing approximately 9.5 and 8 kg, respectively, at the start of the experiment. Dogs were offered 250 g of food for the first 4 weeks and thereafter were given 300 g. Water was presumably available *ad libitum*. There was no analysis of the concentration, stability or homogeneity of the diet although it was stated that "checks on the LD_{50} levels during and at the end of the experiment showed that the values were always within the normal range".

There was no specific mention of observations for mortalities or clinical signs. Bodyweights were recorded weekly. Any uneaten food was weighed. Ophthalmoscopy was performed at an unspecified time. Blood samples were taken prior to experimentation and then at 3, 6, 12 and 24 months. The following haematology parameters were analysed: sedimentation rate, Hb, Hct, MCH, MCV, prothrombin time and erythrocyte, leucocyte, reticulocyte, thrombocyte and differential blood counts. The following clinical chemistry parameters were analysed: ALP, ornithine-carbamyl transferase, sorbitol dehydrogenase, SGOT, SGPT, bilirubin, total protein, globulin, bromsulphalein retention, cholesterol and glucose. Plasma and RBC ChE activities were analysed prior to experimentation and then at weeks 1, 4, 13, 26, 52, 78 and 105. There was no analysis of brain ChE activity. Urine was collected at unspecified times and analysed for the following: protein, glucose, blood, bile pigment, microscopic sediment, urea and creatinine.

All survivors were sacrificed at the end of the 2-year treatment period by exsanguination under sodium-Evipan anaesthesia. These, along with any dogs that died during the study, were autopsied and their internal organs macroscopically examined. The following organs were weighed: thyroid, heart, lungs, liver, spleen, kidneys, adrenals and gonads. The following organs were histopathologically examined: heart, lung, thymus, lymph nodes, liver, gall bladder, spleen, pancreas, kidney, urinary bladder, uterus, prostate, gonads, thyroids, adrenals, salivary glands, oesophagus, stomach, intestines, diaphragm, bone, brain, cervix and pituitary.

No statistical analysis was performed.

Results

Mortalities and clinical signs: A single female from the 0.5 ppm group died due reportedly to "massive pneumonia", while there were no other deaths in any group. There were no clinical signs observed during the study.

Bodyweights and food consumption: Graphically-presented data illustrated that there was no treatment-related effect on bodyweight. Food consumption was unaffected by treatment. Taking into consideration food consumption and bodyweight data, the author calculated that the doses received at dietary levels of 0, 0.5, 1, 2, 5 and 10 ppm were 0, 0.015, 0.029, 0.063, 0.150 and 0.311

mg/kg bw/day, respectively, in males, and 0, 0.014, 0.036, 0.060, 0.171 and 0.338 mg/kg bw/day, respectively, in females.

Ophthalmoscopy: No ophthalmoscopic abnormalities were detected in any dogs.

Haematology, clinical chemistry and urinalysis: There was no treatment-related effect on any haematology, clinical chemistry or urinary parameter.
ChE activity: Results of ChE activity measurements are summarised in the table below. Dose-related inhibition of plasma and RBC ChE activities occurred in both sexes relative to their pre-treatment activity, however, there was no indication in the study report that any results were statistically significant. Toxicologically-significant inhibition of plasma ChE activity occurred in males at and above 2 ppm, and in females at and above 1 ppm. However, the effect in females at 1 ppm only occurred at week 4. At 5 and 10 ppm, plasma ChE inhibition was consistent over the entire study period, while at 2 ppm, toxicologically significant inhibition was reacetyl ChE d at week 4 in females and week 52 in males. Toxicologically significant inhibition of RBC ChE activity occurred in both sexes at and above 5 ppm (from week 13 at 5 ppm and from week 1 and 4 in females and males, respectively, at 10 ppm).

Table 34 ChE activity measurements in dogs

Week	Dietary Level (ppm)					
	0	**0.5**	**1**	**2**	**5**	**10**
Plasma ChE activity in males						
Week 0	7.8	8.1	8.2	7.2	7.0	8.1
Week 1	7.5	8.5	8.3	6.9	3.9 (40%)	3.9 (49%)
Week 4	6.6	7.7	7.1	5.9 (3%)	3.5 (35%)	3.6 (36%)
Week 13	7.0	7.8	6.6 (10%)	5.2 (8%)	3.5 (40%)	3.7 (44%)
Week 26	5.9	7.6	6.3	4.9 (8%)	3.3 (29%)	3.6 (32%)
Week 52	7.5	7.7 (1%)	7.2 (8%)	4.8 (29%)	2.9 (55%)	3.9 (48%)
Week 78	7.9	7.2 (11%)	7.0 (15%)	5.3 (26%)	4.0 (43%)	3.8 (53%)
Week 105	7.7	7.8 (4%)	6.6 (10%)	5.7 (20%)	3.3 (52%)	4.0 (50%)
Plasma ChE activity in females						
Week 0	7.7	9.6	7.4	8.1	7.3	8.6
Week 1	8.1	8.7 (9%)	6.7 (9%)	6.7 (17%)	4.6 (37%)	5.7 (34%)
Week 4	7.5	7.5 (19%)	5.1 (28%)	5.5 (29%)	3.9 (44%)	3.9 (52%)
Week 13	7.9	8.7 (9%)	6.4 (14%)	5.1 (30%)	3.1 (58%)	4.0 (53%)
Week 26	7.8	8.2 (15%)	7.0 (5%)	5.7 (30%)	4.6 (37%)	4.6 (47%)
Week 52	8.5	8.6 (10%)	6.2 (16%)	6.9 (15%)	3.9 (47%)	4.7 (45%)
Week 78	7.7	8.0 (17%)	6.2 (16%)	5.5 (32%)	4.7 (36%)	5.4 (37%)
Week 105	6.5	9.8	5.3 (8%)	4.7 (26%)	4.8 (18%)	4.4 (33%)
RBC ChE activity in males						
Week 0	7.1	7.7	7.0	8.2	7.4	8.0
Week 1	6.7	7.9	7.6	9.4	6.9 (1%)	7.1 (5%)
Week 4	6.9	7.6	8.0	9.6	5.9 (17%)	3.8 (49%)
Week 13	7.3	8.0	7.9	9.2	5.0 (32%)	2.9 (64%)
Week 26	7.7	8.5	8.6	9.2	5.2 (30%)	3.3 (59%)
Week 52	8.1	8.2	9.1	8.5	4.6 (38%)	3.2 (60%)
Week 78	7.5	7.7	8.5	10.2	5.4 (28%)	4.0 (50%)
Week 105	8.0	8.1	8.9	10.2	6.3 (15%)	4.3 (46%)
RBC ChE activity in females						
Week 0	8.7	8.5	6.4	8.9	8.1	9.8
Week 1	7.7	8.0	6.6	8.1 (9%)	7.2 (11%)	6.2 (37%)
Week 4	8.6	8.7	5.8 (10%)	8.6 (4%)	6.6 (19%)	4.3 (56%)
Week 13	7.3	10.3	5.5	8.7	5.0 (22%)	3.8 (45%)
Week 26	7.5	9.9	5.4 (13%)	10.5	5.8 (25%)	5.3 (43%)
Week 52	9.7	11.8	7.3	7.5 (16%)	5.4 (33%)	3.3 (66%)
Week 78	8.3	11.0	6.9	9.6	6.0 (21%)	4.7 (47%)
Week 105	8.4	8.6	6.8	8.9	6.1 (22%)	4.1 (55%)

Results expressed as the mean μmole acetylcholine, with the % inhibition relative to pre-treatment activity contained in parentheses and corrected for any decrease in activity in the control group.

Pathology: There were no treatment-related macroscopic abnormalities detected at autopsy. There was no treatment-related effect on absolute or relative organ weights. There were no treatment-related histopathological abnormalities.

Conclusion: The NOEL in dogs following dietary exposure to fenamiphos for 2 years was 0.5 ppm in females (equal to 0.014 mg/kg bw/day) and 1 ppm in males (equal to 0.029 mg/kg bw/day), based on the toxicologically significant inhibition of plasma ChE activity at and above the next highest dose (0.036 mg/kg bw in females and 0.063 mg/kg bw/day in males). Toxicologically-significant inhibition of RBC ChE activity occurred at and above 5 ppm (0.150 and 0.171 mg/kg bw/day in males and females, respectively). The absence of histopathological examinations and individual animal data were deficiencies to this study and study report, respectively.

Rieth JP (1991) Chronic feeding toxicity study of technical grade fenamiphos (Nemacur®) with dogs. Study No. 88-274-BB. Lab: Mobay Corporation, Health, Environment, Safety and Plant Management Corporate Toxicology Department, South Metcalf, Stilwell, Kansas, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 9th January 1989 to 10th January 1990. Report Date: 20th December 1991.

Jones RD & Greufe NP (1993) Response to USA-EPA review. Supplemental submission to EPA MRID No. 42183601. Original Study No. 88-274-BB. Lab: Miles Inc., Agriculture Division, Toxicology, South Metcalf, Stilwell, Kansas, USA. Sponsor: Miles Inc., Agriculture Division, Kansas City, Missouri, USA. Report Date: 12th July 1993

Van Goethem DL & Elcock LE (1997) Chronic feeding toxicity study of technical grade fenamiphos (Nemacur®) with dogs. Supplemental submission to EPA MRID No. 42183601. Original Study No. 88-274-BB. Lab: Bayer Corporation, Agriculture Division, Toxicology, South Metcalf, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Agriculture Division, Kansas City, Missouri, USA. Report Date: 31st March 1997.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (1989) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement

Guidelines: Study reportedly conducted in accordance with the following guidelines: (1) US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline 83-1, November 1984; (2) US-EPA-TSCA, Health Effects Testing Guidelines, 40 CFR Section 798.3320, July 1988; (3) OECD Guideline for Testing of Chemicals, Section 4, Guideline 452, May 1981; (4) Japan Ministry of Agriculture, Forestry and Fisheries, Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, 59 NohSan No. 4200, January 1985.

Materials and Methods

Technical grade fenamiphos (Batch No. 77-297-55; 88.3% purity; see Appendix II for composition; sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) in 1% (w/w) corn oil was admixed in the diet and fed to 4 Beagle dogs/sex/group for one year at concentrations of 0, 1, 3 or 12 ppm (analytical levels of 0, 0.96, 2.93 and 11.5 ppm, respectively). The dose selection was based on the results of previous chronic and subchronic studies in dogs (Löser 1972b; Hayes 1983). Diets were prepared weekly and stored at –23°C. Analysis of the homogeneity of fenamiphos in the low- and high-dose diets was performed prior to experimentation. The stability of the low- and high-dose diets at room and freezer temperatures (~22 and -23°C, respectively) was determined prior to experimentation. The concentration of fenamiphos in all three diets was analysed every 3 months.

Dogs were sourced from White Eagle Laboratories (Doylestown, Pennsylvania, USA) and acclimatised for approximately 6 weeks prior to experimentation. On receipt, dogs were approximately 6 months old, while pre-treatment bodyweights were 6753-10925 g for males and 5521-9976 g for females. Dogs were housed individually under standard conditions. Food and water were available *ad libitum* throughout all phases of the study.

Observations for mortalities and clinical signs were made daily, with a detailed assessment of clinical signs made weekly. Bodyweights were recorded weekly. Food consumption was recorded daily. Ophthalmoscopic examinations were made prior to the commencement of dosing and sacrifice. Fasted blood samples were collected at three unspecified times prior to the commencement of dosing and then at 3, 6, 9 and 12 months. The following haematology parameters were analysed: Hb, Hct, MCH, MCV, MCHC and erythrocyte, leucocyte, platelet and differential blood counts. Standard clinical chemistry parameters were analysed (see Appendix V) in addition to uric acid, liothyronine and thyroxine. Plasma and RBC ChE activities were analysed prior to experimentation and at 3, 6, 9 and 12 months. Brain ChE activity was analysed at termination. Urine was collected once prior to treatment and then at 3, 6, 9 and 12 months. The following urinary parameters were analysed: appearance, specific gravity, protein, ketones, urobilinogen, microscopic sediment, pH, glucose, occult blood and bilirubin.

At the end of the study, all dogs were sacrificed by an intravenous injection of T-61 (Hoechst-Roussel) and necropsied, which consisted of an examination of all external surfaces, all orifices, body cavities and their viscera and cervical tissues and organs. The following organs were weighed: adrenals, brain, heart, liver, lungs, gonads, pituitary and thyroid (with parathyroids). The standard range of organs and tissues were macroscopically and histopathologically examined (see Appendix VI).

Results were statistically analysed by ANOVA followed by a Student's t-test if significance was attained.

Results

Dietary analysis: Analysis of the top, middle and bottom layers of the mixing bowels used to prepare the 1 and 12 ppm diets indicated that these diets were homogenous (mean concentrations of 0.92$\pm$0.075 and 11.8+0.46 ppm, respectively). Fenamiphos was stable for 14 days at room temperature and 28 days in the freezer (room temperature: 76.6-92.8 and 92.5-116% of the nominal concentrations at 1 and 12 ppm, respectively, over 0, 1, 3, 7, 10 and 14 days; freezer: 78.7-113 and 93.3-122% of nominal concentrations, respectively, over 0, 7, 14, 21 and 29 days). Mean analytical concentrations of fenamiphos were 96-98% of nominal levels in all diets (analytical levels of 0.96$\pm$0.094, 2.93$\pm$0.23 and 11.5$\pm$0.67 ppm at nominal levels of 1, 3 and 12 ppm, respectively).

Mortalities and clinical signs: All dogs survived the duration of the study. There were no treatment-related clinical signs or ophthalmoscopic abnormalities.

Bodyweights and food consumption: Bodyweights and food consumption were unaffected by treatment. Taking into consideration food consumption and bodyweight data, the author calculated that the actual doses received at dietary levels of 0, 1, 3 and 12 ppm were 0, 0.030, 0.089 and 0.308 mg/kg bw/day, respectively, in males, and 0, 0.030, 0.083 and 0.349 mg/kg bw/day, respectively, in females.

Haematology: There was no treatment-related effect on any haematology parameter in females.

At 12 ppm in males, there were a number of significant effects that collectively suggested treatment-related anaemia of marginal toxicological significance. Erythrocyte counts were significantly reduced ($p<0.05$) relative to the control (5.78±0.48, 5.80±0.59, 5.98±0.72, 6.09±0.43 and 6.45±0.21 $10^6/mm^3$ at days –3, 98, 189, 280 and 361, respectively, *versus* 6.05±0.48, 7.13±0.46, 7.35±0.31, 6.90±0.36 and 7.28±0.26 $10^6/mm^3$, respectively, in the control). While values in treated dogs were below the performing laboratory's historical control range (6.51-8.24 $10^6/mm^3$ at 8-10 months and 5.54-8.17 $10^6/mm^3$ at ≥11 months) and suggestive of a toxicological effect, the pre-treatment erythrocyte counts of this group were already lower than this range and are therefore cannot be viewed as treatment-related.

At 12 ppm in males, Hb was significantly reduced ($p<0.05$) at day 98 only (14.1±1.4 *versus* 16.5±0.9 g/dL in the control, with pre-treatment concentrations of 13.6±1.5 and 13.5±1.1 g/dL, respectively). While this finding is below the historical control range of 14.95-19.48 g/dL for 8-10 month old beagles, so too was the pre-treatment Hb, and on this basis, is not considered a treatment-related effect. Hct was significantly lowered ($p<0.05$) at day 98 and 189 (40.7±4.1 and 42.0±5.1%, respectively, *versus* 47.4±2.4 and 49.7±1.8%, respectively, in the control), which was below the respective historical control ranges for age-matched dogs (43.08-55.08% at 8-10 months and 43.07-55.77% for ≥11 months). However, given that pre-treatment Hct was below the historical control range (40.1% in both the control and 12 ppm groups), this finding cannot be viewed as treatment-related.

MCV was significantly elevated ($p<0.05$) relative to the control across the entire study period (69.4±0.8, 70.2±1.1, 70.3±0.7, 68.7±0.3 and 69.6±1.3 μm^3 at days –3, 98, 189, 280 and 361, respectively, *versus* 66.2±1.0, 66.6±1.2, 67.7±1.1, 66.0±1.1 and 66.6±1.0 μm^3, respectively, in the control). However, given that all values fell within the performing laboratory's historical control range for age-matched beagles (62.58-70.51 μm^3 at 8-10 months and 62.54-71.82 at ≥11 months), this elevation was not considered toxicologically significant. At the end of the study (day 361), the MCH of 12 ppm males was significantly higher than the control (24.2±0.5 *versus* 23.0±0.5 pg); this finding was not considered toxicologically significant as it fell within the historical control range of 22.14-25.12 pg for beagles ≥11 months old.

Clinical chemistry: There were a number of statistically significant findings ($p<0.05$) at the highest concentration of 12 ppm that were considered incidental in nature as they fell within the historical control range for age-matched beagle dogs. In high-dose females, significantly elevated ($p<0.05$) SGPT activity occurred at day 280 relative to the control (52±7 *versus* 32±5 IU/L) but as this finding was within the performing laboratories historical control range (0.00-151.03 IU/L for dogs aged ≥ 11 months), it was not considered toxicologically significant. There were no other possible treatment-related effects on any clinical chemistry parameters.

ChE activity: Results of ChE activity measurements are summarised in the table below. Toxicologically- and statistically-significant ($p<0.05$) inhibition of plasma ChE activity occurred at every concentration. When corrected for any loss of activity in the control group, mean plasma ChE inhibition in males over one year was 17-24, 35-52, 53-57% at 1, 3 and 12 ppm, respectively, and 6-23, 27-48 and 52-60%, respectively, in females. Toxicologically- and statistically-significant inhibition of RBC ChE activity occurred in males at 3 and 12 ppm. When corrected for any loss of activity in the control group, mean RBC ChE inhibition in males over one year was 14-22 and 42-52% at 3 and 12 ppm, respectively. While statistically significant inhibition of RBC ChE activity occurred in females at these same concentrations, only the inhibition at 12 ppm was considered toxicologically significant as it was >20% of pre-treatment activity. When corrected for any loss of activity in the control group, mean RBC ChE inhibition in females at 12 ppm was 45-58% over one

year. There was no treatment-related inhibition of brain ChE activity in males, while statistically significant inhibition ($p<0.05$; 17%) occurred in females at 12 ppm.

Table 35 Results of ChE activity measurements in dogs

Finding	Dietary concentration (ppm)			
	0	**1**	**3**	**12**
Males				
Plasma ChE - pretreatment[1]	1.35	1.91	1.59	1.73
Plasma ChE – day 98	1.33±0.29 (1%)	1.44±0.46 (25%)*	0.75±0.14 (53%)*	0.73±0.07 (58%)*
Plasma ChE – day 189	1.20±0.31 (11%)	1.28±0.33 (33%)*	0.75±0.12 (53%)*	0.61±0.04 (65%)*
Plasma ChE – day 280	1.17±0.19 (13%)	1.34±0.43 (30%)*	0.82±0.17 (48%)*	0.59±0.11 (66%)*
Plasma ChE – day 361	1.28±0.20 (5%)	1.43±0.50 (25%)*	0.86±0.14 (46%)*	0.67±0.06 (61%)*
RBC ChE - pretreatment[1]	2.47	2.78	2.74	2.79
RBC ChE – day 98	2.21±0.54 (11%)	2.51±0.42 (10%)	1.84±0.59 (33%)*	1.04±0.15 (63%)*
RBC ChE – day 189	1.88±0.39 (24%)	2.13±0.39 (23%)	1.71±0.46 (38%)*	0.96±0.16 (66%)*
RBC ChE – day 280	2.04±0.45 (17%)	2.36±0.39 (15%)	1.88±0.59 (31%)*	0.98±0.12 (65%)*
RBC ChE – day 361	2.15±0.50 (13%)	2.40±0.40 (14%)	1.98±0.61 (28%)*	1.11±0.12 (60%)*
Brain ChE	7.31±0.2	7.41±0.6	7.02±0.4 (4%)	6.41±0.7 (12%)
Females				
Plasma ChE - pretreatment[1]	1.58	1.60	1.71	1.60
Plasma ChE – day 98	1.57±0.23 (1%)	1.26±0.08 (21%)*	0.99±0.13 (43%)*	0.68±0.17 (57%)*
Plasma ChE – day 189	1.36±0.20 (14%)	1.28±0.20 (20%)	1.01±0.23 (41%)*	0.54±0.10 (66%)*
Plasma ChE – day 280	1.63±0.41	1.26±0.06 (20%)*	0.89±0.06 (48%)*	0.61±0.08 (60%)*
Plasma ChE – day 361	1.52±0.18 (3%)	1.18±0.18 (26%)*	1.00±0.17 (43%)*	0.72±0.09 (55%)*
RBC ChE - pretreatment[1]	3.30	3.05	3.29	2.99
RBC ChE – day 98	3.12±0.38 (5%)	2.75±0.41 (10%)	2.65±0.72 (20%)*	1.12±0.08 (63%)*
RBC ChE – day 189	2.53±0.43 (23%)	2.46±0.39 (19%)	2.26±0.37 (31%)	0.96±0.08 (68%)*
RBC ChE – day 280	2.84±0.27 (14%)	2.62±0.23 (13%)	2.47±0.50 (25%)*	1.11±0.05 (63%)*
RBC ChE – day 361	3.01±0.27 (8%)	2.71±0.43 (11%)	2.73±0.60 (17%)*	1.24±0.05 (58%)*
Brain ChE	7.55±0.3	7.70±0.3	7.08±0.6 (6%)	6.24±0.6 (17%)*

Results expressed as mean IU/mL ± SD, with the % inhibition relative to pre-treatment activity contained in parentheses; 1 = standard deviations not given in the study report; *$p<0.05$

Urinalysis: There was no treatment-related effect on any urinary parameter.

Pathology: Absolute and relative organ weights were unaffected by treatment. There were no treatment-related macroscopic findings.

Histopathology: Atrophy of the prostate was detected in one 12 ppm male (described as slight and diffuse) in addition to a cyst (described as minimal multifocal), while a cyst was also detected in a

second 12 ppm male; no such findings were detected in any other groups. Bayer historical control data (contained in Van Goethem & Elcock 1997) indicated a 0% (0/36) incidence for atrophy and a 14% (5/36) incidence for cysts. The incidences in the current study of 25 and 50%, respectively, are above the historical control incidences. However, given the slight/minimal nature of the findings and that only one male had atrophy, both findings were considered to show an equivocal relationship with treatment.

The occurrence of lymphocytic inflammation of the gall bladder (described as "slight focal") followed a shallow dose-response relationship in males (0, 0, 1 and 3 at 0, 1, 3 and 12 ppm, respectively) and was also increased in females at the highest concentration (1, 1, 0 and 2 at 0, 1, 3 and 12 ppm, respectively). The historical control incidence of this finding is 14% (5/36) in males and 11% (4/36) in females. The authors indicated that the severity of the inflammation did not increase with dose and given that lymphocytic inflammation of the gall bladder had been seen previously in other studies, they considered the finding to be incidental in nature. However, the presence of a dose-response relationship in males and the fact that 3/4 males were affected at the highest dose of 12 ppm and none were affected in the control, mount a compelling case for a treatment-related finding in high-dose males (albeit of unclear toxicological significance).

In females, chronic inflammation of the lung occurred in some treated dogs but was absent in the controls (0, 1, 1, and 2 at 0, 1, 3 and 12 ppm, respectively). The historical control incidence of this finding is 8% (3/36) and therefore the incidences found in the current study of 25, 25 and 50% at 1, 3, and 12 ppm, respectively, are above this value. While the findings in single animals at 1 and 3 ppm can be discounted as treatment-related, the same cannot be said for the highest dose. While the authors have concluded that chronic inflammation of the lung was an incidental finding, the presence of a dose-response relationship and the relatively high incidence at the highest dose, it is considered a treatment-related finding. Vasculitis (described as minimal focal) was detected in 2 dogs at 12 ppm but not in any other group. This finding is considered treatment-related as it is above the historical control incidence of 0%.

No neoplastic lesions were detected in any dogs.

Conclusions: No NOEL was established in this study. The LOEL in beagle dogs following one-year of dietary exposure to fenamiphos was 1 ppm (equal to 0.03 mg/kg bw/day in males and females), based on toxicologically and statistically significant inhibition of plasma ChE activity at and above this level. At and above 3 ppm (equal to 0.089 mg/kg bw/day) toxicologically- and statistically-significant inhibition of RBC ChE activity occurred in males and at 12 ppm in females (equal to 0.35 mg/kg bw/day). Brain ChE activity was significantly inhibited in females at 12 ppm (equal to0.35 mg/kg bw/day). Perturbations in haematology parameters in males at 12 ppm (equal to 0.308 mg/kg bw/day) were suggestive of mild anaemia of marginal toxicological significance. At 12 ppm, chronic inflammation and vasculitis occurred in females and lymphocytic inflammation of the gall bladder occurred in males all of which were considered treatment-related findings of unclear toxicological significance. There was an equivocal occurrence of atrophy and cysts of the prostate in high-dose males.

Supplementary Study

In the absence of a NOEL for plasma ChE inhibition in the above study, and following discussions between the sponsor and the US EPA, a supplementary subchronic toxicity study was conducted and is evaluated below.

Jones RD & Loney ML (1993) A subchronic feeding toxicity study with technical grade fenamiphos (Nemacur®) in dogs. Study No. 91-176-KP. Supplemental submission to EPA MRID No. 42183601. Original Study No. 88-274-BB. Lab: Miles Inc., Agriculture Division, Toxicology, South Metcalf, Stilwell, Kansas, USA. Sponsor: Miles Inc., Agriculture Division, Kansas City, Missouri, USA. Study duration: 29th October 1991 to 28th April 1992. Report Date: 19th February 1993

GLP and QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (1989) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement

Guidelines: Study reportedly conducted in accordance with the following guidelines: (1) US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline 83-1, November 1984; (2) US-EPA-TSCA, Health Effects Testing Guidelines, 40 CFR Section 798.3320, July 1988; (3) OECD Guideline for Testing of Chemicals, Section 4, Guideline 452, May 1981; (4) Japan Ministry of Agriculture, Forestry and Fisheries, Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, 59 NohSan No. 4200, January 1985; and (5) US-FDA, Toxicological Principles for the Safety Assessment of Direct Food Additives and Colour Additives Used in Food, Appendix II Guidelines for Toxicological Testing, October 1982.

Materials and Methods

Technical grade fenamiphos (Batch No. 77-297-55; 88.4% purity; see Appendix II for composition; sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) in 1% (w/w) corn oil was admixed in the diet and fed *ad libitum* to 4 Beagle dogs/sex/group for 6 months at concentrations of 0 or 0.5 ppm. Diets were prepared weekly and stored frozen at approximately –23°C until used. Fresh feed was provided to dogs on a daily basis. Diets were analysed for the concentration, homogeneity and stability of fenamiphos.

Dogs were sourced from White Eagle Laboratories (Doylestown, Pennsylvania, USA) and acclimatised for approximately 4 weeks prior to experimentation. On receipt, dogs were approximately 7 months old, while their pre-treatment bodyweights were unreported. Dogs were housed individually under standard conditions, with water available *ad libitum*.

Observations for mortalities and clinical signs were made daily, with a detailed assessment of clinical signs made weekly. Bodyweights were recorded weekly. Food consumption was recorded daily. Ophthalmoscopic examinations were made prior to the commencement of dosing and sacrifice. Fasted blood samples were collected three times prior to treatment and then every month during treatment for analysis of plasma and RBC ChE activities.

ChE activity data were statistically analysed by ANOVA followed by a Student's t-test if significance was obtained. Clinical observations were statistically analysed by a 2-sample Fisher's Exact.

Results

Dietary analysis: Stability and homogeneity analysis was performed on a 0.4 ppm rather than a 0.5 ppm diet as used in this study. This 0.4 ppm diet was determined to be homogenous, and stable at room temperature (~22°C) for 10 days and at freezer temperature (~-23°C) for 28 days. The mean

analytical concentration of fenamiphos as measured every 4 weeks was 0.408±0.007 ppm, which was approximately 82% of the nominal concentration.

Observations: All dogs survived the duration of the study. There were no treatment-related clinical signs, ophthalmoscopic abnormalities or effects on bodyweight or food consumption. Calculations performed by the authors indicated that the average doses of fenamiphos received by males and female were 0.0108 and 0.0115 mg/kg bw/day, respectively.

ChE activity: There was no statistically- or toxicologically-significant inhibition of either plasma or RBC ChE activity.

Conclusion: The NOEL in beagle dogs following 6 months of dietary exposure to fenamiphos was 0.5 ppm (equal to 0.0108 mg/kg bw/day in males and 0.0118 mg/kg bw/day in females), based on the absence of plasma or RBC ChE inhibition at this concentration.

7. REPRODUCTION STUDIES

7.1 Rats

Löser E (1972c) Bay 68138 generation studies on rats. Report No. 3424. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 3rd May 1972.

Cherry CP, Urwin C & Newman AJ (1972) Pathology report of Bayer 68138 rat breeding study. Addendum to report No. 3424. Report No. 4909/72/344. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Friedrich-Ebertstrasse, Germany. Report date: 15th March 1972.

Materials and Methods

A 50% premix of fenamiphos (unspecified batch No., purity & source) in Silkasil S was admixed in the diet and fed to 3 parental generations of rats (strain FB30) and their offspring throughout all phases of the study. Each experimental group consisted of 10 males and 20 females that were exposed to test concentrations of 0, 3, 10 or 30 ppm (approximately equal to 0, 0.3, 1 and 3 mg/kg bw/day, calculated by dividing by a dietary conversion factor of 10 for rats aged 1-12 weeks). No rationale was given for the choice of these concentrations. Diets were prepared weekly. There was no analysis of the concentration, stability or homogeneity of fenamiphos in the diet although it was reported that the acute oral toxicity of fenamiphos was tested prior to, during and at the end of experimentation and was "within the normal range for a 7-day observation period". The table below summarises the duration of each phase of the study.

Table 36 Design of 3-generation rat study

Phase	Duration
F0 premating exposure	70 days
1st F0 mating	20 days
1st F0 gestation	21 days
F1a lactation until sacrifice	28 days
Waiting period	10 days
2nd F0 mating	20 days
2nd F0 gestation	21 days
F1b lactation; sacrifice of F0 generation	100 days
1st F1b mating	20 days
1st F1b gestation	21 days
F2a lactation until sacrifice	28 days
Waiting period	10 days
2nd F1b mating	20 days
2nd F1b gestation	21 days
F2b lactation (up to 100 days); F1b sacrifice	100 days
1st F2b mating	20 days
1st F2b gestation	21 days
F3a lactation until sacrifice	28 days
Waiting period	10 days
2nd F2b mating	20 days
2nd F2b gestation	21 days
F3b lactation until sacrifice	21 days

Rats were obtained from an unspecified source and at the commencement of dosing were approximately 33 days old and weighed 45-55 g. They were housed individually at room temperature (~22°C), with food and water available *ad libitum*. During mating, two females were housed with one male. There was no mention of the method used to assess pregnancy status or the beginning of gestation. Pup numbers and their bodyweights were recorded immediately after birth. All litters were reduced to 10 pups after 5 days (unspecified basis) and their bodyweights recorded. Lactation was allowed to proceed for 4 weeks after which time the offspring of each first mating were sacrificed (i.e. F1a, F2a and F3a litters). The offspring of each second mating were weaned, allowed to reach sexual maturity (100 days) and mated. At this time, F0, F1b and F2b rats were sacrificed.

There was no mention of any observations for mortalities or clinical signs, however, any rats dying during the study were autopsied. Pups were examined for gross malformations immediately after birth and then during the lactation period. Bodyweights were recorded weekly. Two male and two female F3b pups from each litter were sacrificed at 3 weeks of age by exsanguination under ether anaesthesia and the following organs macroscopically examined: adrenals, gonads, heart, kidneys, liver, lungs and spleen. The following tissues were histopathologically examined: adrenals, heart, kidney, liver, lung, oesophagus, ovaries, pancreas, spleen, testes, thymus, thyroid and uterus (presumably from the same pups, although this was not specified).

Group means were statistically analysed using the Wilcoxon rank sum test

Results

F0 parents

A single 3 ppm female died prior to the second mating due reportedly to "massive pneumonia". One female from the 30 ppm group was sacrificed after the first mating due to the presence of a large tumour on the right of its cervix. No other mortalities occurred. Graphically presented data illustrated that the pattern of bodyweight gain was consistent across all groups. While the average bodyweight of 30 ppm males appeared lower than the other groups, it was not statistically different to the control.

Following both the first and second matings of F0 parental rats (to produce F1a and F1b pups, respectively), there was no treatment-related effect on fertility (No. of females pregnant/mated). Average litter size at birth and after 5 days was unaffected by treatment. Lactation was also unaffected by treatment as pup survival at 4 weeks was consistent across all groups.

F1a and F1b pups

The average bodyweight of pups at birth and over the 4-week lactation period was unaffected by treatment, noting that while average F1b birth weight was slightly lower than the control (6.28 *versus* 6.77 g, respectively) it was not statistically significant. No gross malformations were exhibited at birth or during lactation.

F1b parents

There were no reported mortalities. Graphically-presented data illustrated that female bodyweights were unaffected by treatment. The average bodyweight of 30 ppm males was consistently lower than the control, however, the pattern of bodyweight gain was comparable across all groups. On this

basis and in the absence of any reported statistical difference, the apparent effect on the bodyweight at 30 ppm males is not considered treatment-related.

Following both the first and second matings of F1b parental rats (to produce F2a and F2b pups, respectively), there was no treatment-related effect on fertility (No. of females pregnant/mated). Average litter size at birth and after 5 days was unaffected by treatment for the F2a generation. There was a slight reduction in the litter size at 30 ppm in the F2b generation (11.5 *versus* 13.10 in the control at birth and 10.60 *versus* 12.20 in the control after 5 days), which was not statistically significant and therefore not considered treatment-related. Lactation was unaffected by treatment as pup survival at 4 weeks was consistent across all groups.

F2a and F2b pups

The average bodyweight of pups at birth and over the 4-week lactation period was unaffected by treatment. No gross malformations were exhibited at birth or during lactation.

F2b parents

Mortalities unrelated to treatment occurred in one male and one female in the control groups, and one female in the 3 ppm group. Graphically-presented data illustrated that there was no treatment-related effect on bodyweight.

Following both the first and second matings of F2b parental rats (to produce F3a and F3b pups, respectively), there was no treatment-related effect on fertility (No. of females pregnant/mated). Average litter size at birth and after 5 days, in addition to lactation (indicated by pup survival), were unaffected by treatment.

F3a and F3b pups

The average bodyweight of pups at birth and over the 4-week lactation period was unaffected by treatment. No gross malformations were exhibited at birth or during lactation. A post-mortem examination of F3b pups revealed no macroscopic abnormalities of the internal organs. There were no treatment-related histopathologically abnormalities detected in any of the tissues examined.

Conclusions: The NOEL for parental toxicity, pup toxicity and reproductive toxicity was 30 ppm (~3 mg/kg bw/day), the highest dose tested. The absence of individual animal data was a deficiency to this study.

Eigenberg DA (1991) A two-generation dietary reproduction study in rats using fenamiphos (Nemacur®). Study No. 88-671-BC. Mobay Report No. 5762. Lab: Mobay Corporation, Health, Environment, Safety and Plant Management, Corporate Toxicology Department, South Metcalf, Stilwell, Kansas, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA. Study duration: 8th February to 6th November 1989. Report date: 23rd April 1991.

Eigenberg DA (1997) Supplemental submission in response to the Health Canada review of Study No. 88-671-BC. Agricultural Division Report No. 100692-2. Lab: Bayer Corporation (formerly Mobay Corporation), Agricultural Division, Toxicology, South Metcalf, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Agricultural Division, Kansas City, Missouri, USA. Report date: 24th June 1997.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement

Guidelines: Study reportedly conducted in accordance with the following guidelines: (1) US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline 83-4, November 1984; (2) US-EPA-TSCA, Health Effects Testing Guidelines, 40 CFR Section 798.4700; (3) OECD Guideline for Testing of Chemicals, Section 4, Guideline 416, May 1983; (4) Japan Ministry of Agriculture, Forestry and Fisheries, Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, 59 NohSan No. 4200, January 1985.

Materials and Methods

Technical fenamiphos (Batch No. 77-297-55; 89.5% purity; see Appendix II for composition; sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, Missouri, USA) in 1% (w/w) corn oil was admixed in the diet and fed to 2 parental generations of albino CD Sprague-Dawley rats and their offspring throughout all phases of the study. Each experimental group consisted of 30 rats/sex and were exposed to nominal concentrations of 0, 2.5, 10 or 40 ppm (analytical concentrations of 2.40, 9.12 and 38.9 ppm, respectively). The dose selection was reportedly based on uncited previous studies and in agreement with the US EPA. Diets were prepared weekly and stored frozen at –23°C until use. Fresh diets were offered to rats once a week. Low and high-dose diets were analysed for homogeneity, and stability at room (~22°C) and freezer (-23°C) temperatures. The concentration of fenamiphos in each diet was analysed every 5-7 weeks.

Rats were sourced from Sasco Inc. (Omaha, Nebraska, USA) and were acclimatised for 14 days prior to experimentation. Males and females were 8 and 6 weeks, respectively, at the commencement of dosing. Rats were housed individually under standard conditions, with food and water available *ad libitum*. The duration of each phase of the study is summarised in the table below.

Table 37 Study design of 2-generation rat study

Phase	Duration
F0 premating exposure	10 weeks
F0 mating	Up to 4 weeks
F0 gestation	20 days
F1a lactation	21 days
F1a premating exposure	10 weeks
F1a mating	Up to 4 weeks
F1a gestation	20 days
F2a lactation	21 days

Parental rats (F0 and F1 rats) were exposed to fenamiphos for 10 weeks prior to mating. During this period, bodyweights and food consumption were recorded weekly. Observations for mortality and clinical signs were made twice daily on weekdays and once daily on weekends. More detailed clinical examinations were made on a weekly basis. Two weeks prior to mating, vaginal smears were taken from 10 females/dose to characterise the stage of the oestrus cycle. During mating, one female was housed with one male for up to 21 days and examined each morning for the presence of a vaginal plug or sperm. Unmated females were co-housed for a further week with a male of proven fertility from the same group. Females were then housed individually during gestation and lactation. During gestation, the bodyweight of dams was recorded on days 0, 6, 13 and 20, while food

consumption was recorded weekly. During lactation, the bodyweight of dams were recorded on days 1, 4, 7, 14 and 21, while food consumption was recorded twice during the first week and once during weeks 2 and 3.

At birth, the number of live and stillborn pups was recorded for each litter, with litter sizes recorded from days 0 to 21. Pup weights were recorded on days 0, 4, 7, 14 and 21. All litters were reduced to 8 pups after 4 days on a random basis to ideally yield 4 pups/sex. Excess pups were sacrificed by an injection of T-61 euthanasia solution and necropsied. Those pups designated for ChE activity measurements (see below) were sacrificed by decapitation. Any pups found dead and weanlings not selected for the next generation were necropsied.

Parental males were sacrificed by carbon dioxide asphyxiation following delivery, or where dams had gone past day 24 of gestation. Males were necropsied and testicles and terminal bodyweights recorded. The following organs were preserved for possible histopathological examination: testes, pituitary, seminal vesicles/coagulating gland, prostate gland and any gross lesions. Dams were sacrificed and necropsied after weaning or at day 24 of gestation. Ovary and terminal bodyweights were recorded. The uterus was examined and the number of implantation sites scored. The following tissues were histopathologically examined: pituitary, vagina, cervix, uterus, ovaries and any gross lesions.

Plasma and RBC ChE activities were analysed in ten F0 and F1 adults/sex/dose during week 8 of the premating period. Brain ChE activity in 10 rats/sex/dose was measured at termination. Plasma, RBC and brain ChE activities were analysed in one pup/sex/litter/dose at days 4 and 21 postpartum (i.e. at culling and weaning). In some cases, blood and brain samples were pooled to obtain sufficient quantities for analysis.

The following reproduction indices were calculated: mating index, fertility index, gestation index, birth index, livebirth index and viability index (on day 4 and after day 4) (see Appendix VII for descriptions).

The following statistical tests were performed: ANOVA followed by a Dunnet's test if significance was obtained at $p<0.05$ (bodyweight, food consumption, organ weights and cholinesterase data); the Kruskal-Wallis test followed by the Mann-Whitney test if significance was obtained at $p<0.05$ (litter size, gestational length, viability indices, birth index, live birth index, % of male and females pups, time required for insemination and the number of implantation sites); χ^2-test followed by a Fisher's exact test if significance was obtained at $p<0.05$ (a Bonferroni adjustment was made if necessary) (mating index, fertility index, gestation index and number of litters with stillborns and unknown live or dead pups); and a Fisher's exact test (frequency of histopathological lesions).

Results

Dietary analysis: Analysis of the top, middle and bottom layers of the mixing bowls used to prepare the 2.5 and 40 ppm diets indicated that fenamiphos was homogenously distributed, with all values within 95% of nominal levels. Fenamiphos was stable at room temperature (~22°C) for 14 days, with losses of 10 and 0% in the 2.5 and 40 ppm diets, respectively. When stored at freezer temperature (-23°C), fenamiphos was stable in the 2.5 and 40 ppm diets for 64 days, with no losses reported. Average analytical concentrations of fenamiphos in the 2.5, 10 and 40 ppm diets were 2.40±0.36, 9.12±0.66 and 38.9±2.8 ppm, respectively, which were all within 10% of the nominal concentrations.

Mortalities and clinical signs: There were no treatment-related mortalities or clinical signs in any parental rats.

Bodyweights and food consumption: In F0 rats, there was no treatment-related effect on bodyweight or food consumption during the pre-mating period. In F1 rats, the average bodyweight of the 40 ppm group was significantly lower ($p<0.05$) than the control over the entire pre-mating period; in males, average bodyweight was 9-12% lower than controls, while it was 3-9% lower in females. There was no treatment-related effect on bodyweight at 2.5 or 10 ppm in F1 rats. There was no treatment-related effect on food consumption in F1 rats during the pre-mating period.

During gestation, the bodyweights of F0 dams were unaffected by treatment, while F1 dams in the 40 ppm group had significantly reduced bodyweights ($p<0.05$) at days 0 and 6 (~7% lower than the control group). During lactation, the average bodyweight of 40 ppm dams of both the F0 and F1 generations were significantly lower than the control ($p<0.05$) from lactation days 7 and 4, respectively (up to 9% lower in F0 dams and up to 13% in F1 dams). Bodyweight gain over the lactation period was also significantly lower ($p<0.05$) than the control at 40 ppm in F0 and F1 dams (8±21 *versus* 28±18 g in F0 dams and 14±12 *versus* 38±18 g in F1 dams).

There was no treatment-related effect on food consumption during gestation. Average food consumption was reduced at 40 ppm in F0 dams, but was only significant ($p<0.05$) at lactation day 14 (9% lower than the control). In F1 dams, average food consumption at 40 ppm was significantly reduced ($p<0.05$) during lactation and was up to 19% lower than the control.

Considering bodyweight and food consumption data, the author calculated that the average dose levels ingested during the 10 week pre-mating period were 0.17±0.03, 0.63±0.11 and 2.70±0.49 mg/kg bw/day in F0 males, 0.17±0.04, 0.65±0.14 and 2.94±0.67 mg/kg bw/day in F0 females, 0.20±0.02, 0.73±0.08 and 3.18±0.35 mg/kg bw/day in F1 males and 0.19±0.03, 0.73±0.11 and 3.31±0.54 mg/kg bw/day in F1 females at nominal concentrations of 0, 2.5, 10 and 40 ppm, respectively.

Oestrous cycling: There was no treatment-related effect on oestrous cycling in F0 or F1 parental females.

Reproduction parameters: There was no treatment-related effect on any of the reproduction parameters in either F0 or F1 rats (i.e. mating index, fertility index, insemination lengths, gestation lengths, gestation index, birth index and the number of implantation sites)

Litter parameters: There was no treatment-related effect on pup gender, litter size, the number of litters with stillbirths, live birth index and viability indices in either F1A or F2A pups. There was no treatment-related effect on live birth weight, however, the average bodyweight of high-dose pups was significantly ($p<0.05$) and up to 17% lower than the controls during lactation (from day 14 for F1A pups and day 7 for F2A pups).

ChE activity: There was no treatment-related inhibition of plasma, RBC or brain ChE activities in 4-day old F1A or F2A pups. In 21-day old pups, dose-related inhibition of plasma ChE activity occurred in both sexes, which was statistically ($p<0.05$) and toxicologically significant at 10 and 40 ppm (33 and 56%, respectively, in F1A males; 26 and 75%, respectively, in F1A females; 23 and 60%, respectively, in F2A males and 22 and 56%, respectively in F2A females) (see table below). There was no toxicologically- or statistically-significant plasma ChE inhibition at 2.5 ppm. In 21-day old pups, toxicologically- and statistically-significant inhibition of RBC ChE activity occurred only at 40 ppm (30 and 40% in F1A males and females, respectively, and 25 and 28% in F2A males

and females, respectively) (see table below). There was no treatment-related effect on brain ChE activity in 21-day old F1A or F2A pups.

Table 38 Plasma and RBC ChE activities in 21-day old pups

Parameter	Concentration (ppm)							
	0		2.5		10		40	
	♂	♀	♂	♀	♂	♀	♂	♀
F1A pups								
Plasma ChE	0.70 ±0.12	0.57 ±0.08	0.59 ±0.10 (16%)	0.50 ±0.12 (12%)	0.47 ±0.09* (33%)	0.42 ±0.08* (26%)	0.31 ±0.18 (56%)	0.20 ±0.09* (75%)
RBC ChE	2.80 ±0.29	2.77 ±0.25	2.78 ±0.31 (1%)	2.70 ±0.34 (3%)	2.57 ±0.48 (8%)	2.71 ±0.23 (2%)	1.95 ±0.53* (30%)	1.66 ±0.23* (40%)
F2A pups								
Plasma ChE	0.65 ±0.10	0.59 ±0.11	0.54 ±0.07 (17%)	0.55 ±0.06 (7%)	0.50 ±0.17* (23%)	0.46 ±0.12* (22%)	0.26 ±0.09* (60%)	0.26 ±0.08* (56%)
RBC ChE	3.02 ±0.21	2.87 ±0.20	3.10 ±0.50	3.13 ±0.52	3.33 ±0.71	3.22 ±0.71	2.28 ±0.67 (25%)	2.08 ±0.40* (28%)

Plasma and RBC ChE activities expressed as mean IU/mL ± standard deviation (% inhibition relative to the control); * $p<0.05$

Results of ChE activity measurements in F0 and F1 parental rats are summarised in the table below. In both generations of females, significant ($p<0.05$) dose-related inhibition of plasma ChE activity occurred at every concentration and sampling interval, with the level of inhibition greater than 20% of the control and therefore considered toxicologically significant. In F0 males at day 57 and 111, statistically- ($p<0.05$) and toxicologically-significant inhibition of plasma ChE activity occurred only at 40 ppm. In F1 males, significant inhibition of plasma ChE activity occurred at 40 ppm on day 57 and at 10 and 40 ppm on day 111, with these findings deemed toxicologically significant as they were greater than 20% relative to the control.
Dose-related inhibition of RBC ChE activity occurred in females and was statistically and toxicologically significant ($p<0.05$) in both generations at and above 10 ppm. In 40 ppm males, statistically and toxicologically-significant inhibition of RBC ChE activity occurred in both generations and sampling times. In 10 ppm males, significant ($p<0.05$) inhibition of RBC ChE activity occurred in both generations at most sampling times, however, only the effect on F0 males on day 57 was deemed toxicologically significant as inhibition was >20% relative to the control. At the lowest concentration of 2.5 ppm, no toxicologically significant inhibition of RBC ChE activity occurred in either generation of males.

In both generations of females, toxicologically and statistically significant inhibition of brain ChE activity occurred only at 40 ppm. There was no statistically- or toxicologically-significant inhibition of brain ChE activity in males.

Table 39 % ChE inhibition in F0 and F1 parental rats

Parameter	Concentration (ppm)							
	0		2.5		10		40	
	♂	♀	♂	♀	♂	♀	♂	♀
F0 rats								
Plasma ChE (d57)	0	0	-8	36*	-8	63*	47*	78*
Plasma ChE (d91/111)[1]	0	0	-19	28*	-21	45*	37*	75*
RBC ChE (d57)	0	0	2	6*	22*	25*	54*	56*
RBC ChE	0	0	-3	4	6	39*	39*	51*

Parameter	Concentration (ppm)							
	0		2.5		10		40	
	♂	♀	♂	♀	♂	♀	♂	♀
(d91/111)[1]								
Brain ChE (d91/111)[1]	0	0	-8	-10*	-9	0	1	21*
F1 rats								
Plasma ChE (d57)	0	0	-2	36*	3	66*	52*	85*
Plasma ChE (d91/111)[1]	0	0	-1	33*	31*	60*	67*	81*
RBC ChE (d57)	0	0	6*	3	14*	15*	48*	44*
RBC ChE (d91/111)[1]	0	0	-1	3	16*	36*	48*	49*
Brain ChE (d91/111)[1]	0	0	-2	4	-3	6*	6	29*

Results expressed as the % inhibition relative to the respective control group; *p<0.05; 1 = day 91 for females & day 111 for males

Pathology: In both generations of parental males, there was no treatment-related effect on absolute or relative testes weights. Terminal bodyweight was significantly lower ($p<0.05$) than the control at 40 ppm in F0 males (448.4±41.7 *versus* 492.1±49.1 g) but not F1 males. The terminal bodyweight of both parental generations of females was significantly lower ($p<0.05$) than the control at 40 ppm (274.5±22.0 *versus* 291.5±27.4 g for F0 females; 280.7±23.6 *versus* 303.4±31.0 g for F1 females). Absolute ovarian weight in F0 but not F1 females was significantly ($p<0.05$) lower than the control at 40 ppm (0.101±0.018 *versus* 0.132±0.025 g). In F0 females, relative ovarian weights were significantly reduced ($p<0.05$) across all treatment groups (0.046±0.009, 0.040±0.007, 0.041±0.008 and 0.037±0.007% at 0, 2.5, 10 and 40 ppm, respectively). In the absence of similar findings in F1 females or any pathology of the ovaries, effects on fertility or effects on the ovaries in any other repeat-dose study, the effects on ovarian weight are not considered treatment-related.

In F0 rats, the incidence of oedema of the salivary gland was increased at 40 ppm (1, 1, 0 and 4/30 in males and 0, 0, 0 and 7/30 in females at 0, 2.5, 10 and 40 ppm, respectively), with the increase in females significantly different ($p<0.05$) to the control. The author reported that no oedema of the salivary gland was detected in 7 studies conducted around the same time as the current study. As no oedema of the salivary gland was detected in F1 parental rats, this finding is not considered treatment-related. The author suggested that it may have been caused by an infectious agent. At 40 ppm, two F1 males had calculus, dilation and malformation of the kidney, while one F1 females had a dilated kidney. As no such lesions occurred in F1 rats, these kidney abnormalities were not considered treatment-related. There were no other possible treatment-related abnormalities detected in any parental rats.

There were no treatment-related macroscopic abnormalities observed in pups.

Histopathology: There were no histopathological abnormalities of the reproductive organs and tissues detected in any rats. Examination of those rats exhibiting macroscopic lesions confirmed the occurrence of oedema of the salivary gland in four F0 males in addition to vacuolar degeneration (4/4) and inflammation (2/4). Single F0 males in the control and 2.5 ppm groups also had vacuolar degeneration, oedema and inflammation (2.5 ppm only) of the salivary gland, consistent with the macroscopic observations. The following histopathological findings were noted in the seven F0 females exhibiting oedema of the salivary gland as detected at necropsy: dilatation (1/7), oedema (6/7), giant cells (1/7), haemopoiesis (1/7) and inflammation (5/7). No historical control data are available for these histopathological findings.

Conclusions: The NOEL for reproductive toxicity was 40 ppm (~3 mg/kg bw), the highest dose tested, based on the absence of any reproductive effects at this dose. The LOEL for maternal toxicity was 2.5 ppm (equal to 0.20 mg/kg bw/day) based on the inhibition of plasma ChE activity at and above this dose. The NOEL for male toxicity was 2.5 ppm (equal to 0.17 mg/kg bw/day), based on the inhibition of plasma ChE activity at 10 ppm (equal to 0.65 mg/kg bw/day). The NOEL for pup toxicity was 2.5 ppm (equal to 0.17 mg/kg bw/day), based the inhibition of plasma ChE activity at and above 10 ppm (equal to 0.65 mg/kg bw). RBC ChE activity was inhibited in male and female parental rats at and above 10 ppm (equal to 0.63 and 0.73 and mg/kg bw/day, respectively). Brain ChE activity was inhibited in maternal rats at 40 ppm (equal to 3.2 mg/kg bw/day).

8. DEVELOPMENTAL STUDIES

8.1 Rats

Schlüter G (1981) Evaluation for embryotoxic and teratogenic effects in orally dosed rats. Study No. SRA 3886/015. Report No. 9785. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal, Germany. Study duration: unspecified. Report date: 5th February 1981.

Renhof M (1986) Addendum to Report No. 9785, 5th February 1981: SRA 3886 (Nemacur)/Evaluation for embryotoxic and teratogenic effects in orally dosed rats. Report date: 5th March 1986.

Materials and Methods

Groups of inseminated long Evans rats (FB30 strain; 25/group) were dosed by oral gavage with fenamiphos (Batch No. 808817123; 92.5% purity; unspecified source) in a 0.5% aqueous Cremephor emulsion at 0, 0.3, 1.0 or 3.0 mg/kg bw/day from gestational days (gd) 6-15. The dose volume was 10 mL/kg bw. No rationale was provided for the dose selection.

Rats were sourced from the performing laboratory, were 2.5-3.5 months old and weighed between 181 to 249 g. Except during mating, rats were housed individually under standard conditions, with food and water available *ad libitum*. During mating, two virgin females were housed overnight with one male. Vaginal smears were taken the following day and if sperm was detected, this day was designated as gd 0.

Dams were observed for mortality and clinical signs at unspecified times. Bodyweights were recorded at gd 0, daily during the treatment period and gd 20. Dams were sacrificed on gd 20 by carbon dioxide asphyxiation and all foetuses removed by caesarean section. Foetuses were weighed, sexed and examined for any external abnormalities and any visceral abnormalities. Foetuses were eviscerated and processed for an examination for skeletal abnormalities.

The following statistical tests were performed: Wilcoxin and Mann and Whitney tests (bodyweight; number of implantations, foetuses and resorptions; foetal and placenta weights); χ^2-test (number of foetuses with skeletal abnormalities, number of stunted foetuses); and either a χ^2-test or a Fisher exact test (number of fertilised and pregnant rats).

Results

Dams: At 3 mg/kg bw/day, two dams died and 18 displayed whole-body tremors, which occurred 30 minutes after dosing. There were no mortalities and no clinical signs observed at lower doses or in the control. Average bodyweight gain during the treatment period was approximately 15% lower than the control, but this finding was not statistically significant.

Foetuses: There was no treatment-related effect on litter size, resorptions, average foetal weight or the incidence of stunted foetuses. The average placenta weight of the 3 mg/kg bw/day group was significantly higher ($p<0.05$) than the control group but this was reportedly within the control range, and in the absence of any concomitant effects on foetuses, was not considered a toxicologically significant finding. There were no treatment-related skeletal or visceral variations or malformations.
Conclusions: The NOEL for maternotoxicity was 1 mg/kg bw/day, based on the occurrence of mortalities and clinical signs (whole-body tremors) at 3 mg/kg bw/day. The NOEL for developmental toxicity was 3 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic in Long Evans rats at doses up to 3 mg/kg bw/day.

Clemens GR, Troup CM & Hartnagel Jr RE (1989) Teratology study in the rat with Nemacur Technical. Report No. MTD0108. Lab: Toxicology Department, Miles Inc., Elkhart, Indiana, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, USA. Study duration: unspecified. Report date: 30th August 1989.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement.

Materials and Methods

Fenamiphos (Batch No. 77-297-55; 88.7% purity; sourced from Mobay Corporation, Agricultural Chemicals Division, Kansas City, USA), in a 2% Emulphor vehicle (a polyethoxylated vegetable oil), was administered by oral gavage to groups of 33 pregnant Charles River Crl:CD®BR rats at doses of 0, 0.25, 0.85 or 3 mg/kg bw/day from gd 6-15. The dose selection was based on a previous unpublished range-finding study. The dose volume was 10 mL/kg bw. Dosing solutions were prepared once and stored refrigerated until use. Stability analysis had been undertaken previously, while the concentration in each solution was analysed during the current study.

Rats were sourced from Charles River Breeding Laboratories (Portage, Michigan, USA) and acclimatised for 12 days prior to mating. Females were 12-weeks of age and weighed 215-286 g at the time of mating. Mating took place by housing 2 females overnight with one male, and then examining them the following day for the presence of sperm. The day that sperm were detected was designated as gd 0. Except during mating, rats were housed individually under standard conditions, with food and water available *ad libitum*.

Dams were observed daily for mortalities and clinical signs. Bodyweights were recorded on gd 0, 6, 8, 10, 12, 15 and 20. Food consumption was recorded on gd 1, 6, 7, 12, 16, 17 and 20. Five dams/group were sacrificed by carbon dioxide asphyxiation on gd 16 and analysed for plasma, RBC and brain ChE activities. All remaining dams were sacrificed on gd 20. Blood and brain tissue were collected from 20 of these dams per group for analysis of plasma, RBC and brain ChE activities. The number of corpora lutea, resorptions and implantation scars were recorded for each dam sacrificed on gd 20. Dams were examined for any gross pathological abnormalities, with particular

attention paid to the abdominal and thoracic viscera. Each foetus was assessed for viability, sexed, weighed and subjected to a thorough external examination covering the head, palate, torso, position of the limbs and the number of digits on each paw. Approximately half of the foetuses from each dam were sacrificed by an intracranial injection of barbiturate and subjected to a complete internal examination. These foetuses were then eviscerated and processed for an examination of skeletal development. Brain ChE activity was measured in 20 foetuses/group.

Results were statistically analysed using one or more of the following tests: Dunn, Dunnett's , Fisher's Exact, Healy test or Kruskal-Wallis.

Results

Test article analysis: Analysis of the concentration of the three dosing solutions found that they were within 2% of the nominal concentrations. Stability analysis, conducted on the low- and high-dose solutions, indicated that they were stable for 28 days when stored refrigerated.

Dams: Mortalities and clinical signs were restricted to the high-dose group (3 mg/kg bw/day). Six dams died during the study, with all dams exhibiting tremors within an hour of dosing, which lasted for several hours. Other clinical signs observed in the majority of dams included salivation (89%), lacrimation (61%) and urine soiling of the ventral surface (36%). A smaller proportion of dams exhibited hypo-activity (17%), chromodacryorrhoea (8%) and convulsions (3%).

At 3 mg/kg bw/day, average bodyweight at gd 15 and average bodyweight gain over the treatment period (gd 6-15) were significantly lower than the control group (303.0±4.0 *versus* 316.4±3.7 g and 27±9.8 *versus* 43.7±16.0, respectively). Concomitantly, there was a significant reduction ($p<0.05$) in food consumption measured at gd 12 and 16, relative to the control group (27.7±0.8 and 23.6±0.7 g, respectively, *versus* 26.3±0.6 and 26.3±0.5 g in the control group). Following the cessation of treatment, bodyweight and food consumption were comparable to the control group. There was no treatment-related effect on bodyweight, bodyweight gain or food consumption at 0.25 or 0.85 mg/kg bw/day.

Results of ChE activity measurements are summarised in the table below. At 3 mg/kg bw/day, plasma and RBC ChE activities were significantly inhibited ($p<0.05$) 24 hours after the final dose (on gd 15). As the level of inhibition in both cases was >20% of the control, both findings were considered toxicologically significant and therefore treatment-related. There was no inhibition of plasma ChE activity at the two lower doses. At 0.85 mg/kg bw/day, RBC ChE activity was inhibited by 21% relative to the control, and although not statistically significant, this result is considered toxicologically significant and treatment-related. There was no inhibition of RBC ChE activity at 0.25 mg/kg bw/day. While brain ChE activity was inhibited by 28% at the mid-dose (0.85 mg/kg bw/day), the lack of a dose-response relationship and statistical significance indicated that this parameter was unaffected by treatment. Further, there were reportedly some technical problems during sample preparation that may have affected the results. On gd 20 (~5 days after the cessation of treatment), plasma ChE activity had recovered, while RBC ChE activity remained inhibited by 30% relative to the control at the highest dose. There was no effect on brain ChE activity detected at gd 20.

Table 40 ChE activity measurements in dams

Parameter	Dose (mg/kg bw/day)			
	0	0.25	0.85	3
Plasma ChE gd 15	1468±148	1510±137	1349±98 (8%)	740±112* (50%)
RBC ChE gd 15	572±61	478±40 (16%)	452±31 (21%)	329±35* (42%)
Brain ChE gd 15	2172±308	2064±337 (5%)	1561±97 (28%)	1901±207 (12%)
Plasma ChE gd 20	1367±77	1457±71	1434±73	1304±73 (5%)
RBC ChE gd 20	678±44	655±52 (3%)	632±28 (7%)	473±43* (30%)
Brain ChE gd 20	1602±116	1596±120	1711±112	1562±97 (2%)

Results expressed as mean IU/L (plasma and RBC ChE activities) or mU/g (brain ChE activity) ± 1 SEM, with the % inhibition relative to the control contained in parentheses; * $p<0.05$ = significantly different to the control

No treatment-related gross pathological abnormalities were detected in any dams, including the 6 animals from the 3 mg/kg bw/day group that died. Litter sizes, the number of resorptions and post-implantation losses were unaffected by treatment.

Foetuses: There were no foetal mortalities and no treatment-related effect on foetal weight or sex ratio. Placental weights were comparable across all groups. There was no treatment-related effect on foetal brain ChE activity. There were no treatment-related external or visceral variations or malformations. There was a significant increase ($p<0.05$) in variations of the hyoid body or arch at 0.25 and 3 mg/kg bw/day (13.3, 24.3, 20.5 and 26.0% at 0, 0.25, 0.85 and 3.0 mg/kg bw/day, respectively) but as all findings fell within the performing laboratory's historical control range (2-32%), they were not considered toxicologically-significant. There were no treatment-related skeletal anomalies

Conclusions: The NOEL for maternotoxicity was 0.25 mg/kg bw/day, based on toxicologically significant inhibition of RBC ChE activity at and above 0.85 mg/kg bw/day. At 3 mg/kg bw/day, mortalities, overt signs of toxicity, significantly lower bodyweight gain and food consumption, and inhibition of plasma ChE activity, occurred. The NOEL for developmental toxicity was 3 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic in this particular rat strain.

8.2 Rabbits

Lamb DW (1982) Teratology study with nemacur in rabbits. Study No. 81165. Lab: Hazleton Raltech Inc., Madison, Wisconsin, USA. Sponsor: Mobay Chemical Corporation, Stilwell, Kansas, USA. Study duration: 31st August to 6th October 1981. Report Date: 3rd February 1982

GLP and QA: Statement of compliance with standards of Good Laboratory Practice (US EPA, 21 CFR 58.35); QA statement

Materials and Methods

Fenamiphos (Lot Reference No. 77-297-55; 88.8% purity; sourced from Mobay Chemical Corporation, Stilwell, Kansas, USA) in corn oil was administered by oral gavage to 20 pregnant NZW white rabbits/group at 0, 0.1, 0.3 or 1.0 mg/kg bw/day on days 6-18 of gestation. The dose volume was 0.5 mL/kg bw. The dose selection was based on an unpublished preliminary study (Mobay Chemical Company Report TOX 235) showing that 3.0 mg/kg bw/day fenamiphos was toxic.

Rabbits were sourced from Hoppers Unlimited (Verona, Wisconsin, USA). The age of the rabbits was unspecified. Dams were double mated to males of proven fertility, with the day of mating

designated as gd 1. Dams not falling within the desired bodyweight range of 2500-3700 g were replaced. Mated rabbits were housed individually under standard conditions, with food and water available *ad libitum*.

Throughout the dosing period, dams were observed at least once daily for mortalities and clinical signs. Feed consumption was monitored by visual inspection. Any dams showing signs of premature delivery were sacrificed. Bodyweights were recorded on gd 0, 6, 12, 18, 24 and 29, with the latter corrected for gravid uterine weight. Dams were sacrificed by carbon dioxide asphyxiation on gd 29 and a caesarean section performed. All dams were subjected to a gross external and internal examination. Gravid uterine weights were recorded. Ovaries were macroscopically examined and the number of corpora lutea recorded. The following parameters were recorded: the number and location of live and dead foetuses; sex ratio; early and late resorptions; empty sites and scars; unusual colouration and variations in amniotic fluid or placentae; and any other abnormalities. All viable foetuses were weighed and examined for external abnormalities. Each foetus was examined for visceral abnormalities then eviscerated, fixed stained and examined for skeletal malformations and variations.

The following statistical tests were used to analyse the results: ANOVA followed by a Dunnett's multiple comparison test if significance was obtained (dam bodyweight on gd 0, bodyweight gain between gd 0-29); covariate analysis (dam bodyweights on gd 6 and 29, gravid uterine weight and corrected bodyweight on day 29 using day 0 bodyweight as the covariate; foetal weights using the number of live foetuses as the covariate); Kruskall-Wallis test followed by a Dunn's test if significance was obtained (corpora lutea, number of implants, implantation efficiency, number and percent of live, dead and resorbed foetuses, and sex ratio); χ^2-test (number of litters with gross, visceral and skeletal abnormalities).

Results

Dams: There were no treatment-related mortalities or clinical signs. There was a dose-related decrease in bodyweight gain from gd 0-29 and in gravid uterine weight (see table below). When day 29 bodyweights were corrected for gravid uterine weight, dams in the 0.3 and 1.0 mg/kg bw/day groups were found to loose bodyweight during gestation, however, neither finding was statistically significant, most likely due to the high intragroup variability. An examination of individual animal data revealed that the number of dams in each group with negative bodyweight gain from gd 0-29 was 3/11, 4/10, 5/12 and 8/11 at 0, 0.1, 0.3 and 1.0 mg/kg bw/day, respectively. On this basis, the effect on bodyweight at 1.0 mg/kg bw/day is considered treatment-related. The effect on gravid uterine weight is of questionable toxicological significance due to the high intragroup variability. There were no treatment-related macroscopic abnormalities detected at sacrifice.

Table 41 Bodyweights, bodyweight gain and gravid uterine weights in dams (g)

Parameter	Dose (mg/kg bw/day)			
	0	0.1	0.3	1.0
Bw change gd 0-29	509±223	491±233	338±271	259±248
Gravid uterine weight	422±66	376±66	364±113	338±129
Corrected[1] gd 29 bw	3162±266	3205±195	3156±356	2991±274
Bw change gd 0-29	100±214	115±192	-26±247	-79±272

Results expressed as the mean ± standard deviation1 = gd 29 bw – gravid uterine weight

Foetuses: There were no treatment-related external abnormalities. An examination for visceral abnormalities found an increased incidence of foetuses with the left carotid of the heart arising from

innominate (artery) across all treatment groups in approximately 25% of litters (incidences of 0, 8.5, 7.3 and 9.1% of total foetuses, with 0, 8.1, 6.4 and 10.3% of the litter affected at 0, 0.1, 0.3 and 1.0 mg/kg bw/day, respectively). Data provided with the study report indicated that the historical control litter incidence of this finding is 2.3% (1 litter affected in 43). No historical control data were available on the actual foetal incidence of this abnormality. While there was no clear dose-response effect (due possibly to the closeness of the doses), the absence of such an abnormality in the concurrent control group and given that the litter incidence in all treated groups was higher than the historical control litter incidence, is suggestive of a treatment-related effect. However, the biological significance of this finding is unclear.

There was an increased incidence of the accessory bone of the skull in 8.3-16.7% of treated litters, with 0, 4.5, 2.8 and 12.5% of the litter affected at 0, 0.1, 0.3 and 1.0 mg/kg bw/day, respectively. However, only a small proportion foetuses were actually affected (0, 5.1, 2.6 and 4.8% at 0, 0.1, 0.3 and 1.0 mg/kg bw, respectively). The historical control litter incidence of this abnormality is 10% (3 litters affected of 31), however, no data were available on the actual foetal incidence. In the absence of a dose-response relationship and given that only one or two foetuses were affected, this finding is not considered treatment-related.

Some skeletal abnormalities were detected at 0.3 or 1.0 mg/kg bw/day and included scoliosis and associated rib anomalies (one foetus at 0.3 mg/kg bw/day), absent post-thoracic vertebrae (one foetus at 1.0 mg/kg bw/day) and chain fusion of the sternebrae (5 foetuses from 3 litters at 1.0 mg/kg bw/day). Of these findings, only the chain fusion of the sternebrae was significantly higher ($p<0.05$) than the control when comparing the total incidence of affected foetuses in each group (0, 0, 1.2 and 5.7% at 0, 0.1, 0.3 and 1.0 mg/kg bw/day, respectively). The proportion of litters showing chain fusion of the sternebrae was 0, 0, 8.3 and 25%, with 0, 0, 0.9 and 4.6% of the litter affected at 0.0.1, 0.3 and 1.0 mg/kg bw/day, respectively. The historical control litter incidence of fused sternebrae is 12.9% (4/31 litters; total of 234 foetuses). Given that the litter incidence at the highest dose is above the historical control value (25% *versus* 12.9%) and that there was no similar finding in the concurrent control group, the occurrence of chain fusion of the sternebrae at the highest dose is considered treatment-related but probably secondary to maternotoxicity.

Conclusions: The NOEL for maternal toxicity was 0.3 mg/kg bw/day, based on reduced bodyweight gain to gd 20 at 1.0 mg/kg bw/day. The NOEL for developmental toxicity was 0.3 mg/kg bw/day, based on the occurrence of chain fusion of the sternebrae at 1.0 mg/kg bw.

Becker H (1986) Embryotoxicity (including teratogenicity) study with SRA 3886 in the rabbit. RCC Project No. 065261. Bayer Project No. T4022082. Lab: RCC, Research and Consulting Company Ag, and RCC, Unweltchemie AG, Itingen Switzerland. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: 5th May to 23rd June 1986. Report date: 22nd August 1986.

GLP and QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, Federal Register Vol 43 No. 247 (December 1978 an amended in 1980) and Wegleitung der IKS (Interkantonalw Kontrollstelle fuer Heilmittel) betreffend gute Laborpraqxis fuer nichtklinische Laborversuche (28th April 1980, Berne, Switzerland, amended 23rd June 1982); QA statement

Guidelines: Study reportedly conducted in accordance with the following guidelines: (1) OECD Test Guideline 414 "Teratogenicity" (12th May 1981);and (2) "New and Revised Health Effects Test Guidelines" (November 1984), Office of Pesticides and Toxic Substances, US EPA.

Materials and Methods

Groups of 16 mated female chinchilla rabbits (Kfm:CHIN hybrid, SPF free) were administered fenamiphos (Batch No. 808 511 402=233 590 475; 91.0% purity; unspecified source) in distilled water with 0.5% Cremophor EL by oral gavage from gd 6-18 at 0, 0.1, 0.5 or 2.5 mg/kg bw/day in a dose volume of 4 mL/kg bw. The dose selection was based on a previous range-finding embryotoxicity/teratogenicity study (RCC Project 065250, 21st April 1986). The concentration, homogeneity and stability of the test material were analysed prior to the first dosing and once during the treatment period

Rabbits were sourced from KFM Kleintierfarm Madoerin AG (Fuellinsdorf, Switzerland) and acclimatised for at least 7 days. They were housed with males (1:1) until mating was observed and then housed individually under standard conditions, with food and water available *ad libitum*. Dams were aged between 4 and 6 months, and weighed 2278-3334 g following mating.

Observations for mortalities and clinical signs were made at least twice daily. Bodyweights were recorded daily from gd 0-28. Food consumption was recorded on gd 6, 11, 15, 19, 24 and 28. On day 28, dams were sacrificed by cervical dislocation and foetuses removed by caesarean section. Dams were subjected to a gross macroscopic examination of all internal organs, particularly the uterus and its contents. Gravid uterine weights, the position of the foetuses and the number of corpora lutea were recorded. The uteri of any dams not found to be pregnant were examined for implantation sites. Foetuses were weighed, sexed and examined for any gross external abnormalities. Each foetus was examined for visceral abnormalities then eviscerated, fixed stained and examined for skeletal malformations and variations.

Results were statistically analysed using the following tests: one-way ANOVA (assuming normative data) followed by a Dunnett's t-test if significance was obtained at $p<0.05$; Wilcoxin rank sum test together with a Kruskall-Wallis test; and a Fisher's exact test.

Results

Dosing solutions: Results of the concentration, homogeneity and stability analyses of fenamiphos in the vehicle were variable. Analysis of triplicate samples of the low-, mid- and high-dose solutions found fenamiphos concentrations of 64-80, 14-140 and 60-88% of the nominal concentrations, respectively. On average, analytical concentrations were 71, 84 and 70%, respectively, of the nominal concentrations. In a second analysis, analytical concentrations ranged from 55-130, 46-116 and 53-129% of the nominal concentrations in the low-, mid- and high-dose formulations, respectively. On average, analytical concentrations were 92, 97 and 99% of nominal concentrations, respectively. On the basis of both analyses, it is questionable whether the dosing solutions were homogenous or stable. After 90 minutes at room temperature, the concentration of fenamiphos in the low-, mid- and high-dose formulations were 72.0, 66.4 and 102.6% of the nominal concentrations, respectively.

Dams: There were no mortalities or clinical signs at or below 0.5 mg/kg bw/day. At 2.5 mg/kg bw/day, 4 dams died; three died at gd 8, 10 and 15 (after the 3rd, 5th and 10th doses, respectively) while one died on gd 21, three days after the cessation of treatment. Nine dams, including these 4, exhibited cholinergic signs such as salivation and dyspnoea. Ataxia was observed in 2 of the 4 dams that died during the study, with one of these animals also having diarrhoea. It was reported that clinical signs were evident at approximately 30-60 minutes after dosing and lasted for up to 4 hours.

There was no treatment-related effect on bodyweight gain or food consumption at or below 0.5 mg/kg bw/day. At 2.5 mg/kg bw/day, average bodyweight gain during the treatment period was markedly lower than the control although not significantly different (0.4, 1.7 and 2.3% *versus* 3.7,

3.5 and 2.8% at gd 6-11, 11-15 and 15-19, respectively). At 2.5 mg/kg bw/day, average food consumption during the treatment period was significantly lower ($p<0.05$) than the control (31.6, 34.5 and 22.2% lower at gd 6-11, 11-15 and 15-19, respectively).
There were no treatment-related macroscopic abnormalities detected in dams during the post-mortem examination.

There was no treatment-related effect on post-implantation loss, foetal resorptions or the distribution of live foetuses in the uterus. No dead foetuses were detected in any dam.

Foetuses: There was no treatment-related effect on foetal bodyweight or foetal sex ratio. An external examination revealed no treatment-related abnormalities. It was noted that one foetus in the 2.5 mg/kg bw/day group had encephalocoele and a reduced brain size, but this finding was considered incidental and unrelated to treatment (historical control data provided in the report indicated that the incidence of this finding is one foetus in 147 dams). Besides this foetus, the stage of ossification of the crania was similar across all other foetuses. No visceral abnormalities were detected. While skeletal variations occurred in some pups in each group (delayed ossification; bipartite or abnormally shaped sternebrae; absent, shortened or fused ribs) they bore no relationship with treatment.

Conclusions: The NOEL for maternal toxicity was 0.5 mg/kg bw/day, based on mortalities, cholinergic signs, reduced bodyweight gain and significantly reduced food consumption at 2.5 mg/kg bw/day. The NOEL for developmental toxicity was 2.5 mg/kg bw/day, the highest dose tested. There was no evidence that fenamiphos was teratogenic.

9. GENOTOXICITY

The following Tables summarise the findings of in vitro and *in vivo* genotoxicity studies evaluated as part of the current fenamiphos review. A complete written evaluation of those studies indicating positive or equivocal findings follows.

Table 42 *In vitro* studies

Assay	Strain or cell type	Concentration	Purity & Batch	Positive control	Metabolic activation	Result	Reference
Ames test (Bacterial reverse mutation)	*Salmonella. typhimurium* strains TA 98 TA100 TA1535 TA1537	0, 4, 20, 100, 500 & 2500 µg/plate (Exp 1) 0, 125, 250, 500 & 1000 µg/plate (Exp 2) DMSO solvent	Unspecified purity Batch No. 808817123	Cyclophosphamide [217 µg/plate (TA 1535 & TA 100] Trypaflavin [200 µg/plate (TA 1537 & TA 98)]	+/-	-/- (Cytotoxicity at >500 µg/plate)	Herbold (1979)
		0, 20, 100, 500, 2500 & 12500 µg/plate (Exp 1) 0, 125, 250, 500, 1000 & 2000 µg/plate (Exp 2; repeated) DMSO solvent	92.4% purity Batch No. 816496058	Endoxan (cyclophosphamide) [145 µg/plate (TA1535) or 290 µg/plate (TA100)] Trypaflavine (50 µg/plate; TA1537 & TA98) 2-AA (3 µg/plate) MMS (8 µL/plate; TA1535 & 100) 4-NQO (0.5 µg/plate; TA98)	+/-	-/- (Cytotoxicity ≥500 µg/plate)	Herbold (1985a & b) [QA]
		0, 8, 40, 200, 1000 & 5000 µg/plate	Nemacur GR 10 14.3% fenamiphos Product No. 925470	Sodium azide [10 µg/plate (TA1535)] Nitrofurantoin [0.2 µg/plate (TA100)] 4-nitro-1,2-phenylene diamine [0.5 & 10 µg/plate (TA 1537 &98, respectively)] 2-AA [3 µg/plate]	+/-	-/- (Cytotoxic at 5000 µg/plate)	Herbold (1992) [QA, GLP]
HGPRT mutation (Mammalia	CHO cells	0, 100, 110, 120 & 130 µg/mL (-S9)	85% purity Code No. T2600	EMS (0.2 µL/mL) (-S9)	+/-	-/-	Yang (1985) [QA,

Assay	Strain or cell type	Concentration	Purity & Batch	Positive control	Metabolic activation	Result	Reference
n forward mutation)		0, 170, 190, 210 & 230 µg/mL (+S9) DMSO solvent		Benzo(a)pyrene (2 µg/mL) (+S9)			GLP]
Cytogenetic test	Human lymphocytes	0, 25, 100 & 400 µg/mL DMSO solvent	91.3% purity Batch No. 808511402 - 233590475	Mitomycin C (0.1 µg/mL) (-S9) Cyclophosphamide (10 µg/mL) (+S9)	+/-	+/+	Herbold (1987) [GLP]
Cytogenetic test	Human lymphocytes	0, 25, 50, 75 & 100 µg/mL (-S9) 0, 100, 150, 225 & 350 µg/mL (+S9) DMSO solvent	91.9% purity Batch No. 808511402 - 233590475	Mitomycin C (0.15 µg/mL) (-S9) Cyclophosphamide (15 µg/mL) (+S9)	+/-	+/-	Herbold (1988) [QA, GLP]
Sister Chromatid Exchange	Chinese Hamster V79 cells	0, 2.5, 5.0. 10.0 & 20.0 µg/mL	99.2% purity Unspecified Batch No.	None	-	-	Chen et al (1982a)
Sister Chromatid Exchange	Chinese Hamster V79 cells	0, 10.0, 20.0, 40.0 & 80.0 µg/mL DMSO solvent	99.2% purity Unspecified Batch No	Cyclophosphamide (5 µg/mL)	+	-	Chen et al (1982b)
Unscheduled DNA synthesis	Rat primary hepatocytes	0, 1.5, 5.0, 15, 50 100, 150 & 299 µg/mL DMSO solvent	89.5% purity Reference No. 77-297-55	DMBA (3 & 5 µg/mL)	-	- (Cytotoxicity ≥100 µg/mL)	Curren (1988) [QA, GLP]

Results are expressed as positive (+), negative (-) or equivocal are expressed relative to the presence (+) or absence (-) of exogenous metabolic activation (via the addition of S9 mix, derived from the livers of adult rats that had been induced with aroclor 1254); QA = quality assured study; GLP = statement of compliance with principles of Good Laboratory Practice; CHO = Chinese Hamster Ovary; 2-AA = 2-aminoanthracene; MMS = methyl methane sulphonate; 4-NQO = 4-nitroquinoline-N-oxide; EMS = ethyl methanesulfonate; DMBA = 7,12-dimethylbenz(a)-anthracene; DMSO = dimethyl sulfoxide; unless otherwise indicated, all positive control compounds gave expected results

Table 43 *In vivo* studies

Assay	Species (strain)	Dose or concentration	Purity & Batch	Positive control	Result	Reference
Micronucleus test	Mouse (NMRI) 5/sex/group	0, 0.625, 1.25 & 2.5 mg/kg bw 2 oral doses in 0.5% Cremophor emulsion	92.5% purity Batch No. 808817123	Trenimon (0.125 mg/kg bw) 2 intraperitoneal doses	- (toxicity at 2.5 mg/kg bw)	Herbold (1980a)
Dominant lethal test	Mouse (NMRI) 5 males	0 or 5 mg/kg bw, po	92.5% Batch No. 808817123	None	-	Herbold (1980b)

Results are expressed as positive (+), negative (-) or equivocal; QA = quality assured study; GLP = statement of compliance with principles of Good Laboratory Practice; unless otherwise indicated, all positive control compounds gave expected results

9.1 Evaluation of selected genotoxicity studies

Herbold B (1987) SRA 3886 (common name: fenamiphos, the active ingredient of Nemacur®) Cytogenetic study of human lymphocyte cultures in vitro to test for chromosome damage. Bayer Report No. 15406. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfield, Germany. Study duration: unspecified. Report date: 12th January 1987

GLP & QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA) and OECD Principles of Good Laboratory Practice (Bundesanzeiger, No 42a, 4th February 1983)]

Materials and Methods: The cytogenetic effect of fenamiphos (Batch No. 808511402-233590475; 91.3% purity; Bayer AG, unspecified location) on cultured human lymphocytes was analysed using standard techniques. Fenamiphos was tested at concentrations of 0, 25, 100 or 400 μg/mL in both the presence and absence of an exogenous source of metabolic activation (S9 mix). Fenamiphos was dissolved in dimethyl sulfoxide prior to dilution in culture media. The concentration range was based on a previous unpublished range-finding study. Positive control compounds were 0.1 μg/mL mitomycin C (-S9 mix) and 10 μg/mL cyclophosphamide (+S9 mix). The mitotic index was determined by counting 1000 cells/culture and scoring the number of mitotic and non-mitotic cells. Approximately 200 metaphases were examined at each concentration for the presence of gaps, breaks, fragments, deletions, exchanges and multiple aberrations according to Reiger and Michaelis (1967). Results were statistically analysed using a one-tailed, corrected χ^2-test. A decrease in the mitotic index that was significant at $p<0.01$ and increases in aberration rates at $p<0.05$ were deemed biologically-significant findings.

Results: In the absence of S9 mix, there was a significant ($p<0.01$) concentration-related decrease in the mitotic index at 100 and 400 μg/mL (119, 52 and 0% of the negative control at 25, 100 and 400 μg/mL, respectively). In the presence of S9 mix, the mitotic index was significantly reduced ($p<0.01$) only at 400 μg/mL (<0.1% of the negative control). In both the presence and absence of S9 mix, haemolysis was also detected at 400 μg/mL. Slight reductions in the mitotic index occurred in the presence of either positive control [Mitomycin C: 77% of the negative control ($p<0.05$); cyclophosphamide: 80% of the negative control] but neither were biologically-significant according to the performing laboratory's evaluation criteria.

In the absence of S9 mix, significant increases in aberrations (including and excluding gaps) ($p<0.05$) occurred only at 100 μg/mL [9.5% *versus* 1.0 (including gaps) and 3.0% (excluding gaps) in the negative control], with no increase in exchanges or polyploid metaphases. At 400 μg/mL no metaphase chromosomes were detected and therefore this concentration could not be evaluated. Mitomycin C significantly increased ($p<0.05$) the rate of chromosomal aberrations [11 and 9% (including/excluding gaps, respectively)]. In the presence of S9 mix, there was no increase in aberrations at or below 100 μg/mL. At 400 μg/mL, there was a significant increase ($p<0.01$) in chromosomal aberrations [13.3 and 11.1% (including/excluding gaps, respectively) *versus* 1.5 and 0.5% in the negative control], but no exchanges or increase in the rate of polyploid metaphases. However, only 45 metaphase chromosomes were examined due to the occurrence of haemolysis. Cyclophosphamide caused a significant increase ($p<0.05$ or 0.01) in chromosomal aberrations [6.5 and 5.5% (including/excluding gaps, respectively)].

Conclusion: Fenamiphos is damaging to human chromosomes at cytotoxic concentrations in both the presence and absence of metabolic activation.

Herbold B (1988) SRA 3886 (cn. fenamiphos) In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. Bayer Report No. 16690. Study No. T1025572. Lab & Sponsor: Institute for Toxicology, Bayer AG, Friedrich-Ebert-Strause, Wuppertal, Germany. Study duration: 11th-15th May 1987. Report date 5th June 1988

GLP & QA: Statement of compliance with OECD Principles of Good Laboratory Practice (Bundesanzeiger, No 42a, 2nd March 1983)]; QA statement

Materials and Methods: The cytogenetic effect of fenamiphos (Batch No. 808511402-233590475; 91.9% purity; Bayer AG) on cultured human lymphocytes was analysed using standard techniques. Fenamiphos was tested at concentrations of 0, 25, 50, 75 or 100 μg/mL in the absence of an exogenous source of metabolic activation (S9 mix) and at concentrations of 0, 100, 150, 225 or 350 μg/mL in the presence of metabolic activation. These concentrations were based on findings from the above study of Herbold (1987). Fenamiphos was dissolved in dimethyl sulfoxide prior to dilution in culture media. Positive control compounds were 0.15 μg/mL Mitomycin C (-S9 mix) and 15 μg/mL cyclophosphamide (+S9 mix). The mitotic index was determined by counting 1000 cells/culture and scoring the number of mitotic and non-mitotic cells. Approximately 200 metaphases were examined at each concentration for the presence of gaps, breaks, fragments, deletions, exchanges and multiple aberrations according to Reiger and Michaelis (1967). Results were statistically analysed using a one-tailed, corrected χ^2-test. A decrease in the mitotic index that was significant at $p<0.05$ was deemed a biologically significant finding. A statistically-significant ($p<0.05$) concentration-related increase in the aberration rate was deemed a positive result.

Results: In the absence of S9 mix, there was a significant ($p<0.01$) concentration-related decrease in the mitotic index at every concentration (74, 56, 44 and 39% of the negative control at 25, 50, 75 and 100 μg/mL, respectively). In the presence of S9 mix, there was also a concentration-related decrease in the mitotic index, which was statistically significant ($p<0.01$) at and above 150 μg/mL (82, 56, 54 and 31% of the negative control at 0, 100, 150, 225 and 350 μg/mL, respectively). Both positive control compounds caused a significant reduction ($p<0.01$) in the mitotic index (57 and 54% for Mitomycin C and cyclophosphamide, respectively).

In the absence of S9 mix, there was no treatment-related increase in the incidence of aberrations, while Mitomycin C caused a significant increase ($p<05$) (45.5 and 23% including and excluding gaps, respectively, *versus* 15.5 and 3.5% in the negative control). Mitomycin C also caused a significant increase ($p<0.05$) in exchanges (3% *versus* 0% in the control). In the presence of S9 mix, there was a significant increase ($p<0.05$) in chromosomal aberrations only at the highest concentration of 350 μg/mL (19.0% and 10% including and excluding gaps, respectively, *versus* 10.5 and 3.5% in the negative control). Cyclophosphamide caused significant increases in aberrations, including and excluding gaps (45.5 and 21.5%, respectively), and in exchanges (5% *versus* 0% in the control).

Conclusion: Fenamiphos was cytogenetic to human chromosomes in the presence of metabolic activation at a highly cytotoxic concentration of 350 μg/mL.

10. NEUROTOXICITY STUDIES

10.1 Hens

Kimmerle G (1970) Bay 68138: Subchronic neurotoxicity studies on hens. Report No. 1831. Lab: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld, Germany. Sponsor: unspecified. Study duration: unspecified. Report date: 28th January 1970.

Spicer EJF (1970) Pathology report of Bay 68138 subchronic neurotoxicity tests on hens. Report No. 3773/70/595. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld, Germany. Study duration unspecified. Report date: 27th November 1970.

This study had limited regulatory value due to its lack of reporting detail. Therefore only a brief evaluation follows.

Fenamiphos as a 50% premix containing Silkasil S (unspecified Batch No., purity & source), was admixed in the diet at concentrations of 0, 1, 3, 10 or 30 ppm and fed to 8 hens/group (HNL strain; 15-20 months old; unspecified source) for 30 days. Hens were kept under observation for a further 4 weeks. Hens were housed individually in cages during treatment and in groups during the observation period, with food and water available *ad libitum*. Daily observations were made for signs of neurotoxicity. Bodyweights were recorded weekly. Food consumption was measured weekly during the 30-day treatment period. Whole blood ChE activity was analysed prior to treatment, on the last day of the treatment period and at the end of the post-treatment observation period. Two hens/group were sacrificed following the treatment phase, with all remaining hens sacrificed following the 4 week observation period. The brain, spinal cord and peripheral nerves were processed for histopathological analysis.

There were no treatment-related mortalities. Clinical signs (unspecified) occurred only at 30 ppm but were reportedly unlike typical cholinergic symptoms. No signs of neurotoxicity were reported. Average bodyweight was significantly lower ($p<0.01$) at 30 ppm than the control at day 30 and 58 (1.43 *versus* 1.85 kg and 1.73 *versus* 2.00 kg, respectively). Average food consumption was significantly lower ($p<0.01$) at 30 ppm than the control (1287 *versus* 2804 g). The average dietary intake of fenamiphos ingested by hens was determined to be 0, 0.002, 0.005, 0.016 and 0.026 mg/kg bw/day at 0, 1, 3, 10 and 30 ppm, respectively. At day 30, whole blood ChE activity was inhibited in a dose-related fashion at and above 3 ppm (22, 49 and 65% of the control at 3, 10 and 30 ppm, respectively). No toxicologically significant inhibition of ChE activity occurred at day 58. Histopathological examination revealed no treatment-related abnormalities of the peripheral nerve. Degenerative fibres in the spinal cord occurred in most groups, including the control, and were therefore not treatment-related. Lymphocytic infiltration, including the presence of small aggregates of lymphocytes, occurred in blood vessels of the spinal cord and/or brain at and above 3 ppm (2, 3 and 3/8 hens at 3, 10 and 30 ppm, respectively). In addition, minimal lymphocytic cuffing of vessels of the spinal cord or brain occurred in 3 hens per group at 1 and 10 ppm. The authors considered that these findings were non-specific in nature and possibly related to a viral infection.

Kimmerle G (1971) Nemacur P: Acute neurotoxicity studies on hens. Report No. 2829. Lab: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld, Germany. Sponsor: unspecified. Study duration: unspecified. Report date: 14th June 1971.

This study had limited regulatory value due to its lack of reporting detail. Therefore only a brief evaluation follows.

Technical grade fenamiphos (unspecified purity, Batch No. & source) was dissolved in polyethylene glycol and administered to groups of 10 White Leghorn hens (16-18 months old; 1.6-2.1 kg bw; unspecified source) as a single oral gavage dose of 1.0, 2.5, 3.75, 5.0, 7.5 or 10 mg/kg bw. Five positive control hens received 500 mg tri-ortho-cresylphosphate (TOCP) orally. To evaluate potential neurotoxicity, hens were observed for 3 weeks post-treatment. Deaths occurred at and above 3.75 mg/kg bw within 1-2 hours of dosing (2/10, 6/10, 7/10 and 2/2 at 3.75, 5.0, 7.5 and 10.0 mg/kg bw, respectively). Cholinergic signs occurred "quickly" in all hens at and above 2.5 mg/kg bw and had resolved within 24 hours in survivors. There were no signs of neurotoxicity (e.g. lameness or paralysis). The NOEL was 1.0 mg/kg bw based on the occurrence of clinical signs and deaths at and above 2.5 mg/kg bw. In a parallel experiment, atropine (50 mg/kg bw; intraperitoneal) was administered prior to dosing with 3.75, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 or 25.0 mg/kg bw fenamiphos. Deaths occurred at and above 10.0 mg/kg bw (2/10, 6/10, 7/10, 2/2 and 2/2 at 10.0, 12.5, 15.0, 17.5 and 25.0 mg/kg bw, respectively), while cholinergic signs occurred at and above 5.0 mg/kg bw in the majority of hens. The NOEL was 3.75 mg/kg bw based on the occurrence of cholinergic signs at and above 5.0 mg/kg bw. No clinical signs consistent with delayed neuropathy occurred in fenamiphos-treated groups but "typical" features developed in the TOCP-treated hens (unsteady gait, lameness and paralysis).

Flucke W & Kaliner G (1987) SRA 3886 Technical (common name: fenamiphos) Delayed neurotoxicity studies on hens following acute oral administration. Report No. 16187. Study No. T1020919. Lab: Bayer Ag, Institute for Toxicology, Friedrich-Ebert-Strasse, Wuppertal, Germany. Sponsor: Mobay Corporation, Agricultural Chemicals Division, unspecified location. Study duration: October to November 1985. Report date: 5th November 1987.

GLP & QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement

Guidelines: Statement of compliance with US EPA guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals. Revised edition November 1984)

Materials and Methods

Fenamiphos (Batch No. 808511402-233590475; 91.3% purity; unspecified source) was administered by oral gavage to 30 white leghorn hens (strain Lohmann Selected Leghorn), under atropine protection, at a dose of 25 mg/kg bw. Two doses were administered, separated by an interval of 3 weeks. The dose was chosen based on previous acute oral neurotoxicity study in hens (Bayer Report No. 14230) and other preliminary tests on hens under atropine protection. Atropine sulphate was given 10 minutes prior to dosing (100 mg/kg bw, intramuscular) and again at approximately 7 hours after dosing (30 mg/kg bw, subcutaneous). Survivors received additional injections of atropine at approximately 24 and 30 hours (50 and 30 mg/kg bw, respectively). A negative control group of 6 hens received the vehicle only [deionised water and Cremophor EL (2% v/v)]. A positive control group of 5 hens received 375 mg/kg bw of triorthocresyl phosphate (TOCP) by oral gavage in deionised water and Cremophor EL (2% v/v). All dosing solutions were administered at 5 mL/kg bw. The stability and homogeneity of fenamiphos in the dosing formulation was analysed.

Hens were sourced from Brinkschulte (Snenden, Germany) and acclimatised for approximately 4 weeks prior to experimentation. They were approximately 7-10 months old and weighed 1.28-1.69 kg at the start of the study. Hens were housed under standard conditions, with food and water available *ad libitum*.

Hens were allowed to recover for 21 days after each dose of fenamiphos, vehicle or TOCP. Hens were observed for mortalities and clinical signs several times on the days of treatment and then on a daily basis. The time of death was recorded in each case. Motor co-ordination was examined by prodding hens for 2-3 minutes at least twice weekly and then scoring them accordingly (0 = normal; 1 = slightly abnormal gait; 2 = ataxia/disturbance of motor coordination; 3 = severe ataxia/paresis; 4 = complete paralysis). Bodyweights were recorded prior to treatment and then weekly. Any hens dying during the study were necropsied. All survivors from the fenamiphos and negative control groups were exsanguinated under Evipan-Sodium anaesthesia and the following tissues prepared for histopathological examination: sciatic nerves with their tibial and common peroneal branches, brain and spinal cord. Positive control hens were sacrificed and processed on day 17 due to the occurrence of severe clinical signs. These tissue were examined from all positive and negative control hens, and from only 6 fenamiphos-treated hens.

No statistical analysis was performed.

Results

Analytical chemistry: Fenamiphos formulated in deionised water and Cremophor EL (2% v/v) was determined to be stable at an unspecified temperature for 19 hours. This same formulation was also determined to be homogenous.

Mortalities and clinical signs: Forty-three percent (13/30) of hens died 1-4 days after the first dose of fenamiphos, with signs of toxicity evident from 25 minutes to 14 days post-dose. The signs exhibited by all hens included staggering gait, ruffled feathers, tachypnoea, reduced activity, flaccid/drooping wings and a spasmodic state. A few hens also displayed sternal and lateral recumbency and salivation. Following the second dose of fenamiphos, one hen died 2 days post-dose and clinical signs were observed in all hens from 45 minutes to 7 days. These signs were consistent with those observed after the first dose of fenamiphos. There were no deaths in either the positive or negative control groups, however, clinical signs (ataxia to paresis) were observed from 7-17 days after dosing with TOCP, which reportedly became progressively more severe. All positive control hens were sacrificed in a moribund condition on day 17.

Bodyweights: Fenamiphos-treated hens had a 13% loss of bodyweight during the week following the first doses, which did not recover before the second treatment or for the remainder of the study. TOCP-treated hens also had a marked loss of bodyweight, which continued until their sacrifice during week 3 (21%). Over the 6 week experimental period, control hens had a slightly lower average bodyweight compared to the pre-treatment period ($\leq$3%).
Motor co-ordination examinations: No signs of neurotoxicity were detected in either the fenamiphos-treated or negative control hens. Seventeen days after administration, hens treated with TOCP exhibited signs typical of delayed neurotoxicity including ataxia/disturbance of motor co-ordination (3/5), severe ataxia/paresis (1/5) or complete paralysis (1/5).

Gross pathology: There were no gross pathological abnormalities detected in negative or positive control hens. In the 14 fenamiphos-treated hens that died during the study, the following gross abnormalities were noted: distended lungs (14); fluid-filled lungs (10); mottled or dark lungs (5), mottled liver (5); mottled, pale or marbled kidneys (3); distended and/or fluid present in the crop (7) and in some cases the pericardium (5); and occasional reddening of the mucosa of the glandular stomach and/or duodenum (2). There were no gross abnormalities detected in fenamiphos-treated hens that survived to scheduled sacrifice and therefore it is unlikely that these findings were treatment-related but could have been due autolysis.

Histopathology: There were no treatment-related neurodegenerative effects detected. In all hens treated with TOCP, fibre degeneration in the sciatic nerve (mild to severe) and the lumbar and cervical segments of the spinal cord (mild to moderate) were detected. In addition, two hens had degeneration of the thoracic segments of the spinal cord. It was reported that the myelin of degenerated fibres was fragmented, the sheath cells activated and the segments of the fibre sheaths expanded.

Conclusions: Fenamiphos was overtly toxic but did not cause neuropathy in hens at a concentration of 25 mg/kg bw.

Flucke W (1988) SRA 3886 Technical (common name: fenamiphos) Study of the effect on the neurotoxic esterase (NTE) following oral administration to hens. Report No. 17388. Study No. T0021656. Lab: Bayer AG, Institute for Toxicology, Friedrich-Ebert-Strasse, Wuppertal, Germany. Sponsor: Mobay Corporation, Agricultural Chemicals Division, unspecified location. Study duration: January-February 1986. Report date: 21st November 1988.

GLP & QA: Statement of compliance with standards of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA) and OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981)]; QA statement

Materials and Methods

Fenamiphos (Batch No. 808511402-233590475; 91.3% purity unspecified source) was administered by oral gavage to adult white leghorn hens (strain Lohmann Selected Leghorn; 9/group) under atropine protection at a dose of 0 or 25 mg/kg bw/day. Fenamiphos was formulated in Cremophor EL (2% v/v) and deionised water, and the dose volume was 5 mL/kg bw. This formulation was determined to be stable for a period of 24 hours, and homogenous. Atropine sulphate was given 10 minutes prior to dosing (100 mg/kg bw, intramuscular) and again at approximately 7 hours after dosing (30 mg/kg bw, subcutaneous). Survivors received additional injections of atropine at approximately 24 and 30 hours (50 and 30 mg/kg bw, respectively). A positive control group of 3 hens received 100 mg/kg bw of TOCP by oral gavage in deionised water and Cremophor EL (2% v/v). Hens were sourced from Brinkschulte (Snenden, Germany) and were acclimatised for 6 days prior to experimentation. They were approximately 6 months old and weighed 1.25-1.70 kg at the start of the study. Hens were housed under standard conditions, with food and water available *ad libitum*. Bodyweights were recorded prior to dosing and then at days 1, 2 and 7. Three hens/group were sacrificed by decapitation at 24 and 48 hours, and at 7 days after dosing for analysis of neurotoxic esterase activity (NTE) in the brain and spinal cord. No statistical analysis was performed.

Results

One fenamiphos-treated hen died within the first 24 hours of dosing, with all remaining hens surviving to sacrifice. In those 3 hens sacrificed 24 hours after dosing, brain NTE activity was inhibited by 0, 27 and 25% relative to the control, but there was no inhibition of NTE activity in the spinal cord. Forty-eight hours after dosing, there was no inhibition of NTE activity in brain, with only one hen having inhibition of spinal cord NTE activity (26% relative to the control). Seven days after treatment, only slight inhibition of NTE activity was detected in treated hens in either the brain (~6%) or spinal cord (~1%). In those hens treated with the positive control (TOCP), NTE activity was inhibited by up to 100% in the brain and 92% in spinal cord at 24 hours, up to 92 and 81%, respectively, at 48 hours, and up to approximately 50% of the control at 7 days after dosing. Based on the high variability in NTE activity due reportedly to the assay method, the relatively low level of inhibition relative to the positive control and that there was no consistent inhibition between

brain and spinal cord, the findings in fenamiphos-treated hens are not considered toxicologically significant but incidental in nature.

Conclusions: A single oral dose of 25 mg/kg bw fenamiphos failed to inhibit NTE activity in hens.

10.2 Rats

Dreist M (1995) SRA 3886 (common name: fenamiphos) Acute oral neurotoxicity screening study in wistar rats. Report No. 24408. Study No. T6058166. Lab & Sponsor: Institute of Toxicology Agrochemicals, Bayer AG, Freidrich-Ebert-Strasse, Wuppertal, Germany. Study duration: 22nd August to 16th September 1994. Report date: 25th October 1995.

GLP & QA: Statement of compliance with principles of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA), OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981) and principles of GLP according to Annex 1 ChemG (Bundesgesetzblatt, Part I, July 29, 1994)]; QA statement

Guidelines: Study conducted according to US-EPA-FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS (1991) EPA 540/09-91-123, PB 91-154617.

Materials and Methods

Fenamiphos (Batch No. 809335134; 95.2% purity; sourced from Bayer AG, unspecified location) was administered as a single oral gavage dose to fasted Wistar rats (12/sex/dose; strain Hsd Win:WU) at 0, 0.4, 1.6 or 2.4 mg/kg bw. The dose selection was based on a previous range-finding study (Study No. T0058052), where the NOEL for clinical signs was 0.4 mg/kg bw for males and 1.3 mg/kg bw for females. Dosing was staggered over 4 days to accommodate the Functional Observational Battery (FOB). Satellite groups of 6 rats/sex/dose were treated with the same concentrations of fenamiphos then sacrificed after 50 minutes for the analysis of plasma, RBC and brain ChE activities. Fenamiphos was formulated in demineralised water and Cremophor® EL (2% v/v). The stability and homogeneity of fenamiphos in this vehicle was determined prior to the commencement of the study.

Rats were sourced from Harlan Winkelmann (GmbH Borchen, Germany) and were acclimatised for approximately 6 days prior to dosing. Rats were approximately 8-10 weeks of age and weighed approximately 160 g for males and 150 g for females. Rats were housed individually under standard conditions, with food and water available *ad libitum*.

Cage-side observations for mortality and clinical signs were made daily. A FOB was performed on all rats one week prior to, at 25-65 minutes and 7 and 14 days after dosing. All rats were observed and scored for the standard FOB parameters (see Appendix VIII). Following the FOB, all rats were assessed for locomotor activity sometime between 95-165 minutes after dosing (in addition to one week prior to treatment and at 7 and 14 days post-treatment). Measurement of motor activity took place at 10 minute intervals over 70 minutes using an automated maze system, which consisted of 8-9 figure eight mazes fitted with 8 infrared emitter/detector pairs. The number of beam interruptions occurring during each 10 minute interval was recorded using a Universal Maze Monitoring System (Colombus Instruments, Columbus, Ohio, USA). Performance of the FOB and locomotor analysis were conducted blinded and in a "semi-random" order.

Excluding the satellite group, all rats were subjected to a complete gross necropsy, which consisted of an examination of all organs, body cavities, cut surfaces, external orifices and surfaces for any gross abnormalities. Six rats/sex/dose were exsanguinated under phenobarbital anaesthesia and the

following tissues prepared: brain (plus optic nerve), spinal column with spinal cord, eyes, hind and forelimbs with bilateral skeletal muscles and peripheral nerves, skull with gasserian ganglion, the tail and any macroscopic abnormalities. Brain and terminal bodyweights were recorded. All survivors not needed for neuropathology were sacrificed by diethyl ether inhalation. Tissues from control and high-dose rats were processed for histopathological examination.

Results were analysed using the following statistical tests: rank test of Mann and Whitney, and Wilcoxon (clinical pathology data, terminal body and organ weights); ANOVA (motor and locomotor activity; repeated measures ANOVA and a one-way ANOVA followed by a Dunnett's test if a significant difference was detected (session activity data); 2-way repeated measures ANOVA and a repeated measures ANOVA, followed by a Dunnett's test (interval data); 2-way repeated measures ANOVA and a repeated measures ANOVA (continuous FOB data); General Linear Modelling or Categorical Modelling, with *post hoc* comparisons using Dunnett's test and an Analysis of Contrasts (categorical data collected in the FOB); and a one-sided Fisher test (pupil response on day 0).

Results

Analytical chemistry: Solutions of fenamiphos in the vehicle at concentrations of 0.001 and 0.2% were stable at room temperature for 4 days. Fenamiphos was also homogenously distributed in these same solutions. Analytical concentrations of fenamiphos were within 91.3-96.3% of the nominal concentrations.

Mortalities and clinical signs: At 2.4 mg/kg bw, 4 males and one female died at 21-31 minutes after dosing. Three replacement males treated with this same dose also died. None of these rats were tested in the FOB. There were no mortalities at lower doses. Observations for clinical signs made after the FOB and motor/locomotor activity tests (4-8 post dose) detected no clinical signs.

Bodyweight: There was no treatment-related effect on bodyweight. Based on bodyweight and food consumption data, the author calculated that the actual doses received by rats were 0, 0.37, 1.52 and 2.31 mg/kg bw, respectively.

FOB: Results of FOB findings are summarised in the Tables below. Significant ($p<0.05$) treatment-related effects occurred on the day of dosing at 1.6 and 2.4 mg/kg bw in both sexes and included muscle fasciculations, piloerection, incoordinated and stilted gait, constricted pupils and the presence of lacrimal, nasal and/or oral red stains. These effects were reportedly consistent with acute cholinergic toxicity. In addition, forelimb and hind-limb grip strength in males and forelimb grip strength in females were significantly reduced at 2.4 mg/kg bw. None of these FOB findings were evident at 7 or 14 days post-dose.

Table 44 Summary of FOB findings in male rats

Parameter	Dose (mg/kg bw)			
	0	**0.4**	**1.6**	**2.4**
Home cage observations				
Piloerection	0/12	0/12	0/12	7/8*
Gait abnormalities (incoordination)	0/12	0/12	1/12 Severity = 1	1/8* Severity = 1 4/8* Severity = 2
Involuntary motor - clonic Muscle fasciculations	0/12	0/12	8/12* Severity = 1 1/12* Severity = 2	3/8* Severity = 1 4/4* Severity = 2
Pupil response (constricted)	0/12	0/12	1/12	3/8*

Parameter	Dose (mg/kg bw)			
	0	**0.4**	**1.6**	**2.4**
Stains				
Lacrimal	0/12	0/12	0/12	1/8
Nasal	0/12	0/12	0/12	2/8*
Oral	0/12	0/12	1/12	4/8*
Open field observations				
Piloerection	0/12	0/12	0/12	7/8*
Involuntary motor - clonic				
Repetitive chewing	0/12	0/12	9/12*	4/8*
Muscle fasciculation	0/12	0/12	1/12*	4/8*
Gait abnormalities (incoordination)	0/12	0/12	4/12 Severity = 1	2/8* Severity = 1 4/8* Severity = 2
Neuromuscular observations				
Grip strength				
Forelimb	0.94±0.07	0.94±0.13	0.82±0.14	0.66±0.18*
Hind-limb	0.49±0.06	0.49±0.11	0.45±0.07	0.37±0.08*

Results expressed as the number of rats showing the effect/total number of rats; * p<0.05;

Table 45 Summary of FOB findings in female rats

Parameter	Dose (mg/kg bw)			
	0	**0.4**	**1.6**	**2.4**
Home cage observations				
Piloerection	0/12	0/12	0/12	5/11*
Gait abnormalities (incoordination)	0/12	0/12	0/12	4/11* Severity = 1
Involuntary motor - clonic Muscle fasciculations	0/12	0/12	1/8 Severity = 1	3/11* Severity = 1 3/11* Severity = 2
Pupil response (constricted)	0/12	0/12	0/12	5/11*
Stains (Oral)	0/12	0/12	0/12	3/11* Severity = 1 1/11* Severity = 2
Open field observations				
Piloerection	0/12	0/12	0/12	5/11*
Involuntary motor - clonic Muscle fasciculation	0/12	0/12	3/12 Severity = 1	4/11* Severity = 1 3/11* Severity = 2
Gait abnormalities (incoordination)	0/12	0/12	0/12	5/11* Severity = 1
Neuromuscular observations				
Grip strength				
Forelimb	1.02±0.10	1.00±0.06	1.04±0.11	0.86±0.18*

Results expressed as the number of rats showing the effect/total number of rats; *p<0.05

Motor and locomotor activities: There was no statistically-significant effect on motor activity. Control rats were found to have a low level of motor activity on day 0 relative to their pre-treatment activity (males: 192±60 *versus* 423±93; females: 198±94 *versus* 401±121) in addition to their activity on days 7 and 14. The author attributed this decrease to the overnight fasting prior to testing on day 0. As a consequence, the decrease in motor activity seen with every dose of fenamiphos also at day 0 cannot be interpreted as a treatment-related finding. The motor activity of all groups was comparable on days 7 and 14 relative to pre-treatment activity. There was no treatment-related effect on locomotor activity.

ChE activity: The results of plasma and RBC ChE activity measurements in the satellite groups of rats are summarised in the table below (there was no treatment-related effect on brain ChE activity). Toxicologically-significant inhibition of plasma ChE activity (i.e. >20% relative to the control) occurred at every concentration in both sexes, with statistical significance achieved at and above 1.6 mg/kg bw in males and at every dose in females. Toxicologically- and statistically-significant inhibition of RBC ChE activity occurred in males at every dose and in females at and above 1.6 mg/kg bw.

Table 46 Plasma and RBC ChE activity measurements in rats

Parameter	Dose (mg/kg bw)							
	0		0.4		1.6		2.4	
	Males	Females	Males	Females	Males	Females	Males	Females
Plasma ChE	0	0	23	55**	64**	77**	61**	85**
RBC CHE	0	0	24*	4	70**	51*	76**	80**

Results expressed as the % inhibition relative to the control; *p<0.05; **p<0.01

Gross Pathology: Black discolouration of the liver and pale discolouration of the spleen were detected in all 8 rats that died shortly after dosing with 2.4 mg/kg bw (4 original and 3 replacement males; one female). In addition, one male also had brownish discolouration of the lung. There were no other treatment-related gross abnormalities. There were no treatment-related effects on terminal bodyweight or absolute or relative brain weights.

Histopathology: Hyperaemia (7/7) and Kupffer cell foci (3/7) of the liver were detected in rats that died at the highest dose (only 7 of the 8 dead rats appear to have been examined). Brown/black pigment deposits were detected in the male that showed gross brownish discolouration of the lungs. There were no microscopic lesions detected in skeletal muscle or neural tissue from high-dose rats. Consequently, there was no examination of muscle or neural tissue at the two lower doses.

Conclusion: The LOEL following a single oral dose of fenamiphos to rats was 0.4 mg/kg bw, based on the inhibition of plasma and RBC ChE activities at and above this dose. Mortalities cholinergic signs, FOB abnormalities, discolourations/pigment deposition of the liver, lungs and spleen were evident at the highest dose of 2.4 mg/kg bw. There was no evidence that fenamiphos caused neuropathy.

Dreist M & Popp A (1996) SRA 3886 (common name: fenamiphos) Subchronic neurotoxicity screening study in wistar rats (Thirteen-week administration in the diet). Report No. 24948. Study No. T9058277. Lab & Sponsor: Department for Short-Term Rodent Studies and Neurotoxicology. Bayer AG, Freidrich-Ebert-Strasse, Wuppertal, Germany. Study duration: 23rd January to 9th May 1995. Report date: 29th March 1996.

GLP & QA: Statement of compliance with principles of Good Laboratory Practice [US EPA, 40 CFR Part 160 (FIFRA), OECD Principles of Good Laboratory Practice, c(81)30 (Final) Annex 2 (Paris, May 1981) and principles of GLP according to Annex 1 ChemG (Bundesgesetzblatt, Part I, July 29, 1994)]; QA statement

Guidelines: Study conducted according to US-EPA-FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS (1991) EPA 540/09-91-123, PB 91-154617.

Materials and Methods

Fenamiphos (Batch No. 809335134; 95.5-95.7% purity; sourced from Bayer AG, unspecified location) was admixed in the diet and fed to Wistar rats (strain Hsd Cpb:WU; 12/sex/dose;) at nominal concentrations of 0, 1, 10 or 50 ppm for 13-14 weeks. The dose selection was based on the

results of previous subchronic and chronic dietary studies (Hayes 1986a & b). Diets, which contained 1% peanut oil, were prepared weekly (on Thursday) and offered to rats twice weekly (Friday and Monday). Due to the instability of fenamiphos in the diet over 6 days at room temperature, the half of the diet to be given on Monday was stored frozen at -20°C until use. The stability and homogeneity of fenamiphos in the diet were analysed prior to experimentation. Regular checks were made of the concentration of fenamiphos in the diet (weeks 1, 7 and 14).

Rats were sourced from Harlan Winkelmann (GmbH Borchen, Germany) and were acclimatised for approximately 6 days prior to dosing. Male rats weighed approximately 160 g and females approximately 120 g. The age of the rats was unspecified although they were described as being "young adult". Rats were housed individually under standard conditions, with food and water available *ad libitum*.

Observations for mortalities and clinical signs were made at least twice daily. Detailed physical examinations were made daily for the first three weeks and weekly thereafter. Bodyweights and food and water consumption were recorded weekly. All rats were observed and scored for the standard FOB parameters prior to the commencement of dosing and then at weeks 4, 8 and 13 (see Appendix VIII). Following the FOB, all rats were assessed for locomotor activity, which took place at 10 minute intervals over 70 minutes using an automated figure-eight maze system fitted with 8 infrared emitter/detector pairs. The number of beam interruptions occurring during each 10 minute interval was recorded using a Universal Maze Monitoring System (Colombus Instruments, Columbus, Ohio, USA). Performance of the FOB and locomotor analysis were conducted blinded and in a "semi-random" order.

All rats were subjected to an ophthalmoscopic examination prior to treatment and at termination. Plasma and RBC ChE activities were measured in 6 rats/sex/dose during weeks 4 and 14, with brain ChE activity measured at week 15 [the terminal sacrifice of all rats used for ChE activity measurements was postponed from week 14 to 15 due to an error in formulating the low-dose diet, which was higher than the nominal concentration (2.5 ppm *versus* 1 ppm)].

All rats were subjected to a complete gross necropsy, which consisted of an examination of all organs, body cavities, cut surfaces, external orifices and surfaces for any gross abnormalities. Six rats/sex/dose (i.e. those not used for ChE activity measurements) were exsanguinated under phenobarbital anaesthesia and the following tissues processed for histopathological examination: brain (plus optic nerve), spinal column with spinal cord, eyes, hind and forelimbs with bilateral skeletal muscles and peripheral nerves, skull with Gasserian ganglion, and any macroscopic abnormalities. Brain and terminal bodyweights were recorded. Only tissues from control and high-dose rats were processed for histopathological examination. All survivors not needed for neuro-histopathology were sacrificed by diethyl ether inhalation during week 15.

Results were analysed using the following statistical tests: Dunnett's test (bodyweight, food consumption and organ weight data); adjusted Welsh test (ChE activity); ANOVA (motor and locomotor activity); repeated measures ANOVA and a one-way ANOVA followed by a Dunnett's test if a significant difference was detected (session activity data); 2-way repeated measures ANOVA and a repeated measures ANOVA, followed by a Dunnett's test (interval data; continuous FOB data); and General Linear Modelling or Categorical Modelling, with *post hoc* comparisons using Dunnett's test and an Analysis of Contrasts (categorical data collected in the FOB).

Results

Analytical chemistry: With the exception of the error in the low-dose diet, which was considered by the authors to be an isolated occurrence, analytical levels of fenamiphos were within 85-114% of nominal concentrations (average analytical concentrations of 0, 1, 10 and 40 ppm at nominal levels of 0, 1, 10 and 50 ppm, respectively). Analysis of 3 samples from a 0.5 ppm and 100 ppm diet indicated that they were homogenous (coefficients of variation of 7.3 and 1.7%, respectively). On this basis, fenamiphos was considered to be homogenously distributed in the diet over a concentration range of 0.5 to 100 ppm. Stability analysis of a 0.5 and 100 ppm diet found analytical concentrations were approximately 90% of nominal concentrations after 4 days at room temperature, decreasing to 80-86% after 6 days and 50-77% after 10 days. On this basis it was concluded that within the concentration range of 0.5-100 ppm fenamiphos is stable in the diet for at least 6 days at room temperature. It was also stated that fenamiphos is stable when stored frozen at -20°C for at least 7 weeks, although no data were provided to substantiate this claim.

Mortalities and clinical signs: There were no deaths. Muscle fasciculations were observed from weeks 1-3 in all high-dose females but were not evident in males or at lower doses. There were no other treatment-related clinical signs.

Bodyweights, food and water consumption: Bodyweight and food and water consumption were unaffected by treatment. Based on bodyweight and food consumption data, the average doses of fenamiphos ingested at nominal levels of 0, 1, 10 and 50 ppm were calculated by the authors at 0, 0.06, 0.61 and 3.13 mg/kg bw/day, respectively, in males, and 0, 0.08, 0.80 and 3.98 mg/kg bw/day, respectively, in females.

Ophthalmoscopy: There were no treatment-related ophthalmoscopic abnormalities.

FOB: In 50 ppm females, forelimb grip strength was significantly lower ($p<0.05$) than the control during week 13 (1.29±0.15 *versus* 1.44±0.13). There was no such effect during weeks 4 or 8, or in males. Foot splay was significantly lower than the control in 10 ppm females during week 4 (59±9 *versus* 73±11) and week 8 (66±12 *versus* 83±18), and in 10 and 50 ppm females during week 13 (66±10 and 70±11, respectively, *versus* 86±17). However, given the lack of a dose-response effect and that the foot splay of 10 and 50 ppm females was comparable to their pre-treatment levels (67±12 and 69±12, respectively), these findings were not considered treatment-related. There was no treatment-related effect on any other FOB parameter.

Motor and locomotor activity: There was no treatment-related effect on motor or locomotor activity.

ChE activity: Results of ChE activity measurements are summarised in the table below. Dose-related inhibition of plasma and RBC ChE activities occurred in both sexes at weeks 4 and 15. In males, significant inhibition ($p<0.01$; ~70%) of plasma ChE activity occurred at 50 ppm, with toxicologically-significant inhibition (i.e. >20% relative to the control) occurring at and above 10 ppm (30% at week 4 and 38% at week 15). In females, significant inhibition ($p<0.01$) of plasma ChE activity occurred at and above 10 ppm (>70%), with toxicologically-significant inhibition occurring at every dose. Significant inhibition ($p<0.01$; 90%) of RBC ChE activity occurred in males at 50 ppm, with toxicologically-significant inhibition also occurring at 10 ppm during week 15 (25%). In females, significant inhibition ($p<0.01$; 90%) of RBC ChE activity occurred at 50 ppm, with toxicologically significant inhibition occurring at and above 10 ppm (25% at week 4 and 30% at week 15. There was no toxicologically or statistically significant inhibition of brain ChE activity in males, while there was significant inhibition ($p<0.01$; 12%) in females at 50 ppm.

Table 47 ChE activity measurements in rats

Parameter	Concentration (ppm)			
	0	**1**	**10**	**50**
Males				
Plasma ChE week 4	0.44±0.102	0.39±0.052 (11%)	0.31±0.06 (30%)	0.14±0.037 (68%)**
Plasma ChE week 15	0.52±0.156	0.44±0.111 (15%)	0.32±0.051 (38%)	0.16±0.049 (69%)**
RBC ChE week 4	0.74±0.197	0.83±0.160	0.70±0.123(5%)	0.09±0.071 (88%)**
RBC ChE week 15	0.71±0.136	0.67±0.101 (6%)	0.53±0.05 (25%)	0.05±0.005 (93%)**
Brain ChE activity	12.54±0.763	11.87±0.577	11.26±2.275	11.37±0.494 (9%)
Females				
Plasma ChE week 4	1.29±0.392	0.92±0.259 (29%)	0.38±0.101 (71%)**	0.15±0.056 (88%)**
Plasma ChE week 15	1.85±0.566	1.26±0.445 (32%)	0.43±0.09 (77%)**	0.17±0.05 (91%)**
RBC ChE week 4	0.81±0.189	0.85±0.207	0.61±0.135 (25%)	0.11±0.082 (86%)**
RBC ChE week 15	0.69±0.106	0.78+0.157	0.55+0.218 (30%)	0.03+0.014 (96%)**
Brain ChE activity	11.94±0.400	12.59±0.333	11.74±0.525	10.48+0.421 (12%)**

Results expressed as the mean kU/L (plasma and RBC ChE activities) or U/g (brain ChE activity) ± 1 SD, with the % inhibition relative to the control contained in parentheses; * significantly different to the control at $p<0.05$; ** significantly different to the control at $p<0.01$

Gross pathology: There were no treatment-related gross abnormalities detected at necropsy and no effect on absolute or relative brain weights.

Histopathology: There were no treatment-related histopathological abnormalities.

Conclusions: The NOEL in males following 15 weeks of dietary exposure to fenamiphos was 1 ppm (equal to 0.06 mg/kg bw/day), based on toxicologically-significant inhibition of plasma and RBC ChE activities at and above 10 ppm (0.61 mg/kg bw/day). No NOEL could be established for females due to toxicologically-significant inhibition of plasma ChE activity at every dose. Transient muscle fasciculations occurred in females at the highest dose of 100 ppm (equal to 3.98 mg/kg bw/day). There was no evidence that fenamiphos caused neuropathy up to 100 ppm (equal to 3.13 and 3.98 mg/kg bw/day in males and females, respectively).

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Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 19 of 23] [Bayer; sub: 11022, A3162/32, Box 21]

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Thyssen J (1974b) ®Nemacur-sulfoxide acute toxicity in rats. Report No. unspecified. Lab & sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 9 of 23]

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Abbink J, Weber H & Ecker W (1988) [Phenyl-1-^{14}C]Nemacur: Absorption, distribution, excretion and metabolism in laying hens. PF Report No. 3101. Study No. M 1850224-2. Lab: Bayer AG, Monheim, Institute for Metabolism Research, Leverkusen, Bayerwerk, Germany. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Research and Development Department, Mobay Research Park, South Metcalf, Stillwell, Kansas, USA. Unpublished. [Bayer; sub: CR68-1, Vol 16 of 23] *(Study conducted on a species that is not an appropriate model for human metabolism)*

Abbink J, Weber H & Ecker W (1989) [Phenyl-1-^{14}C]Nemacur: Absorption, distribution, excretion and metabolism in a lactating goat. PF Report No. 3100. Study No. M 1850219-5. Lab: Bayer AG, Monheim, Institute for Metabolism Research, Leverkusen, Bayerwerk, Germany. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Research and Development Department, Mobay Research Park, South Metcalf, Stillwell, Kansas, USA. Unpublished. [Bayer; sub: CR68-1, Vol 15 of 23] *(Study conducted on a species that is not an appropriate model for human metabolism)*

Cain KS, Hanlon CM & Hundal JS (1989) Metabolism of ^{14}C-fenamiphos in tobacco plants and ^{14}C-residues identified in cigarette smoke, Report No. 99711. Lab & Sponsor: Mobay Corporation, Agricultural Chemical Division, Research and Development Department, South Metcalf, Stilwell, Kansas, USA. Unpublished. [Bayer; sub: CR68-1, Vol 12 of 23] *(Plant residue study not relevant to the hazard assessment of fenamiphos)*

Coulston F & Wills JH (date unspecified) Summary of research by the Institute of Comparative and Human Toxicology, Albany Medical College, on phenamiphos. Unpublished. [Bayer; sub: CR68-1, Vol 12 of 23] *(Study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment)*

Dubois KP, Flynn M & Kinoshita F (1967) The acute toxicity and anticholinesterase action of Bayer 68138. Report No 20810. Lab: Toxicity Laboratory, University of Chicago, Chicago, Illinois, USA. Sponsor: unspecified. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment)*

Dubois KP & Flynn M (1968) The subacute parenteral toxicity of Bay 68138 to rats. Report No 22178. Lab: Toxicity Laboratory, University of Chicago, Chicago, Illinois, USA. Sponsor: unspecified. Unpublished. [Bayer; sub: CR68-1, Vol 12 of 23] *(Administration route (intraperitoneal) not relevant for human risk assessment; study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment)*

Dreist M (1996) Method validation study in rats for the acute and subchronic neurotoxicity screening battery. Study No. T3060610. Report No. 24770. Lab: department for Short-Term rodent Studies and Neurotoxicology, Bayer AG, Friedrich-Ebert-Str, Wuppertal, Germany. Sponsor: Bayer AG, Toxicology, Friedrich-Ebert-Strasse, Wuppertal, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 12 of 23] *(Method validation study not relevant to the hazard assessment of fenamiphos)*

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Development, Institute for Metabolism Research, Leverkusen, Germany. Sponsor: Bayer AG, Crop Protection Development, Institute for Metabolism Research, Leverkusen, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 16 of 23] *(Study conducted on a species that is not an appropriate model for human metabolism)*

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Heimann KG (1984) SRA 3886 (fenamiphos) determination of acute toxicity in rats. Report No. unspecified. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment; study report in German)*

Khasawinah AM (1971) Metabolism of ®Nemacur (ethyl 4-(methylthio)-m tolyl isopropylphosporamidate) in tobacco plants. Report No. 29142. Lab: Chemagro Corporation, Research and Development Department, unspecified location. Sponsor: Bayer AG, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 14 of 23] [Bayer; sub: 219, A3162/8, Box 89] *(Plant residue study not relevant to the hazard assessment of fenamiphos)*

Khasawinah AM (1972b) Metabolism of ®Nemacur in snap beans grown in closed glass chambers. Report No. 34992. Lab: Chemagro Corporation, Research and Development Department, unspecified location. Sponsor: Bayer AG, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 14 of 23] [Bayer; sub: 219, A3162/8, Box 89] *(Plant residue study not relevant to the hazard assessment of fenamiphos)*

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Khasawinah AM (1973b) The metabolism of ®Nemacur in cabbage. Report No. 39120. Lab: Chemagro Corporation, Research and Development Department, unspecified location. Sponsor: Bayer AG, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 14 of 23] *(Plant residue study not relevant to the hazard assessment of fenamiphos)*

Khasawinah AM (1973c) The metabolism of ®Nemacur in carrots. Report No. 36005. Lab: Chemagro Corporation, Research and Development Department, unspecified location. Sponsor: Bayer AG, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 14 of 23] *(Plant residue study not relevant to the hazard assessment of fenamiphos)*

Kimmerle G (1972) Acute toxicity of SRA 3886 in combination with S276 and with E154 to rats. Report No. 3438. Lab & Sponsor: Farbenfabriken Bayer AG, Institut fur Toxikologie, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment – particularly the identity of S276 and with E154)*

Knaak JB, Ackerman CR, Yee K, Fredrickson AS & Lee (unspecified date) Reentry research: dermal dose red cell cholinesterase-response curves for fenamiphos, fenamiphos sulfoxide and sulfone. Report No. 69689. Lab/Sponsor: Californian Department of Food and Agriculture, Sacramental, California, USA. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Study unsuitable for regulatory purposes due to the lack of reporting detail to allow an independent scientific assessment; poor study design – the only toxicological endpoint measured was RBC ChE activity; no individual animal data)*

Lamb DW & Landes AM (1978) *In vitro* inhibition of cholinesterase by ®Nemacur and Nemacur metabolites. Report No. 66068. Lab/Sponsor: Mobay Chemical Corporation, Chemagro Agricultural Division, Kansas City, Missouri, USA. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(In vitro study not relevant to the human risk assessment of fenamiphos in the presence of adequate in vivo data on ChE inhibition)*

Loeser E (1970) Bay 68138: Subchronic toxicological investigation on dogs. Report No. 2008. Lab & Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: 219, A3162/8, Box 89] *(Study unsuitable as only one dose level was tested)*

Löser E & Kimmerle G (1971) Acute and subchronic toxicity of ®Nemacur active ingredient. Lab/Sponsor: Institut für Toxikologies, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany. Pflanzenschutz-Nachrichten Bayer 24/1971, 1 [Bayer; sub: CR68-1, Vol 13 of 23] *(This summary report was a compendium of various toxicity studies conducted by Bayer and was not amenable to an independent scientific assessment)*

Mavournin KH, Blakey DH, Cimino MC, Salamone MF & Heddle JA (1990) The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. Mutation research **239**:29-80. [Bayer; sub: CR68-1, Vol 13 of 23] *(This study report contained no specific data on fenamiphos)*

Mawdesley-Thomas LE & Urwin C (1970b) Pathology report of Bay 68138 sub-chronic toxicological studies in dogs (feeding experiment of 3 months) (Addendum to report No. 1655.) Report No. 3354/70/166. Lab: Huntingdon Research Centre, Huntingdon, England. Sponsor: Institute for Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Parent report No. 1655 was not submitted for evaluation. Therefore the data in this report on its own has no regulatory value)*

Mihail F (1980) SRA 3886 and curaterr: Evaluation for acute combination toxicity. Report No. 9294. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Unpublished. [Bayer; sub: CR68-1, Vol 13 of 23] *(Study not relevant as curaterr is not an approved active in Australia. There are no registered Australian products containing fenamiphos and curaterr)*

Pauluhn J (1986) Comparative studies on the effects of cholinesterase-inhibiting substances: influence of test substance intake via the respiratory passages on bronchial tone and on cholinesterase activity in plasma and in erythrocytes of rats. Report No. 14488. Lab & sponsor: Bayer Institute for Toxicology, Wuppertal-Elberfeld, Germany. Unpublished [Bayer; sub: 11022, A3162/32, Box 21] *Study not relevant to the hazard assessment of fenamiphos*

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APPENDICES

APPENDIX I: List of Clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
albumin	clotting parameters (clotting time, prothrombin time)	appearance
ALP (alkaline phosphatase)	erythrocyte count	specific gravity
bilirubin (total)	haematocrit (packed cell volume)	glucose
calcium	haemoglobin	ketones
chloride	leucocyte differential count	sediment (microscopic)
cholesterol (total)	leucocyte total count	occult blood
cholinesterase activity	platelet count	pH
creatinine (blood)	reticulocyte count	protein
gamma-glutamyl transpeptidase	MCH	volume
globulin	MCHC	bilirubin
glucose (blood)	MCV	urobilinogen
serum lactate dehydrogenase	blood smear	reducing substances
phosphorus		
potassium		
protein (total)		
SGPT (serum alanine aminotransferase)		
SGOT (serum aspartate aminotransferase)		
sodium		
triglycerides		
urea nitrogen (blood)		
creatinine phosphokinase		

APPENDIX II: Organs for weight determination and histopathological examination

Organs Weighed	Tissues Examined		
Adrenals Brain Gonads Heart Kidneys Liver Spleen Thyroid (w/parathyroid)	Adrenals aorta blood smear bone bone marrow brain (3 levels) caecum colon duodenum epididymes eyes eyes (optic nerve) gall bladder Harderian glands head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	heart ileum jejunum kidneys lacrimal gland liver lungs lymph nodes mammary gland muscle (smooth) muscle (skeletal) nerve (peripheral) oesophagus ovaries pancreas pituitary	prostate rectum salivary gland seminal vesicle skin spinal cord (cervical thoracic, lumbar) **spleen** sternum stomach testes thymus thyroid (w/parathyroid) trachea urinary bladder uterus vagina Zymbal's gland gross lesions

APPENDIX III: Reproductive and Developmental Indices

$$\textbf{Male/female mating index } (\%) = \frac{\text{number of males/females with confirmed mating*}}{\text{number of males/females placed with females/males}} \times 100$$

* defined by females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females/males}} \times 100$$

* defined by a female giving bith to a litter or with pups/fetuses in utero

$$\textbf{Female fertility index (\%)} = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$$

* defined as the number of females that gave birth to a litter or with pup/fetuses in utero

** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$$

* defined as the number of females that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of liveborn pups on the day of birth}} \times 100$$

* before standardisation of litters (i.e. before culling)

$$\textbf{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}}.$$

* after standardisation of litters (i.e. after culling)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \times 100$$

$$\textbf{Pre-implantation loss (\%)} = \frac{\text{number of corpora lutea – number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\textbf{Post-implantation loss (\%)} = \frac{\text{number of implantations – number of live foetuses}}{\text{number of implantation}} \times 100$$

APPENDIX IV: Standard FOB parameters

Observations	Parameters
Home cage observations	Posture, piloerection, gait abnormalities, involuntary motor movements, vocalisations and any other abnormalities
Handling observations	Ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, pupil size, pupil response, lacrimation, salivation, stains and any other abnormalities
Open field observations	Piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behaviour, gait abnormalities, vocalisations, arousal, rearing, defecation, urination and any other abnormalities
Sensory observations	Approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch, eye blink response, hindlimb extension, olfactory orientation
Neuromuscular observations	Hindlimb extensor strength, hindlimb foot splay, grip strength hind- and forelimb and rotarod performance
Physiological observations	Catalepsy, body temperature, bodyweight