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FOR AGRICULTURAL AND VETERINARY CHEMICALS

AUSTRALIA

CHEMICAL REVIEW PROGRAM

REVIEW OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS OF METHAMIDOPHOS

prepared by the

Office of Chemical Safety

of the

Department of Health and Ageing

Canberra

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ABBREVIATIONS

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight

bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume

mL	Millilitre
µL	Microlitre

Concentration

ppb	Parts per billion
ppm	Parts per million

Clinical chemistry, haematology

ACh	Acetylcholine
AChE	Acetylcholinesterase
A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BChE	Butyrylcholinesterase
BUN	Blood urea nitrogen
CE	Carboxylesterase
ChE	Cholinesterase
CPK	Creatine phosphatase (phosphokinase)
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NTE	Neuropathy target esterase
LNTE	Lymphocyte neuropathy target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine

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TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy

CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry

DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GLC	Gas liquid chromatography
HPLC	High pressure liquid chromatography
^{1}H-NMR	Proton nuclear magnetic spectroscopy
ITLC	Instant thin layer chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
RIA	Radioimmunoassay
SA	Specific activity (radioactivity)
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
GD	Gestation Day
GLP	Good Laboratory Practice
LD	Lactation Day
LOEL	Lowest Observed Effect Level
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OP	Organophosphorus pesticide
OPIDN	Organophosphate Induced Delayed Neurotoxicity
OPIDP	Organophosphate Induced Delayed Polyneuropathy
PND	Postnatal Day

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
APVMA	Australian Pesticides and Veterinary Medicines Authority
CAC	Codex Alimentarius Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
FAO	Food and Agriculture Organisation of the UN
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
NCI	National Cancer Institute
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
NOHSC	National Occupational Health and Safety Commission
NTP	National Toxicology Program
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

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EXECUTIVE SUMMARY

Methamidophos is an organophosphate insecticide for use in the control of a broad range of insects in food crops. In Australia, methamidophos is the active ingredient in 2 registered products and MRLs have been established in bananas, brassicas, citrus fruits, cotton seed, meat, eggs, lettuce, macadamia nuts, capsicums, potatoes, soya beans, sugar beet, tomatoes and tree tomatoes. Methamidophos is included in Schedule 7 of the SUSDP and has an ADI of 0.0006 mg/kg bw/d, based on a NOEL of 0.06 mg/kg bw/d for the inhibition of cholinesterase activity in a 1-yr dog study. As a consequence of its severe acute toxicity potential international trade of commodities treated with methamidophos are required to conform with the Prior Informed Consent (PIC) Procedure as agreed under the 1998 Rotterdam convention.

Methamidophos is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under Australia's Chemicals Review Program. Following the data call-in process a number of additional data submissions on the toxicology of methamidophos were received from industry and the public, and these data together with all previously submitted registration data and relevant published data, have been assessed in detail.

Methamidophos was nominated for review because of its high potential to cause toxicity by acute and chronic exposure and reports of human poisonings, some of which involved the development of organophosphate-induced delayed neuropathy (OPIDN). Furthermore, at the time of its nomination, the toxicology database on methamidophos contained a high proportion of studies that pre-dated good laboratory practice and agreed toxicological testing guidelines, including a number of potentially invalid studies. Following a review of all the toxicity data it was concluded that there should be no change to the approval status of methamidophos active constituent or to the ongoing registration of existing methamidophos products in Australia. The review recommended revision of the existing ADI and established an ARfD for the first time. The revised ADI of 0.0003 mg/kg bw/d was established on the same endpoint as previously but was based on the NOEL in a 2-month dietary study in rats and the application of a 100-fold safety factor. The ARfD of 0.003 mg/kg/bw was based on a NOEL of 0.3 mg/kg bw for inhibition of cholinesterase activity in an acute neurotoxicity study in rats and the application of a 100-fold safety factor.

Following a review of all the toxicity data it was concluded that there should be no change to the current SUSDP schedule entry for methamidophos. An amendment has been made to the Safety Directions, to accommodate the formulation types for products currently available in Australia.

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TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	Very rapid (T_{max} approx. 10 min.). Based on the low concentrations of radioactivity present in faeces (2-5%) after 48 h absorption was almost complete.
Distribution	Highest concentrations were detected in the liver.
Potential for accumulation	Very low.
Rate and extent of excretion	Rapidly excreted in urine (70-77%) and expired air (25%), most eliminated within 24 h.
Metabolism	Rapid; almost completely metabolised within 24 h; 6 metabolites identified in urine, accounting for 98% of the excreted radioactivity.
Toxicologically significant compounds (animals, plants and environment)	Methamidophos. Impurities in the active and plant metabolites no more acutely toxic than parent.

Acute toxicity (for 73-76% ai. except where otherwise indicated)

Rat oral LD_{50} (mg/kg bw)	15-19
Worst oral LD_{50} in other species	15 in mice
Rat dermal LD_{50} (mg/kg bw)	108
Worst dermal LD_{50} in other species	69 in rabbits
Rat inhalation LC_{50} (mg/m^3)	63, 377 (MMAD ~0.6 μm and <5 μm, respectively)
Worst inhalation LC_{50} in other species	Deaths observed at 30 mg/m^3 in mice
Skin irritation	No irritation observed at non-lethal doses
Eye irritation	Slight irritant
Skin sensitisation	None (Buehler)

Short-term toxicity

Target/critical effect	Cholinesterase inhibition (brain, RBC, plasma)
Lowest relevant oral NOEL (mg/kg bw/d)	0.03 (8-week rat feeding study), 0.04 (13-week dog feeding study)
Lowest relevant dermal NOEL (mg/kg bw/d)	1 (3-week rat study)
Lowest relevant inhalation NOEC (mg/m^3)	1 (13-week rat study; head-only exposure)

Genotoxicity	Not mutagenic; weak clastogen *in vitro* and *in vivo*

Long-term toxicity and carcinogenicity

Target/critical effect	Cholinesterase inhibition (brain, RBC, plasma)
Lowest relevant NOEL (mg/kg bw/d)	0.06 (1-y dietary study in dogs)

Carcinogenicity	No evidence of oncogenic potential

Reproductive toxicity

Reproduction target/critical effect	Reduced bw gain, survival to 21days and cholinesterase activity in neonates
Lowest relevant reproductive NOEL (mg/kg bw/d)	0.2 (2-generation study in rats)

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Developmental toxicity	
Developmental target/critical effect	Reduced litter and placental weights, increased incidence of skeletal variations at maternotoxic doses in rats.
Lowest relevant developmental NOEL (mg/kg bw/d)	0.14 (in rats)
Delayed neurotoxicity	Evidence of OPIDN and intermediate syndrome in humans. NTE inhibition observed in brain, spinal cord and sciatic nerve but no evidence of OPIDN at non-lethal doses in experimental animals.
Immunotoxicity	No data
Dermal absorption (*in vivo*)	~40% in rats at 5, 50 or 500 μg/cm^2 after 10 or 24 h. ~5% in humans at 3 μg a.i./cm^2/8 h with an EC formulation (Tamaron SL 600).

Summary	NOEL (mg/kg bw/d)	Study	Safety factor
ADI 0.0003 mg/kg bw/d [based on inhibition of ChE activity in plasma, RBC and brain]	0.03	2-mo dietary study in rats (Christenson *et al*, 1991)	100
ARfD 0.003 mg/kg bw [based on inhibition of ChE activity in RBC and brain]	0.3	Acute neurotoxicity study in rats (Sheets *et al*, 1994a)	100

SUMMARY TOXICOLOGY REPORT

Introduction

Methamidophos is an organophosphate insecticide, for use in the control of a broad range of insects in food crops. In Australia, methamidophos is the active ingredient in 2 registered products and MRLs have been established in bananas, brassicas, citrus fruits, cotton seed, meat, eggs, lettuce, macadamia nuts, capsicums, potatoes, soya beans, sugar beet, tomatoes and tree tomatoes. Methamidophos is included in Schedule 7 of the SUSDP and has an ADI of 0.0006 mg/kg bw/d, based on a NOEL of 0.06 mg/kg bw/d in a dog 1-yr study.

This report presents a re-evaluation of previously submitted data together with new data from a submission to the Office of Chemical Safety under the Chemical Review Program (CRP).

Metabolism and Toxicokinetics

In rats, orally administered methamidophos was rapidly and almost completely absorbed from the gastrointestinal tract. Methamidophos was distributed to all tissues, with highest concentrations in the liver and carcass and low levels in adipose tissue. In goats given 0.7 mg/d ^{14}C-labelled methamidophos po for 3 d, the distribution of radioactivity in tissues was highest in liver, kidney, muscle and fat. Amino acids were the main radiolabelled components

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in muscle, and non-saponifiables (e.g. cholesterol), free fatty acids and glycerol the main radiolabelled fractions in fat. Approximately 7.6% of the administered radioactivity was excreted in milk. Methamidophos showed low potential for accumulation in the tissues examined (Krautner, Marsh and Downs, 1997).

Urine was the major route of excretion; approximately 70-77% was excreted in urine and approximately 20% in faeces, mostly within 24 h. There is evidence that methamidophos is degraded to CO_2 and excreted *via* the lungs, but the pathway has not been elucidated, nor could the formation of volatile intermediates (e.g. methyl mercaptan, methane, dimethyl sulfide) in the biotransformation of methamidophos be demonstrated in rats given single oral doses of 1 mg/kg bw methamidophos (Klein, 1997).

Most methamidophos was metabolised within 24 h. At 24 h after oral administration of 15 mg/kg bw radiolabelled methamidophos to rats, only 1.4% of the dose was recovered as unchanged methamidophos. Major metabolites in urine were phosphoric acid (27.5%), O,S-dimethyl thiophosphoric acid (22.5%), O-methyl thiophosphoric acid amide (18%), S-methyl phosphoramidothioic acid (12.1%), S-methyl thiophosphoric acid (11.2%) and unknown (7.3%) (Fakhr, Abdel-Hamid & Afifi, 1982). By day 4, the majority of radioactivity was present as inorganic phosphate (Crossley & Tutass, 1969). Methamidophos is metabolised primarily by hydrolysis, with cleavage of the P-N bond yielding dimethyl phosphoric acid derivatives, as demonstrated by Tutass (1968)

Methamidophos crosses the placenta and is transferred in milk. Pregnant rats were administered 8 mg/kg bw ^{14}C-labelled methamidophos po on GD18. The concentration in placentae, foetuses and uteri, measured between 10 min and 48 h s were similar to maternal heart levels at 10 min, but declined rapidly so that at 48 h, foetal levels were 1/10th of maternal heart levels. Methamidophos was transferred in the milk of lactating rats; approximately 2% was recovered in the offspring of lactating rats given a single dose of 8 mg/kg bw (Salama, 1990). Goats given 3.75 mg/d ^{14}C-labelled methamidophos po for 7 days excreted 18% *via* urine, 5% *via* faeces and 3% *via* milk, with the balance (25%) presumed to have been *via* expired air (Lee & Crossley, 1972).

In vitro studies showed that the permeability of rat skin to methamidophos was higher than that of human skin. The cumulative *in vitro* penetration of human skin by methamidophos technical (0.03, 0.9 or 9.4 mg/cm^2) or methamidophos formulated as Tamaron SL 600 concentrate (0.02 or 7.7 mg/cm^2) during 24 h continuous exposure did not exceed 1% of the applied dose. When applied to rat skin, the cumulative *in vitro* penetration reached a maximum of 9.8% of the applied dose at 0.9 mg/cm^2 methamidophos technical and 29.7% of the applied dose for the formulation concentrate (up to 121 times greater than penetration of human skin). The enhanced penetration of methamidophos across rat skin when formulated as Tamaron SL 600 may be due to the excipients in the formulation (van de Sandt *et al*, 1998).

In vivo dermal absorption of methamidophos was greater in rats than humans. Following application to rat skin at 0.005, 0.05 or 0.5 mg/cm^2 (0.2, 2 or 17 mg/kg bw), a maximum of 15-20% of the administered dose was absorbed at 2 h, 36-42% at 10 h and 40-44% at 24 h (Bagos et al, 1991a & 1991b). The amount of radioactivity absorbed following a single 8 h dermal exposure to 240 µg ^{14}C-labelled methamidophos formulated as Tamaron SL 600, was 30%, 11% and 5% in rats, monkeys and humans, respectively (Fuller *et al*, 2000; Selim, Van Lier and Verheggen, 2000).

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Acute Studies

Technical methamidophos (73-76% purity) has high acute oral toxicity in laboratory animals. The oral LD_{50}s in rats and mice ranged between 15 and 33 mg/kg bw. In rats and mice, the LD_{50}s were slightly higher in non-fasting animals than in fasting animals (Mihail, 1981b,c). The median lethal doses were similar for a range of methamidophos purities between 73% and 95%, and there was no difference in the acute oral toxicity of enantiomers compared with racemic methamidophos (Flucke *et al*, 1990b). In guinea pigs, rabbits, cats and dogs the lethal dose was within the range for mice and rats. In general, the signs of acute methamidophos toxicity were similar in all species and are typical of organophosphate poisoning. Clinical signs occurred within 5-10 minutes and included tremors, salivation, dyspnoea and, rarely, clonic convulsions. Animals that died had pulmonary congestion and oedema, and distension of the stomach and intestines with gas. Surviving animals recovered completely within 7 d. (eg. Cavalli, Hallesy & Spence, 1968d).

Methamidophos (73-76% purity) has high acute dermal toxicity in rats (LD_{50}s 108-179 mg/kg bw) and rabbits (LD_{50} 69-122 mg/kg bw). Shortly after application, rabbits had clinical signs of salivation, ataxia and tremors, and deaths usually occurred within 24 h (Rittenhouse & MacGregor, 1977a). However, in a dermal irritation study by Levy, Wong & MacGregor (1979), after application of 0.1 mL methamidophos (approx. 30 mg/kg bw), rabbits had increased respiration, ataxia, salivation, miosis, tremors, diarrhoea and collapse, and 5/9 rabbits died within 24 h of application of the test material, suggesting that the LD_{50} in rabbits may be similar to other species.

The inhalational toxicity of methamidophos (73-76% purity) was also high in rats (LC_{50}s 63 mg/m^3/4 h; MMAD ~0.6 µm, 377 mg/m^3/1 h; MMAD <5 µm). In mice and rabbits, deaths were recorded at 30 mg/m^3. In guinea pigs, no deaths occurred following exposure to 30 mg/m^3/4 h or 102 mg/m^3/1 h. Dermal application of 0.5 mL methamidophos technical to intact or abraded skin of rabbits caused slight to well-defined erythema which resolved by 72 h in survivors (Rittenhouse & MacGregor, 1977a). Application of 0.1 mL methamidophos technical to intact or abraded skin of rabbits caused slight to well-defined erythema and slight oedema which resolved by 72 h in intact skin and by 96 h in abraded skin (Levy, Wong & MacGregor, 1979). In studies in which no deaths occurred, no signs of irritation were observed in rabbits following application of 0.003 mL methamidophos technical to the intact or abraded skin for 24 h (Hixson *et al*, 1980b), or following application of 0.5 mL methamidophos technical to intact skin for 4 h (Martins, Machemer & Pauluhn, 1990). Eye irritation studies showed that methamidophos is a slight eye irritant. Application of 0.1 mL methamidophos technical (74.2% ai.) caused slight conjunctival erythema and discharge which resolved by 72 h (Martins, Machemer & Pauluhn, 1990). Application of 0.01 mL methamidophos technical (73.2% ai.) to the rabbit eye caused conjunctival erythema, chemosis and discharge which had not resolved by d 13, and iritis lasting 4 days. Washing the eye did not reduce the severity or duration of the irritation (Hixson *et al*, 1980b). In another study, application of 0.1 mL methamidophos technical, of unspecified purity, caused conjunctivitis, iritis and corneal opacity which had not fully resolved until d 14 (Rittenhouse & MacGregor, 1977b); this study was considered unsuitable for regulatory purposes because it was not GLP-compliant and the purity of the methamidophos was not given. Methamidophos technical did not cause skin sensitisation in guinea pigs in modified Buehler test (Korenga *et al*, 1984) and a 42.7% LC formulation (in propylene glycol) was negative in a standard Buehler test (Porter *et al*, 1987).

Short-Term Repeat-Dose Studies

In a pilot study, mice were fed methamidophos technical (75% ai.) in the diet at 0, 2, 10, 50 and 100 ppm for 6 wk. Plasma, RBC and brain ChE activity (tested at weeks 1, 5 and 6) was

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inhibited in all treatment groups, by approximately 20% at 2 ppm (0.3 mg/kg bw/d) and 90% at 50 ppm (15 mg/kg bw/d) (Foster *et al*, 1980).

In a pilot study, rats were fed 0, 1, 2, 4, 8, 16, 32 or 64 ppm methamidophos technical (75% ai.) in the diet for 34 days. There were no deaths or clinical signs. Bodyweight gains were reduced by 40% in the males at 64 ppm. Plasma, RBC and brain ChE activities were significantly inhibited at 4 ppm and above, and the effects were greater in females than in males. The NOEL was 2 ppm (0.2 mg/kg bw/d) based on inhibition of plasma, RBC and brain ChE activity at higher doses (Abernathy *et al*, 1980).

Rats were fed methamidophos technical (77.68% ai.) at 0, 0.5, 1, 2 or 4 ppm in diet for 8 wk. There were no deaths or clinical signs. Plasma, RBC and brain ChE activities were inhibited at 1 ppm and above. The degree of inhibition did not change substantially after d 14, and females were more affected than males. The NOEL for ChE inhibition was 0.5 ppm (0.03 mg/kg bw/d) (Christenson *et al*, 1991).

Rats received dermal applications of methamidophos technical (76.9% ai.) at 0, 1, 15 or 50 mg/kg bw/d, 6 h/d for 21 d. There were no deaths or clinical signs. Male rats had reduced bodyweight gain at 15 and 50 mg/kg bw/d. Plasma, RBC and brain ChE activities were inhibited by 25-55% at 15 mg/kg bw/d and by 60-75% at 50 mg/kg bw/d. The NOEL for ChE inhibition was 1 mg/kg bw/d (Sheets, Gastner & Hamilton, 1997).

Rabbits received dermal applications of methamidophos technical (64.5% ai.) at 0, 0.5 or 5 mg/kg bw/d, 6 h/d, 5 d/wk for 3 wks. Although there were no deaths or clinical signs, plasma and RBC ChE activities were inhibited by 11-44% at 5 mg/kg bw/d The NOEL was 0.5 mg/kg bw/d (Heimann, Nash & Lorke, 1981b).

Rats received inhalation (head-only) exposure to 0, 1.4, 5.4 or 33 mg/m^3 methamidophos aerosol (75.7 ai.), 6 h/d for 5 d. Although there were no deaths at any concentration, clinical signs in rats at 33 mg/m^3 included tremors, ungroomed coats, reduced mobility and weakness of the rear extremities. Plasma, RBC and brain ChE activities were inhibited by ≤13% at 5.4 mg/m^3 and by 20% (RBC), 83% (plasma) and 67% (brain) at 33 mg/m^3. The NOEC for ChE inhibition was 5.4 mg/m^3 (Pauluhn & Machemer, 1987a).

Rats received inhalation (head-only) exposure to 2.6, 12 or 48 mg/m^3 methamidophos aerosol (73% ai.), 6 h/d, 5 d/wk for 3 wks. Although there were no deaths at any concentration, clinical signs in rats at 48 mg/m^3 included tremors, ungroomed coats and reduced mobility. Bodyweight gains were reduced in males at 48 mg/m^3. At 2.6 mg/m^3, plasma ChE was inhibited by 21% in females. At 12 mg/m^3, ChE was inhibited by 26% and 66% in males and females, respectively, in plasma and, in the females, by 21% in RBC and 39% in brain. At 48 mg/m^3 ChE activities were significantly inhibited in plasma (m 72%, f 88%) and brain (m 62%, f 68%). A NOEC could not be established in this study, due to ChE inhibition in plasma at the lowest test concentration (Pauluhn & Machemer, 1987b).

Subchronic Studies

Rats were fed methamidophos technical (50% ai.) at 0, 2, 6, 20 or 60 ppm in the diet for 3 months. There were no treatment-related deaths. At 60 ppm, there were clinical signs (slightly reduced activity and weakness) and bodyweight gains were slightly reduced. Plasma and RBC

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ChE activity were inhibited at 6 ppm (about 15-20%) and above. The NOEL was 2 ppm (approx. 0.3 mg/kg bw/d) (Loser & Lorke, 1970a; Groning & Lorke, 1976a).

In a published study, rats were fed methamidophos (90.4% purity) (0, 1, 2.5, 5, 10 or 20 ppm) in the diet for 3 months. Clinical signs at 20 ppm included reduced activity, ruffled coat and huddled posture. Whole blood ChE activity was inhibited in male rats at 2.5 ppm (≥20%). (Guo *et al*, 1986d).

Dogs were fed methamidophos (0, 1.5, 5 or 15 ppm) in the diet for 3 months. There were no deaths and no clinical signs were observed. Plasma and RBC ChE activity were inhibited (≥20%) at 5 and 15 ppm. The NOEL was 1.5 ppm (0.04 mg/kg bw/d) (Loser & Lorke, 1970b; Groning & Lorke, 1976b).

Rats received inhalational (head only) exposure to methamidophos (73.4% ai.) at 1, 5.4 or 23 mg/m^3 6 h/d, 5 d/wk) for 13 wks. There were no deaths. Rats at 23 mg/m^3 had slight to moderate muscle tremors during the entire exposure period, and decreased bodyweight gain in the males during wks 2-13 and females during wks 4-11. Statistically significant inhibition of RBC, plasma and brain ChE activity was observed at 5.4 and 23 mg/m^3. The NOEC was 1.0 mg/m^3. (Pauluhn & Machemer, 1988 & 1990).

Chronic Studies

In an oncogenicity study, mice were fed 0, 1, 5 or 25 ppm methamidophos technical (70% ai.) in the diet for 2 years. There were no treatment-related deaths or clinical signs. Food consumption and bodyweight gain were each reduced by ≤10% at 25 ppm. There was no effect of treatment on the incidence of neoplasms. In a study in which no clinical chemistry or urinalysis measurements were performed, the NOEL was 5 ppm (0.7 or 0.8 mg/kg bw/d in males and females, respectively), based on decreased food consumption and bodyweight gain at the higher dietary concentrations (Hayes *et al*, 1984a).

In a chronic toxicity/oncogenicity study, rats were fed 0, 2, 6, 18 or 54 ppm methamidophos technical (70% ai.) in the diet for 2 years. There were no treatment-related deaths and the only clinical signs were increased urination at 18 and 54 ppm. Bodyweight gains were reduced in males at 18 ppm and both sexes at 54 ppm. There was no effect of treatment on the incidence of neoplasms. A dose-related inhibition of ChE activity was observed in treated rats, which was significant (≥ 20%) in the plasma, RBC and brains of males and females at 6 ppm and above. On this basis, the NOEL was 2 ppm (0.09 and 0.11 mg/kg bw/d for males and females respectively) (Hayes *et al*, 1984b).

In a chronic toxicity study, dogs were fed 0, 2, 8 or 32 ppm methamidophos technical (71.5% ai.) in the diet for 12 months. There were no treatment-related deaths or clinical signs. A dose-related inhibition of ChE activity was observed in all treatment groups after the first 2 wks of treatment. The degree of inhibition was significant (≥ 20%) in the plasma, RBC and brain of males and females at 8 ppm and above. On the basis of significant (≥ 20%) plasma, RBC and brain ChE inhibition at 8 ppm (0.22 mg/kg bw/d), a NOEL of 2 ppm (0.06 mg/kg bw/d) was established (Hayes *et al*, 1984c).

Reproductive Studies

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Rats were fed 0, 3, 10 or 33 ppm methamidophos technical (70.5% ai.) in the diet from 100 days prior to mating throughout 2 generations. At 33 ppm, the parenteral generations had reduced premating bw and reduced gestational and lactational bw gain. Lactational bw gain was also reduced in the F_{2a} and F_{2b} pups. The production of F_{2b} litters was further characterised by a decreased proportion of spermatozoa-positive dams that delivered live pups, reduced litter size at birth, and reduced viability and lactation indices. The NOEL for parental toxicity and reproductive toxicity was 10 ppm (0.5 mg/kg bw/d), based on reduced bodyweight gain in the parents and pups, and reduced viability in the offspring at higher doses (Hixson *et al*, 1984b).

Rats were fed 0, 1, 10 or 30 ppm methamidophos technical (73% ai.) in the diet from 140 (m) or 70 (f) days prior to mating, throughout 2 generations. In adults, methamidophos caused a deficit in premating bw in F_0 males at 30 ppm and in F_1 males and females at 10 and 30 ppm. Maternal bw gain during gestation of the F_{2a} and F_{2b} litters was decreased at 30 ppm. During lactation, maternal food consumption was reduced at 30 ppm and maternal bw was decreased at 10 and 30 ppm. In neonates, bw and/or bw gain were decreased at 10 and 30 ppm. Survival of pups to 21 days was decreased for the F_0/F_{1a}, F_0/F_{1b} and F_1/F_{2b} generations at 30 ppm. In adults, statistically significant inhibition of RBC ChE occurred at 1 ppm in F_0 and F_1 males at termination. At 10 and 30 ppm, plasma, RBC and brain ChE inhibition was observed in both sexes of F_0 and F_1 adults at 8 wk and study termination. In neonates, plasma, RBC and brain ChE were consistently inhibited to a statistically significant extent at 10 and 30 ppm. A NOEL for parental toxicity was not established due to the RBC ChE inhibition in males at 1 ppm. The NOEL for reproductive toxicity was 10 ppm (0.2 mg/kg bw/d), based on reduced bodyweight gain and inhibition of ChE activity in pups at higher doses (Eigenberg, Freshwater & Lake, 1998; Astroff & Eigenberg, 1998; Moore, 1999).

Developmental Studies

In a dose-ranging study, pregnant rats were administered methamidophos technical (75% ai.) at 0, 0.1, 0.3, 1, 3.3 or 10 mg/kg bw/d, by gavage, during gestation days 6-20. There were no deaths. Clinical signs (twitching, salivation, lacrimation) were observed at 3.3 and 10 mg/kg bw/d. There was a dose-related decrease in maternal bodyweight gain (20% at 0.1 mg/kg bw/d and 50% at 10 mg/kg bw/d), and mean foetal bw was reduced by about 20% at 10 mg/kg bw/d. (Breeden *et al*, 1980).

Mated female rats were administered methamidophos technical (70.5% ai.) at 0, 0.3, 1 or 3 mg/kg bw/d, by gavage, during gestation days 6-15. There were no deaths. Clinical signs (twitching, hyperactivity, salivation, lacrimation and excessive urination) were observed in all rats at 3 mg/kg bw/d, starting within the first 2 days of treatment and persisting until GD20. At 3 mg/kg bw/d, maternal food consumption and bodyweight gain were reduced by about 40% and mean foetal bodyweights were about 6% lower than controls. The NOEL for maternal and developmental toxicity was 1 mg/kg/day (Hixson, Mallicot & Lamb, 1984a).

In a published study, mated female rats were administered methamidophos (90.4% purity) at 0, 0.1, 0.5 or 2 mg/kg bw/d, by gavage, during gestation days 6-15. Unspecified clinical signs were observed in an unspecified number of rats at 2 mg/kg bw/d. At 2 mg/kg bw/d, maternal food consumption and bodyweight gain were reduced by about 40% and mean foetal bodyweights were about 6% lower than controls (Guo *et al*, 1986g).

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In a published study, mated female rats were administered methamidophos (60% ai.) at 0, 1 or 2 mg/kg bw/d, by gavage, during gestation days 6-15. There was a significant dose-related increase in the incidence of embryo lethality, malformed foetuses, anencephaly and anotia and a dose-related decrease in the number and viability of foetuses. Mean foetal bw was depressed by about 40% in both treated groups. Cyanosis and congestion were observed in foetuses of dams at 2.0 mg/kg (Hanafy, Atta & Hashim, 1986a).

In a dose-ranging study, mated female rats were administered methamidophos technical (75.6% ai.) at 0, 0.04, 0.1, 1 or 4 mg/kg bw/d, by gavage, during gestation days 6-20. At 4 mg/kg bw/d, maternal food consumption was reduced, there was significant mean maternal bw loss and mean litter weights were significantly less than controls. There was a dose-related inhibition of plasma, RBC and brain ChE activities which was >20% at ≥0.1 mg/kg bw/d (plasma) and ≥1 mg/kg bw/d (RBC and brain). There were no other treatment-related effects on developmental or reproductive parameters and no malformations or variations were observed in any litter (Astroff *et al*, 1996a).

In the main study, mated female rats were administered methamidophos technical (75.9% ai.) at 0, 0.04, 0.1 or 4 mg/kg bw/d, by gavage, during gestation days 6-15. The analytical doses were 0, 0.05, 0.14 and 5.49 mg/kg bw/d. There were no deaths. At 4 mg/kg bw/d, clinical signs (muscle twitching, tremors and salivation) were observed in the dams for a few hours after dosing, food consumption was reduced and there was significant maternal bw loss. Maternal plasma, RBC and brain ChE activities were inhibited by 80-90% at 4 mg/kg bw/d. At 4 mg/kg bw/d, mean litter and placental weights were significantly less than controls, and there was an increased incidence of skeletal variations (incomplete or unossified bones). No malformations or variations were observed in any litter. The maternal and developmental NOEL was 0.14 mg/kg bw/d (Astroff *et al*, 1996b).

Inseminated female rabbits were administered methamidophos technical (62% ai.) at 0, 0.1, 0.5 or 2.5 mg/kg bw/d, by gavage, during gestation days 6-18. There were no treatment-related deaths. Maternal bw gain was reduced by 30-50% in all treatment groups, but there were no maternal clinical signs and there was no effect of treatment on foetal development. No malformations or variations were observed in any litter (Machemer & Lorke, 1979). A maternal NOEL was not established. The developmental NOEL was 2.5 mg/kg bw/d

Inseminated female rabbits were administered methamidophos technical (76% ai.) at 0, 0.1, 0.5 or 2.5 mg/kg bw/d, by gavage, during gestation days 6-18. The analytical doses were 0, 0.2, 0.65 or 2.47 mg/kg bw/d. There were no treatment-related deaths. At 2.5 mg/kg bw/d, hyperactivity was observed at 2-4 h after treatment. Maternal bw gain was reduced by 20% at 2.5 mg/kg bw/d and 13% at 0.5 mg/kg bw/d in all treatment groups, but there was no effect of treatment on foetal development. No malformations or variations were observed in any litter. The NOEL for maternal toxicity was 0.2 mg/kg bw/d. The developmental NOEL was 2.5 mg/kg bw/d (Hoberman *et al*, 1996).

Genotoxicity Studies

Gene Mutation in bacteria, fungi and cultured mammalian cells

Several reverse mutation assays in bacteria and forward mutation assays in mammalian cells were uniformly negative, with and without metabolic activation. There were no significant increases in the numbers of revertant colonies of *S. typhimurium* at up to 5, 10 or 12.5

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mg/plate methamidophos (Herbold & Lorke, 1980a; Machado *et al*, 1982; Guo *et al*, 1986g; Herbold, 1994). The HGPRT locus forward mutation assay, using Chinese hamster ovary cells, was negative at up to 0.2 μL/mL (Harbell *et al*, 1990) and up to 5000 μg/mL (Bigger *et a.*, 1993)

DNA damage and repair

In vitro tests for DNA damage were negative at up to 10000 μg methamidophos in a Pol test, using *E coli* (Herbold & Lorke, 1983) and at ≤1.0 μL/mL in an assay for unscheduled DNA synthesis using primary rat hepatocytes (Curren *et al*, 1988). *In vivo* methylation of guanine (7- methylguanine) was demonstrated in male mice given 1.6, 4.4 and 5.3 mg/kg bw ^{14}C-methamidophos (Zayed & Mahdi, 1987).

Chromosomal effect assays (in vitro)

Methamidophos caused chromosomal aberrations in mouse spleen cells at concentrations of 0.25, 0.5, 1.0, 2.0 μg/mL (Amer & Sayed, 1987). Equivocal results were obtained in the red muntjac lung fibroblast line (KIZ-7901) at 4.2, 14.1, 42.3 μg/mL (Guo *et al.*, 1986g); there was no effect on chromosome aberration rate but the number of aberrant types increased - chromatid interchanges, multicentric fragments and disintegration fragments appearing in addition to simple fragments and fragment pairs. Equivocal results were also obtained in Chinese hamster ovary (CHO) cells. Methamidophos (up to 5000 μg/mL) was negative in tests with metabolic activation but equivocal results were obtained without activation (Murli & Spicer, 1990). Tests for sister chromatid exchange (SCE) inV79 cells at up to 80 μg/mL were negative with metabolic activation (Chen *et al*, 1982a) and equivocal without metabolic activation; there was no increase in the number of SCE per cell but a cell-cycle delay was seen at all doses after 27 h of treatment (Chen *et al*, 1982b). SCE assays in Red muntjac (*Muntiacus muntjak*) diploid cells at up to 42.3 μg/mL (He *et al*, 1982; Guo *et al*, 1986g) or White Swiss mouse spleen cells at up to 2.0 μg/mL (Amer & Sayed, 1987) were weakly positive in the absence of metabolic activation.

Gene mutation in vivo

Dominant lethal mutation tests were negative in mice administered methamidophos as a single oral dose of 5 mg/kg bw (Herbold & Lorke, 1980b), or up to 8.9 mg/kg bw/d po for 5days (Eisenlord *et al*, 1984), or up to 2 mg/kg bw/d po to males for 7 d pre-mating (Guo *et al*, 1986g).

Chromosomal Effect Assays (in vivo)

Micronucleus tests were negative in mice administered methamidophos as a single doses of 5 or 10 mg/kg bw po (Herbold & Lorke, 1981), up to 12 mg/kg bw po (Esber & Carver, 1983), 5 mg/kg sc (Guo *et al.*, 1986g), or 8 mg/kg bw ip (Herbold, 1996). However, Amer & Sayed (1987) obtained weakly positive results in micronucleus tests in mice administered single doses of 6 mg/kg bw ip, 4.5 mg/kg bw 2/wk for 2 wk ip, 50 or 100 ppm in the diet, or dermal applications of 12 or 24 mg/kg bw 2/wk for 2 wk.

Amer & Sayed (1987) also obtained weakly positive results in *in vivo* tests for sister chromatid exchange in mice given 6 or 8 mg/kg bw ip. Other tests for nucleated marrow cells

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and chromosome aberrations were negative at maximum doses of 3 mg/kg bw po x 2 and 5 mg/kg sc x 2 at 24 h intervals in mice 20 ppm in the diet of rats for 12 wk (Guo *et al*, 1986g).

Neurotoxicity Studies

Hens

In an acute toxicity/delayed neurotoxicity study, White Leghorn hens were administered methamidophos technical (74% ai.) by gavage at 0, 30, 34 and 51 mg/kg bw on days 0 and 21, simultaneously with 50 mg/kg bw im atropine. The doses of the test substance equalled or exceeded its LD_{50} in this strain of hen (30 mg/kg po), established in the first part of this study. The incidence of mortality was positively correlated with dose, while the latency of death decreased with increasing dose. Unspecified signs of acute toxicity occurred "in most cases" within the test groups but disappeared within 24 h. A "few" hens vomited following treatment. No signs of delayed neurotoxicity were observed in treated hens and there were no significant histopathological findings (Kruckenberg *et al*, 1979).

Thyssen, Eben & Lorke (1982) carried out four separate studies: a pilot study using methamidophos technical (74.6% ai.) at 0 or 30 mg/kg bw/d for 5 d; clinical and neurotoxic esterase (NTE) determinations using 0 or 25 mg/kg bw/d for 5 d; clinical determinations using 0, 30 or 35 mg/kg bw/d for 5 d, repeated after 10 d; and clinical and NTE determinations using Tamaron™ Fl (52.4% methamidophos technical in glycol monomethyl ether) at 35.7 mg/kg bw/d for 5 d po (equivalent to 25 mg/kg bw/d for 5 d po of pure methamidophos technical). The substances were administered to White Leghorn hens by gavage. Atropine sulfate at 50 mg/kg bw im was simultaneously administered with each treatment. At 25 and 30 mg/kg bw/d, deaths occurred and hens showed acute cholinergic signs which persisted for 9-10 d after the last treatment. There was severe (>60%) NTE inhibition in brain, spinal cord and sciatic nerve for 5 d after the end of treatment, still evident in brain at 38 d after treatment with methamidophos technical and in sciatic nerve 38 d after treatment with Tamaron™ Fl. However, neither methamidophos technical nor Tamaron™ Fl caused clinical signs of delayed neurotoxicity in chickens.

White Leghorn hens received 2 topical applications, 3 weeks apart, of 200 mg/kg bw Methamidophos technical (73.3% ai.) applied to the comb. Atropine sulfate was given at 50 mg/kg bw im immediately before application of the test substance and sc at the end of the application and on the two following days. After both administrations of test-substance all hens showed clinical signs (staggering gait, lassitude (drooping wings), reduced mobility extending to prostration, fluffed plumage, dyspnoea and apathy), persisting for 10 min to 7 d.. The mortality rate after the first and second administrations were 33% and 5%, respectively. A decline in bw at wk 1 and 4 in the test substance treated hens was probably treatment-related. Signs of delayed neurotoxicity were not observed and brain and nerve histology was unremarkable (Flucke, Kaliner & Machemer, 1985b). In a second study (Flucke *et al*, 1988), a dermal dose of 200 mg/kg bw methamidophos technical (73% ai.) to hens caused biologically significant inhibition of brain and spinal cord NTE activity that did not reverse fully within 7 d of treatment. However the degree of inhibition observed (up to 61% in brain and 46% in spinal cord) appeared to be insufficient to cause delayed neurotoxicity.

In a test for delayed neurotoxicity, White Leghorn hens received 50 mg/kg bw po Methamidophos (90.4% purity). The hens were protected with 5 mg/kg bw atropine sulfate im and 25 mg/kg bw pralidoxime iodide im 15 min before and 3 and 6 h after dosing with

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methamidophos. No clinical signs of delayed neurotoxicity or neurohistological abnormalities were observed in methamidophos treated hens (Guo *et al*, 1986e).

In a 3-month subchronic delayed neurotoxicity study, methamidophos technical (76% purity) was administered by gavage to White Leghorn hens at 0, 0.3, 1, 3 mg/kg bw/d for 5 d/wk for 3 mo po. Hens at 3 mg/kg showed somnolence and slight emaciation from d 10 onwards, but there was no sign of ataxia or changes in motor activity or locomotion co-ordination, and there were no treatment-related deaths. At 3 mg/kg the mean bw of hens was statistically significantly lower than that of controls from wk 3 to wk 14. There were no signs of delayed neuropathy. At 1 and 3 mg/kg there was a treatment-related and dose-dependent inhibition of plasma butyryl ChE (BuChE) and spinal cord neuropathy target esterase (NTE). Brain NTE activity was statistically significantly less (17%) than controls at 3 mg/kg. The highest degree of NTE inhibition recorded was 42% in spinal cord at 12 wk at 3 mg/kg bw/d. There were no remarkable gross necropsy or histopathological findings. On the basis of bw depression, clinical signs, biologically significant inhibition (≥ 20% inhibition) of plasma BuChE activity and NTE inhibition at 1 mg/kg bw/d and above, this study established a NOEL for neurotoxicity of 0.3 mg/kg bw/d (Sachsse et al, 1987).

In an exploratory study on the potential for methamidophos racemate and enantiomers to induce OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy), White Leghorn hens were given single doses of 100, 200 or 400 mg/kg bw methamidophos racemate or enantiomers, by gavage. Atropine sulfate and a combination of atropine sulfate and pralidoxime chloride (2-PAM) were administered concurrently. Clinical signs and deaths occurred in each treatment group; however, the variation between antidote regimes chosen for each treatment group, preclude any meaningful comparison. All (+)-methamidophos groups lost bw, and the hens at 400 mg/kg bw did not fully recover their initial bw at termination on day 28. The hens treated with (-)-methamidophos or the racemate at 400 mg/kg bw also lost bw but returned to their initial bw at termination. The (+)- enantiomer caused low intensity, reversible, delayed neurotoxicity at 200 mg/kg bw and marked, irreversible, delayed neurotoxicity at 400 mg/kg bw. Racemate methamisophos also induced low to moderate intensity delayed neurotoxicity at 400 mg/kg bw. There was no evidence of OPIDP following oral dosing with 400 mg/kg bw (-)-methamidophos. There were no gross lesions at termination in any group (Flucke *et al*, 1990b).

In a study of the effect on NTE in hens following oral administration of methamidophos racemate and enantiomers, White Leghorn hens received single oral gavage doses of methamidophos racemate and enantiomers (95.5% purity) at 50, 100, 200, 300 and 400 mg/kg bw. Atropine sulfate and a combination of atropine sulfate and pralidoxime chloride (2-PAM) were administered concurrently. The inhibition of NTE by (+)-methamidophos was similar to the racemate and was substantially greater than (-)-methamidophos. Reactivation of inhibited NTE by (+)-methamidophos occurred to a greater extent than (-)-methamidophos and was independent of the extent of inhibition and the time after dosing. Lymphocyte NTE inhibition was completely reversible after 7 d (Flucke, Eben & Machemer, 1990c).

In a subchronic dermal neurotoxicity study, White Leghorn hens received methamidophos technical (76.3% ai.) at 0, 0.5, 1.5, 4.5 mg/kg bw, applied topically to the comb, 1/d 5 times/wk. Treatment-related signs were observed at 4.5 mg/kg bw. All hens exhibited apathy, ruffled feathers and staggering gait from 5 h after the 1st treatment to termination. Reduced food consumption and disturbances of the gastrointestinal tract (discoloured faeces, diarrhoea) were also noted at unspecified incidences at variable times during treatment. Mean bws were

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reduced at 4.5 mg/kg bw by up to 20% over the treatment period but fully reversed during the recovery period. At 4.5 mg/kg, low level NTE inhibition was observed in brain and spinal cord at 4 and 13 wks, ranging from 7.8% to 21.4%. Inhibition (about 40%) of plasma ChE occurred at 4.5 mg/kg bw, but this had fully reversed by the end of the recovery period. There was no evidence of delayed neurotoxicity in any group, and there were no treatment-related gross pathological or histopathological findings at necropsy. On the basis of biologically significant inhibition (≥ 20%) of plasma ChE, a NOEL was established at 1.5 mg/kg bw/d (Bomann *et al*, 1992).

Lotti *et al* (1995b) reported comparative biochemical and clinical studies in hens treated with single doses of R-(±), D-(+) and L-(-) methamidophos (>98% purity) to elucidate which of them initiates, protects from or promotes OPIDP. Hens received pre-treatment with atropine sulfate (20 mg/kg bw ip) and then single gavage doses of 50 or 120 mg/kg bw R-(±) methamidophos, 25 or 60 mg/kg bw D-(+) methamidophos, or 60 or 120 mg/kg bw L-(-) methamidophos. D-(+) methamidophos was a less potent inhibitor of AChE than L-(-) methamidophos but was a more potent inhibitor of NTE. The time-course of AChE and NTE inhibition in brain, spinal cord and sciatic nerve over 1-13 d was determined, using doses that gave comparable inhibition. With both enantiomers, maximum AChE inhibition occurred during days 1-2. Following dosing with D-(+) methamidophos, AChE activity was fully restored by d 4. However, following dosing with L-(-) methamidophos, restoration of AChE activity was slower, reaching to 70-77% of control activity by d 13. Inhibition of NTE activity by D-(+) and L-(-) methamidophos was maximal at d 1 and d 4, respectively, and restoration of activity was somewhat slower in L-(-) methamidophos-treated hens. A single dose of 120 mg/kg po of R-(±) methamidophos induced 72-76% NTE inhibition at 24 h after dosing, but no clinical signs of unpromoted OPIDP were observed at 15-20 d after dosing. On this basis, the minimum neuropathic dose of R-(±) methamidophos was >120 mg/kg bw po. A single dose of 60 mg/kg bw po D-(+) methamidophos induced 81-83% NTE inhibition at 24 h after dosing. Mild unpromoted clinical signs of OPIDP were observed at 15-20 d after dosing. On the basis of these clinical signs, the minimum neuropathic dose for D-(+) methamidophos was considered to be 60 mg/kg bw po. A single dose of 120 mg/kg bw po L-(-) methamidophos induced 79-91% NTE inhibition at 96 h after dosing. Mild unpromoted clinical signs of OPIDP were observed 15-20 d after dosing. On the basis of these signs the minimum neuropathic dose for L-(-) methamidophos was considered to be 120 mg/kg bw po.

Kellner, Sanborn & Wilson (2000) examined the effect of impurities and chirality on methamidophos-induced NTE aging, *in vitro* and *in vivo*. Hens received 35 mg/kg bw im technical methamidophos (73.1% purity), 30 mg/kg bw im analytical methamidophos (99.4% purity), 25 mg/kg bw D-(+) methamidophos or 25 mg/kg bw L-(-) methamidophos. All birds were dosed with 20 mg/kg bw atropine and 50 mg/kg bw 2-PAM im before being dosed with methamidophos. *In vivo*, methamidophos technical (73.1% purity) and methamidophos analytical (99.4% purity) showed a similar capacity to inhibit NTE (61 and 58% total inhibition respectively) within 24 h of dosing. Methamidophos technical produced a significantly higher proportion of aged-inhibited NTE (18%) than methamidophos analytical (6%). *In vitro*, methamidophos technical and methamidophos analytical also showed a similar capacity to inhibit NTE in chick embryo brain after 1 h of incubation. The total inhibition of NTE by methamidophos technical was not affected by metabolic activation (60% with metabolic activation, 64% without) but the proportion of aged-inhibited NTE formed with metabolic activation (11%) was twice as high as that formed without activation (5%). The proportion of aged-inhibited NTE formed with methamidophos analytical was not affected by metabolic activation (5% with or without). The *in vitro* studies suggested that bioactivation

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was required for the enhanced aging potential of methamidophos technical. The enhancement of the NTE aging potential of methamidophos technical *in vivo* and *in vitro* was attributed to the presence of impurities. *In vivo*, the capacity of D-(+) methamidophos to inhibit NTE was 2.8 times greater than that of L-(-) methamidophos within 24 h of dosing (39 and 14% total inhibition respectively). For D-(+) and L-(-) the proportions of aged-inhibited NTE were 10% and 14% respectively. *In vitro*, the capacity of D-(+) methamidophos to inhibit NTE was 2.2 times greater than that of L-(-) methamidophos within 1 h of incubation (73 and 33% total inhibition respectively). For D-(+) and L-(-) the proportions of aged-inhibited NTE were 4 and 6% respectively. Metabolic activation did not influence these proportions. Metabolic activation had no discernible effect on the NTE inhibition/aging potential of analytical racemic methamidophos or the resolved isomers suggesting that, under the conditions of this study, they were poor substrates for microsomal mixed-function oxidase systems and for glutathione-dependent transferases.

In an acute oral neurotoxicity screening study with technical grade methamidophos (75.6% purity), rats received single doses of 0, 1, 3 or 8 mg/kg bw by gavage.
Cholinergic signs appeared after treatment on d 0. Males at 3 and 8 mg/kg bw had muscle fasciculations and ataxia and, at 8 mg/kg, males showed tremors and salivation. Females at 8 mg/kg bw, had muscle fasiculations, ataxia and salivation. All signs had reversed within 5 d.
Treatment-related behavioural and motor activity effects were observed after treatment on d 0 at all dose levels in males and at 3 and 8 mg/kg bw in females. There was significant ($\geq$ 20%), dose-dependent inhibition of plasma, RBC and brain ChE at 1 mg/kg and above in both sexes. There was no histological evidence of injury to the central or peripheral nervous system at the highest dose of 8 mg/kg bw (Sheets *et al*, 1993).

Since a NOEL could not be established in the above study, a supplementary acute oral neurotoxicity study was conducted in rats using single gavage doses of 0, 0.3, 0.6 mg/kg bw po. There were no deaths and no treatment-related clinical signs (including behavioural signs and motor activity) in any group. There was significant (>20%) inhibition of plasma, RBC and brain ChE activities at 0.6 mg/kg bw. The NOEL was 0.3 mg/kg bw methamidophos, based on inhibition of plasma, RBC and brain ChE at the higher dose (Sheets *et al*, 1994a).

In a subchronic dietary neurotoxicity study in rats, Fischer 344 rats received methamidophos technical (75.6% purity) at 0, 1, 12 or 60 ppm in the diet for 14 wk. At 12 and 60 ppm, rats showed clinical signs (muscle fasciculations, increased reactivity, tremors perianal stain, urine stain and lacrimation), that appeared during the 1st 2 wk of exposure and generally persisted throughout the rest of the exposure period. Significant decreases in forelimb grip strength were observed at 60 ppm in males at wk 4 - 13 and in females at wk 8 and 13. Statistically significant decreases in motor activity were seen in males over the whole treatment period at 60 ppm, and in females at wk 4 at 12 and 60 ppm. Plasma and RBC ChE activities at 4 and 14 wks, and brain ChE activity at 14 wk after treatment were significantly ($\geq$ 20% inhibition) decreased in males and females at 12 and 60 ppm. There were no treatment-related gross lesions in any group and no treatment-related histological findings at 60 ppm. The NOEL for neurotoxicity was 1 ppm in diet (0.07 mg/kg bw/d), based on plasma, RBC and brain ChE inhibition and neurobehavioural signs at the higher doses (Sheets *et al*, 1994b; Sheets *et al*, 1996).

Developmental neurotoxicity studies

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A study by Wang & Huang (1987) was designed to examine the effects of perinatal methamidophos exposure in female mice at lower levels (1/6-1/60 LD_{50}) on the development of cerebral cortex and behaviour in their offspring. Pregnant mice were administered methamidophos po at 0, 0.4, 2.2 and 4 mg/kg bw/d from GD 16 to LD 21 (weaning of offspring). Methamidophos produced evidence of cortical injury and behavioural toxicity in the offspring. Some physical and reflex developmental parameters (eye opening, righting reflex, suspension reflex, auditory startle reflex) were delayed at 2.2 and 4 mg/kg bw/d. The swimming scores and the number of T-water maze trials were significantly different from controls at each dose of methamidophos. At 2.2 and 4.0 mg/kg bw/d methamidophos at PND 30, neuron density in the cerebral cortex was significantly higher than controls. A NOEL could not be established in this study.

Other Human Studies

Occupational Exposure

Four Bayer internal letters indicated that occasional temporary inhibition of ChE to a minor degree was observed in production workers making SRA 5172/Tamaron™ (Kollert, 1981); however, staff members engaged in the formulation of Tamaron™ were medically examined regularly and no damaging effects on their well-being had been observed (Miksche, 1981).
Methamidophos produced at the Elberfeld plant had been processed into liquid formulations on a scale of 600 - 800 metric tons annually at the FVL II Plant in Dormagen for about 15 yr. Under the protection of the occupational hygeine measures practiced, no cases of dermal or mucous membrane irritation had been observed in employees (Faul, 1990). Slight transient inhibition of ChE activity had been observed occasionally in employees engaged in the production of Tamaron™. No further details were provided (Sitt, 1990).

In the period 12 June 1968 to 1 September 1970, there were no adverse effects reported in 67 exposures to Monitor (a product containing 50% methamidophos); 54 of the reports concerned individuals who were exposed following their activities in the field, including mixing formulations for spraying and/or applying spray to crops either by knapsack or by air. The other 13 reports involved exposure in research laboratories or in manufacturing plants. (Ospenson *et al*, 1968)

Two women (aged 45 and 55 y), engaged in lettuce seed extraction in a French agricultural research station, were hospitalised following exposure to methamidophos (Orthotox) that had been applied by thermal fogging to lettuce grown within hoop-houses. Both patients presented at hospital bearing the odour of the pesticide, and were decontaminated. They showed typical signs of acute anticholinesterase poisoning (pallor, salivation, sweating, nausea or vomiting, headache and abdominal pain and/or swelling and arterial hypotension). However, there was no evidence of depressed plasma or RBC ChE activity in either patient, one of whose blood was sampled 4 h after the onset of symptoms. The second patient was not assayed until at least 24 h after admission. Following resolution of the acute cholinergic crisis, each patient developed proximal neurological signs that were diagnosed as intermediate syndrome (parasthesia and bursts of pain in the thighs of the first patient, while the second patient lost visual acuity and muscular strength and had hyper-reflexivity of the upper limbs). On d 30, retrobulbar optical neuritis developed in the second patient, recovering by 2 mo post-exposure. Subsequently, both patients developed sensory and peripheral neuropathy, which the study authors attributed to OPIDN. The time course of onset was different in each case. The first patient developed numbness of the distal legs and calves from d 9, while parasthesia,

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diminished sensation, cramps in the hands and legs and hyporeflexivity became evident in the second patient from mo 2 onwards. By 7 mo post-exposure, intense sensory impairment and motor dysfunction had developed in the legs and hands of both patients, accompanied by behavioural and mood disorders. The condition persisted for 16 mo in each patient, after which there was improvement in the clinical picture, neuromuscular function, psychometric tests and sensory threshold measurements. At 18 mo, the first patient was well but symptoms had not resolved totally in the second (De Haro *et al,* 1999).

McConnell, Keifer & Rosenstock (1994) examined inpatient discharge diagnoses relating to workers from Nicaragua's Northern Pacific coastal plain who were previously hospitalised for acute organophosphate poisoning. The authors had previously reported evidence of persistent central nervous system effects among this population (Rosenstock et al., 1991). Between 1 July 1986 and 31 July 1988, 52 male patients, aged 15-44 yr at the time of admission, were identified as having been treated for occupational organophosphate poisoning. Thirty six agreed to participate in the study. The 36 treated patients all had medical record documentation of symptoms consistent with severe organophosphate insecticide poisoning and all received atropine during treatment. Each previously poisoned person was matched by sex and age (within 5 yr) to a sibling or friend in the same community who had never been medically treated for a pesticide poisoning. During 1989, the matched pairs were brought to the regional capital for examination. Of the 36 previously poisoned participants, 21 were classified as previously poisoned with methamidophos (group M). The remaining 15 persons reported never having been treated for methamidophos poisoning and were poisoned with other organophosphates or mixtures of organophosphates and other pesticides (group NM). The group poisoned with methamidophos had higher mean vibrotactile thresholds than the group poisoned with other material and the control group.

Poisoning Incidents

Senanayake & Johnson (1982) reported 10 cases, seen over 3 years, of acute polyneuropathy after poisoning by methamidophos (Tamaron™) in Sri Lanka. At the time of the poisonings Tamaron™ was believed to have contained 60% w/v methamidophos in ethylene glycol monomethyl ether with an unidentified dispersing agent (5%) and small amounts of several compounds related to methamidophos, principally an unidentified isomer and the *N*-methyl analogue of methamidophos. All patients were unconscious on admission to hospital. Seven of the cases were attempted suicides by ingestion. Of the other 3, one person had sucked the insecticide through a tube from a spraying machine, another had spilled the formulation on his body while opening the bottle and another had become intoxicated while spraying the insecticide. The amounts ingested (where known) ranged from 30-80 mL. All patients survived. All of the patients had shown an acute cholinergic crisis soon after exposure to the insecticide with a polyneuropathy developing 2-3 wk later. Six patients followed for 8 wk or more also had evidence of pyrimidal tract involvement (weakness, hyperactive tendon reflexes and Babinski responses). No ChE assays were performed on these patients.

In a review of the article by Senanayake & Johnson (1982), Pauluhn & Machemer (1984a) referred to the evidence available at the time: that compounds structurally related to methamidophos had not induced neurotoxicity; studies on the structure-effect relationship of organophosphates had shown that only those organophosphates which possess specific structural characteristics have a neurotoxic effect; and hens had shown reversible inhibition of NTE following methamidophos administration, but no clinical or histopathological findings indicative of delayed neurotoxicity had ever been observed. The review authors emphasised

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that the diagnosis had been made solely on the basis of signs and symptoms of poisoning, but ChE activity had not been determined and a positive identification of the substance involved was lacking. It was calculated that one patient who had survived treatment, consumed a dose of methamidophos 65 times greater than the known oral LD_{50}. The severity of the poisoning was estimable only from the observation that the patient was unconscious for more than 24 h and was administered very high doses of atropine (270 mg within 24 h). It was claimed that, in the absence of precise monitoring of the cardiovascular system, cardiovascular effects including cerebral oedema could not be ruled out. This can cause a change in muscle tone resulting in increased spasticity suggestive of pyramidal tract involvement. The electromyographic examination was not conclusive and did not permit a diagnosis of alkyl phosphate-induced polyneuropathy. The review authors further reported that individual cases of Tamaron™ poisoning in Germany had induced coma for up to 3 d from which the patients recovered without delayed effects.

Senanayake (1985) described 27 isolated cases of acute polyneuropathy over a 6 yr period, occurring after exposure to 3 organophosphorus compounds marketed in Sri Lanka. The cases were 22 males and 5 females aged 14-33 yr. Methamidophos was involved in 25 of the cases. The method of exposure was ingestion with suicidal intent (19), accidental ingestion (5) and accidents during spraying (3). Neuropathy developed following the acute cholinergic crisis after a delay of 15-22 d. The primary signs were acute weakness of feet and hands in 27 cases, with calf pain preceding the weakness in 20 and paraesthesia of limbs in 13. Physical signs were absent ankle jerk in 27 cases, foot drop in 22, claw hand in 11 and sensory (pain) impairment in 11. The dominant hand was more affected in 17. Two patients had external ocular palsies. Four patients, in late stages, had mild pyramidal signs. Electromyography of distal muscles of the limbs showed varying degrees of denervation. Stimulation of common peroneal and/or tibial nerves failed to evoke motor action potentials (MAP) in 21 cases. Median nerve stimulation failed to evoke MAP in 4. Sensory conduction in the digital branches of the median nerve was impaired in 2. Signs of clinical improvement appeared any time after 2 wk from the onset. Four patients recovered completely in 6 wk to 2 yr. The other patients were under observation for signs of complete recovery at the time of the author's preparation of this abstract.

Senanayake & Karalliede (1987), described an “intermediate syndrome” in 10 patients, affected by organophosphorus poisoning, who were admitted to the University Medical Unit at Peradeniya, Sri Lanka, during the previous 3 yr. The only case involving methamidophos was a 22 yr old male, who had taken an unspecified dose of methamidophos with suicidal intent, had developed cholinergic signs that included miosis, salivation, sweating and fasiculations, and was unconscious on admission. Upon treatment with pralidoxime and atropine, the patient recovered from the cholinergic crisis within 24-48 h. However, within 4 d, an intermediate syndrome had developed in the methamidophos-affected patient which lasted for 32 d and overlapped the development of polyneuropathy. The initial clinical signs were weakness and respiratory insufficiency, which required intermittant positive-pressure ventilation. The syndrome was characterised by weakness of the muscles innervated by cranial nerves. Marked weakness of neck flexion was a feature and was shown as an inability to raise the head from the pillow. Other features were weakness of the proximal limb muscles with normal strength in the distal muscles, weakness of shoulder abduction and hip flexion and the absence of knee and ankle reflexes. Sensory impairment was not observed. The regression of signs followed a characteristic pattern in those who survived (7/10). Cranial nerve palsies (palatal, facial and external ocular in that order), were the first to regress. Then followed an improvement of the respiratory impairment and recovery of strength in the

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proximal limb muscles. Neck flexion was the last function recovered. The period of recovery ranged from 5-18 d after the onset of weakness except in the methamidophos-affected patient whose neck flexion capacity took 32 d to recover. By that time he had developed weakness of the distal muscles of the limbs due to delayed neuropathy.

Two female patients, 1 of which was a 22 yr old primigravada admitted to the Teaching Hospital, Peradeniya, Sri Lanka, 3 h after ingestion of methamidophos with suicidal intent. The amount ingested was not specified. The patient presented with mental confusion, pin point pupils and generalised muscle fasciculations. It was estimated that she was 36 wk pregnant. Treatment included gastric lavage, atropine and pralidoxime. The patient was rational and free of muscle fasiculations within 24 h. Eighty h after admission, she became restless, dyspnoeic and cyanosed with a respiratory rate of 50/min and tidal volume of 150 mL. Mechanical ventilation was required for 6 d. She was clinically normal afterwards. Forty d after poisoning, the patient delivered a male child with a bw of 2.85 kg and an Apgar score of 8 at 1 min. At birth the child was clinically free of congenital or neurological abnormalities (Karalliedde, Senanayake & Ariaratnam, 1988)

A 32 y old male attempted suicide by swallowing 200 mL of Tamaron^{R} containing 228 g/L of methamidophos. The estimated dose was 600 mg/kg bw. The patient displayed severe cholinergic toxicity and became comatose within 2-3 h. Mechanical ventilation, treatment with atropine, pralidoxime and benzodiazepine were provided for 4 d. Plasma and erythrocyte ChE activities were < 10% of unspecified normal values and began to recover from d 4. Lymphocytic neuropathy target esterase (LNTE) was 1.0, 1.6, 5.9, 16.4 and 12.5 nmoles/min/g of protein at 22, 43, 84 h and 9 and 37 d after intoxication, respectively, equivalent to inhibition of approximately 89, 82 and 35% at 22, 43 and 84 h respectively. Methamidophos concentration in plasma peaked at 6.2 µg/mL at 22 h after ingestion. On d 10 the patient was well and neurological examination and electrophysiological parameters were normal. However, on d 25 the patient complained of leg weakness and on d 36 neurologic examination showed discrete peripheral neuropathy in lower limbs. In tibial and peroneal nerves, motor nerve conduction velocities (NCV) were moderately reduced (40-44 m/sec) whereas amplitudes of motor action potential (MAP) were severely affected (0.1-1 mV). MAPs were also reduced in the median nerves (3-6 mV). Sensory NCV, somato-sensory and visual evoked potentials were normal (Moretto *et al*, 1994).

Sun, Zhou & Xue (1998) conducted an epidemiologic survey of OPIDP among 104 patients who had been poisoned with methamidophos and were admitted to hospital between 1987 and 1992, in China. Ingestion was the route of poisoning in 95% of cases. Intake of 20 mL or more was associated with severe poisoning in the majority of cases. OPIDP were diagnosed in 14/104 (13.5%) cases of oral methamidophos poisoning. The intake volume ranged from 10-100 mL with a mean volume of 33 mL for methamidophos alone (7 cases) and 23 mL for a 2:1 mixture of methamidophos and dimethoate (6 cases). All 14 had acute cholinergic signs and 12 had coma of 1-24 h duration. In all OPIDP cases, blood ChE activity was severely inhibited (data not provided), and the disappearance of cholinergic signs coincided with the restoration of ChE to "normal" levels. The clinical manifestations of OPIDP were sensory disturbances (numbness and paresthesia in the distal limbs) followed by dyskinesia (weakness, difficulties in raising arms and legs, dysbasia, loss of capacity to stand and walk, difficulty with fine movements of hands and fingers. Functional disturbances of the autonomic nervous system e.g. chilling of the skin and excessive perspiration, neurasthenic syndrome: insomnia, headache and dizziness were also noted. Thirteen patients recovered, mostly within 2 y. One patient was still affected with OPIDP 64 mo after diagnosis.

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A 16-y old male ingested approximately 100 mL of a pesticide containing methamidophos. On admission to hospital, the patient was unconscious, tachypneic and had diffuse rhonchi, miosis and prominent fasciculations of pectoral and abdominal musculature. Acute signs included recurrent sialorrhea, blurred vision, generalised weakness, fasciculations, nausea and vomiting. At the time of his discharge from hospital (8 d after admission) his neurological examination was normal except that his pinch and grip strengths were reduced and vibrotactile threshold was mildly elevated. Approximately 5 d after discharge, the patient began to experience pain in the gluteal region, thighs and calves, followed by weakness of the lower extremities, difficulty walking, and weakness of grip. Measurements of grip and pinch strength 52 d after poisoning demonstrated considerable deterioration from those obtained at the discharge examination, but vibrotactile threshold was normal. Nerve conduction studies demonstrated reduced amplitude of ulnar nerve distal compound muscle action potential. Sensory nerve conduction studies were normal. ChE activity approximately 24 h after ingestion but before treatment with the ChE reactivator pralidoxime, was low in RBC and in plasma. RBC ChE was still mildly depressed at 52 d. LNTE activity was low at 3 d. Autoantibodies to NF triplet proteins, GFAP and MBP were detected in serum collected 3 d and 52 d after poisoning. During this period there was a marked increase in IgG to GFAP and to NF 200, but a moderate decrease in autoantibodies to GFAP and NF 68 (McConnell *et al*, 1999).

Chan (2001) reported on methamidophos contamination of vegetables in Hong Kong. In 1987, 64 patients presented to the emergency department of the Prince of Wales Hospital after eating vegetables contaminated with methamidophos. Sixty percent developed signs within 1-3 h of exposure. Common features included nausea (95%), vomiting (86%), chills (75%), sweating (67%), dizziness (63%), abdominal pain (56%), diarrhoea (45%), headache (27%), increased lacrimation (27%), weakness (25%) and miosis (19%). These patients required symptomatic treatment only.

Other Studies

Mechanistic studies

The toxicity of methamidophos is due to the parent compound and not to a metabolite, and correlates with levels of methamidophos in the CNS and PNS. The distribution and clearance, and correlation with inhibition of ChE activity was measured in rats administered single iv doses of 8 mg/kg bw ^{14}C-labelled methamidophos. Highest levels of radioactivity in most tissues were found approximately 1 min. after iv dosing. The levels in the CNS and PNS were similar and generally lower than most other tissues, reaching maximum levels in 20-60 min, coinciding with maximum severity of clinical signs and maximum inhibition of ChE activity in the CNS. By 8 h, clinical signs had disappeared and ChE activity in the CNS was >50% of control activity; however, plasma levels were still depressed (27% of controls). (Gray, Thompson & Fukuto, 1982).

Vilanova, Johnson & Vicedo (1987) examined the interaction of some unsubstituted phosphoramidate analogs of methamidophos with acetylcholinesterase and neuropathy target esterase in hen brain. The capacity of S-methyl esters and dichlorophenyl analogs to inhibit AChE increased progressively with increasing chain length. *S*-methyl esters were up to 450 times more active against AChE than against NTE and the dichlorophenyl esters were up to 10 times more active against NTE than against AChE. When AChE was inhibited by methyl,

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ethyl, *n*-butyl or *n*-hexyl compounds from either series, there was marked spontaneous reactivation (75-100%) and no apparent aging at 19 h at 37°C. With inhibition of AChE by *O-iso*-propyl SMe some spontaneous reactivation with complete aging of all remaining inhibited AChE occurred during 19 h. There was no spontaneous reactivation or aging of NTE.

In a study of the interaction of methamidophos with hen and human acetylcholinesterase and neuropathy target esterase, the brains and sciatic nerves of hens given 1.5-2.3 kg bw methamidophos (resolved optical isomers, >99.9% purity) were excised and homogenised with samples of human cortex and nucleus caudatus. Human and hen brain AChE and NTE activity were assayed by incubation of the homogenates with D-(+) methamidophos or L-(-) methamidophos. The respective rate constants for the inhibition of AChE by D-(+) methamidophos in hen and human brain homogenates were 0.13×10^3 and 0.24×10^3 M^{-1} min^{-1}; for the inhibition of NTE by D-(+) methamidophos they were 59 (51 sciatic nerve) and 88 M^{-1} min^{-1} respectively. The respective rate constants for the inhibition of AChE by L-(-) methamidophos in hen and human brain homogenates were 2.8×10^3 and 5.7×10^3 M^{-1} min^{-1}; for the inhibition of NTE by L-(-) methamidophos they were 3.0 (5.0 sciatic nerve) and 3.2 M^{-1} min^{-1} respectively. The time-course of both hen and human NTE inhibition by D-(+) or L-(-) isomers of methamidophos was observed to follow first order kinetics extrapolating through the origin, indicating that the inhibition of NTE was progressive and irreversible. In contrast, the time course of AChE inhibition by the two isomers did not follow first order kinetics because of spontaneous reactivation of AChE. Approximately 20% of human and 12% of hen AChE inhibited by D-(+) had spontaneously reactivated at 6 h. No further reactivation was observed. On the basis of the rate constants, the L-(-) isomer was 22 to 24 times more potent inhibitor of AChE than the D-(+) isomer in hen and human brain homogenates respectively, but the D-(+) isomer was a more potent inhibitor of NTE than the L-(-) isomer; 20 and 28 times more potent in hen and human brain homogenates respectively. Each isomer was a more potent inhibitor of AChE than NTE in both hen (D-(+) 2.2; L-(-) 930 times as potent) and human (D-(+) 2.7; L-(-) 1780 times as potent) brain homogenates. For each isomer, the rate constants for inhibition of AChE in human brain homogenates were twice those for hen brain homogenates. The respective rate constants for the spontaneous reactivation of AChE inhibited by D-(+) methamidophos in hen and human brain homogenates were 0.69×10^{-3} and 0.83×10^{-3} min^{-1}. The respective rate constants for the spontaneous reactivation of AChE inhibited by L-(-) methamidophos in hen and human brain homogenates were 1.52×10^{-3} and 6.50×10^{-3} min^{-1}. The rate of spontaneous reactivation of AChE inhibited by L-(-) methamidophos was 2 and 8 times greater than that following inhibition by the D-(+) isomer in hen and human brain homogenates respectively (Bertolazzi *et al*, 1991).

In a published study, Robinson & Beiergrohslein (1982) examined the *in vitro* inhibition of human erythrocyte and plasma ChE activity by methamidophos. Methamidophos was incubated with plasma at concentrations of 4.01, 7.22, 8.67 or 10.80 M $\times 10^{-5}$, or with erythrocytes at 1.91, 4.78, 6.69, 9.56 M $\times 10^{-5}$. Methamidophos was a comparatively weak inhibitor of human ChE. ChE activities in RBC and plasma decreased during the 7-12 min observation period. The rate of inhibition of RBC ChE (3.14×10^3 /M/min) was greater than that of plasma ChE (1.85×10^3 /M/min). Although the inhibition rate constant for methamidophos in plasma ChE is similar in rats and humans, the inhibition rate constant for RBC ChE in rats is 2.8 times higher than in humans.

Robinson & Beiergrohslein (1980) showed that the rate constants for the *in vitro* inhibition of RBC and brain ChE inhibition were not significantly different, but were about 5 times greater than the rate constant for inhibition of plasma ChE. *In vitro* inhibited brain and plasma ChE

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spontaneously reactivated. Inhibited brain ChE took 4 h to reactivate to 50% of control activity compared to 14 h for inhibited plasma ChE. After 25 h the inhibited brain and plasma enzyme activities had regained 88 and 65% of control activity, respectively The addition of pralidoxime to *in vitro* inhibited enzyme preparations partially restored enzyme activity (maximum 37 and 27% restoration of brain ChE and plasma ChE respectively at 0.5 h). Complete reactivation of inhibited enzyme was not observed. The ability to restore ChE activity progressively declined with time over 0.5-25 h. The addition of a 2nd dose of pralidoxime at the various test intervals did not further increase the rate of return of the ChE activity. The ip LD_{50} for methamidophos in rats was 15 mg/kg bw. All rats that died after dosing with methamidophos did so within 15 min after dosing. In these rats mean brain ChE activity after death was reduced to 12.4% of control activity. In those that survived the LD_{50} dose, brain, plasma and stomach ChE was observed to be 49, 49 and 19% of control activity respectively 24 h after dosing. Atropine and pralidoxime protected against the lethality of methamidophos, increasing the ip LD_{50}s to 60 and 52 mg/kg respectively. All rats treated with methamidophos and atropine that died, did so within 15 min of dosing. Most of the deaths observed in rats given methamidophos plus pralidoxime occurred between 1-2 h after dosing.
In vivo inhibited plasma, stomach and brain ChE activity spontaneously reactivated. During the 1st 24 h after a 12.5 mg/kg bw dose of methamidophos, the rates of restoration of activity were 2.1%/h, 1.9%/h and 0.8%/h for brain, plasma and stomach ChE respectively. At 48 h after dosing, plasma, stomach and brain ChE had returned to at least 60% of control values. Pralidoxime did not improve reactivation rates.

Guo *et al* (1986b) determined a threshold dose for inhibition of whole blood ChE activity in rats. Inhibition of whole blood ChE (21-28%) was first observed at 0.5 h following oral administration of 1 mg/kg bw of methamidophos (90.4% purity). On this basis the po threshold dose eliciting inhibition was 1 mg/kg bw. Inhibition of whole blood ChE (42-45%) was first observed at 4 h following dermal application of 30 mg/kg. It was not demonstrated at 20 mg/kg, indicating the relatively poor dermal absorption of methamidophos. Sixty per cent inhibition of whole blood ChE activity was observed at 0.5 h following the intragastric administration of 5.0 mg/kg bw methamidophos. At 2 h the degree of inhibition was 78%. The degree of inhibition had recovered "substantially" at 24 h and "within 48-72 h" it had fully reversed.

Easter & Rosenburg (1986) examined the cholinesterase inhibition potential of analytical grade methamidophos (99.1% purity) and methamidophos technical (74.7% purity) following topical application of a single dose to rats at 0, 1.0, 2.5, 6.25, 15.6 mg/rat for 24 or 72 h. At 15.6 mg/rat 3/5 analytical grade methamidophos females died from 6 h to 1 d and 2/5 methamidophos technical females died at 1.5 h post dosing, the latter without pre-mortality signs. Cholinergic signs (tremors, fasciculations, salivation, dyspnoea, ataxia, lacrimation and collapse) were observed in most or all rats treated at 15.6 mg/rat and in females treated at 6.25 mg/rat but were absent at lower doses. The treatment of male and female rats with a single dose of dermally applied analytical grade methamidophos or methamidophos technical resulted in dose-dependent inhibition (up to 15.6 mg/rat) of RBC and brain AChE and plasma BChE at 24 and 72 h after exposure. For each grade of methamidophos the degree of inhibition at 24 h was greater than at 72 h. Females were more sensitive to the effects of methamidophos. For both grades of methamidophos and for all types of ChE, the degree of inhibition at 24 h was up to 85% greater in females than males. This observation parallels the more extensive appearance of clinical signs and mortality in females at 6.25 and 15.6 mg/rat after 24 h exposure to both grades of methamidophos. The ID_{50} (dose corresponding to 50% of mean control activity) for all types of ChE and both grades of methamidophos was least in

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females at 24 and 72 h except for RBC AChE at 72 h in those treated with analytical grade methamidophos. The capacity of methamidophos technical to produce ChE inhibition was greater than that of analytical grade methamidophos. The ID_{50} values for methamidophos technical were less than those for analytical grade methamidophos except for RBC AChE in rats treated with methamidophos technical.

In a published study, Camara *et al* (1997) reported that methamidophos (99.8% purity) exhibited species selectivity in its anticholinesterase activity, being a more potent inhibitor of ChE from rat skeletal muscle than ChE from frog skeletal muscle. ChE I_{50} values for methamidophos for rat diaphragm muscle, brain and hippocampal homogenate were 30, 29 and 20 μM respectively suggesting that methamidophos is a weak inhibitor of ChE. At 100 μM methamidophos caused 80% inhibition of rat skeletal muscle ChE in 2 h. Upon removal of methamidophos from the system, spontaneous reactivation of ChE from 20% to 90% control activity occurred in 4 h. On the basis of this data, the inhibition half life for ChE inhibition was 1.2 h and the spontaneous reactivation half life was 2.2 h. Reversal of inhibition took twice as long as inhibition. The results suggested that the effect of methamidophos on neuromuscular transmission is most likely to be solely an inhibition of ChE because it reversed a partial blockade of twitch tension in rat skeletal muscle induced by d-tubocurarine and decreased the ability of rat skeletal muscle to maintain tension during tetanus (indicating an accumulation of ACh in the synaptic cleft). Further, it failed to induce any change in spontaneous or evoked presynaptic release of ACh from motor nerve terminals in skeletal muscle of rat, and showed no signs of direct postsynaptic effects on muscle nicotinic ACh receptors in skeletal muscle of rat. Methamidophos failed to induce any pre- or postsynaptic effects unrelated to ChE inhibition in rat hippocampal neurons because neither the miniature postsynaptic currents mediated by GABA and AMPA-type glutamate response nor NMDA-induced macroscopic currents were affected by it. It did not have any significant effect on NMDA-induced whole cell current and did not affect the α-bungarotoxin-sensitive neuronal nicotinic ACh receptor. In contrast, paraoxon induced a significant increase in spontaneous transmitter release as evidenced by an augmentation in frequency of GABA and glutamate-mediated miniature postsynsaptic currents. Also, paraoxon is able to block the ion channels of glycine, $GABA_A$, NMDA and neuronal nicotinic ACh receptors (Rocha *et al.*, 1996).

Wong & Kodama (1979) characterised the *in vitro* inhibition of rat and monkey red blood cell and brain acetylcholinesterase by acephate, technical acephate and methamidophos and their mixtures. The *in vitro* I_{50} values for methamidophos (99.6%.purity) in rats were determined to be 5.0×10^{-6} M and 9.0×10^{-7} M for brain and RBC AChE respectively. The *in vitro* I_{50} values for methamidophos in Rhesus monkeys were determined to be 3.5×10^{-6} M and 9.0×10^{-6} M for brain and RBC AChE respectively. On the basis of the determined I_{50} values, methamidophos was 1.4 times more potent in inhibiting brain AChE in monkey as in rat and 10 times less potent in inhibiting RBC AChE in monkey than in rat.

Biochemical, histochemical and histopathological studies

Rats received methamidophos (60% purity) at single doses of 20 mg/kg bw or daily doses of 2 mg/kg bw/d for 14 d or 10 wk, by gavage. Methamidophos administered as a large single dose (20 mg/kg bw) caused signs of cholinergic stimulation characterised by salivation, diarrhoea, dyspnoea and paralysis. Prolonged administration of small daily doses of methamidophos (2 mg/kg bw/d for 10 wk) caused persistent tremors and induced the development of numerous (mandibular) abscesses. A single dose (20 mg/kg bw) or daily doses (2 mg/kg bw/d for 14 d)

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of methamidophos caused elevation in AST, ALT, and AP, pathological changes in the liver of rats, depletion of liver glutathione, nephrotoxicity and subsequent elevation of BUN, and increased the concentration of blood glucose and cholesterol (Hanafy, Arbid & Afify, 1991).

Methamidophos technical grade impurities toxicity studies

O,O-dimethyl phosphoramidothioate is an intermediate used in the manufacture of technical methamidophos and is the impurity in technical methamidophos with the highest concentration (≤ 9%). The reported lowest oral LD_{50}s in mice and rats were 430 and 410 mg/kg bw; the dermal LD_{50} in rats was 4360 mg/kg bw and the inhalation LC_{50} was >14.5 mg/m^3. Acute clinical signs reported by (Cavalli *et al.*, 1968j) were paresis, hyporeflexia, areflexia, anaesthesia and hypothermia. Death from respiratory failure occurred between 2-16 h after dosing. Survivors were severely depressed for as long as 48 h after dosing. No pathological abnormalities were noted at necropsy.

Monomethyl sulfate ion is an impurity present at ≤ 4%. In rats methyl sulfate has an oral LD_{50} of 205 mg/kg bw, an sc LD_{50} of 100 mg/kg bw, a 4 h inhalation LC_{50} of 45 mg/m^3 and in mice an oral LD_{50} of 140 mg/kg bw and an inhalation LC_{50} of 280 mg/m^3. Clinical signs of acute toxicity included somnolence and respiratory depression. Methyl sulfate is also a severe irritant to skin and eye of rabbits.

O,O,S-trimethyl phosphorothioate (OOS-TMP) is present in methamidophos technical at < 1%. The lowest oral LD_{50}s in mice and rats are 134 and 60 mg/kg bw, respectively. Mallipudi *et al*, (1979) described the acute clinical signs as being: at 200 mg/kg bw po, bw loss, severe diarrhoea, excessive urination and bleeding from mouth, nose and ear. The signs were less severe but observable at 40 mg/kg bw po. At 15 mg/kg bw po the signs were absent except for bw loss. Serum CE and serum ChE activities were significantly inhibited for extended periods. CE was initially more sensitive to inhibition than ChE. Mean serum CE at 4 h and 2 and 6 d after a single dose of 15 mg/kg bw po was 56, 76 and 61% control activity respectively. Activity was restored to 100% between 6-14 d in survivors. At the same dose, mean serum ChE at 4 h and 2 and 6 d was 67, 56 and 60% control activity respectively. Activity was restored to 100% between 6-14 d in survivors. The study authors considered that inhibition of CE was responsible for the toxicity potentiating property of this and other trialkyl esters present as impurities in malathion technical.

Verschoyle & Cabral (1982) described 2 phases of acute toxicity in rats. Within the 1st 30 min signs of poisoning were typically cholinergic and included fasciculations, salivation, urinary incontinence and occasionally chromodacryorrhea. Mortalities during this phase did not occur. Recovery from cholinergic signs was observed by 24 h but at this time rats began to show pilo-erection and panting respiration. They lost up to 20% of their bw in 4 d. The majority of deaths occurred within 3-6 d after dosing. In rats cholinergic signs occurred only at oral doses higher than the oral LD_{50} (60 mg/kg bw). The compound was less toxic to mice which showed a mixed response with deaths occurring both in the early cholinergic phase and in the later pulmonary phase. At a dose of 50 mg/kg bw po female Wistar (LACp) rats showed a statistically significant ($p \leq 0.01$) doubling in wet and dry relative lung wt 4 d after dosing. There was no difference between treated and control rats in lung water content. The results were similar for Sprague-Dawley male rats given 60 mg/kg bw po and female F344 rats given 70 mg/kg bw po. Histological examination of enlarged lung tissue showed extensive cellular proliferation with progressive diffuse interstitial thickening which peaked at d 4 after dosing. This caused loss of alveolar space and consolidation of large areas of all

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lobes. In surviving rats the lesion had regressed by d 8. Pretreatment with the H_2 receptor antagonist cimetidine (SKF 525A) increased the oral LD_{50} of the compound from 50 to 252 mg/kg bw.

Imamura *et al*, (1983) reported that *O,O,S*-trimethyl phosphorothioate markedly alters the morphology of the bronchiolar epithelium of male Sprague-Dawley rats when given as a single oral dose in corn oil at 20 mg/kg bw. The oral dose of 20 mg/kg bw resulted in few acute cholinergic signs and the rats survived for at least 28 d. Loss of bw and red staining around the nose and eyes were observed 24 h after treatment and were present for at least 7 d. Because signs were most apparent at 3 d after dosing, the morphology of the bronchiolar epithelium and extracellular bronchopulmonary lavage LDH activity were investigated at that time. The morphology of the bronchiolar epithelium in treated rats was altered. The Clara cells were 50% fewer in number and 30% larger in diameter than control Clara cells. The number of ciliated cells also increased but the cilia appeared morphologically normal. Significantly higher ($p < 0.05$) mean LDH levels were found in tracheal lavage fluid from treated rats than from control rats (12 cf 6 U/100 mL respectively). The increase may have been due to LDH release from cells damaged by the compound in the bronchopulmonary tree.

Rodgers, Imamura & Devens (1985) reported on the effects of 0.5 or 5.0 mg/kg bw/d *O,O,S*-trimethyl phosphorothioate (> 99.8% purity) for 14 d on the generation of cell-mediated and humoral immune responses in female C57B1/6 mice ($H\text{-}2^b$). There was no significant loss of bw in treated mice. Administration of 0.5 mg/kg bw/d of *O,O,S*-trimethyl phosphorothioate resulted in an increase in thymic lymphocyte number and wt and enhanced humoral immune responses, mitogen responses and interleukin 2 (IL-2) production. The capacity for generation of a cytotoxic T-lymphocyte (CTL) response was not changed. At a dose of 5 mg/kg bw/d, there was no change in humoral or CTL immune responses. There was an increase in IL-2 production and mitogenic responses. Overall, treatment with low amounts of *O,O,S*-trimethyl phosphorothioate elevated immune responses in the mouse. The study authors emphasised that this was in contrast to the findings of their unpublished data which showed that unspecified acute amounts of this compound caused suppression of CTL and antibody responses.

The principal aim of a study by Koizumi, McCloud & Imamura (1986) was to establish a profile of reproductive toxicity in rats caused by *O,O,S*-trimethyl phosphorothioate at a relatively low oral dose when it is given early in gestation (GD 8-10). A pair-fed study was conducted to separate indirect effects on the gonadotrophic hormone system caused by decreased food consumption and loss of bw induced by *O,O,S*-trimethyl phosphorothioate, from other mechanisms of action. For the reproductive study, virgin female Sprague-Dawley rats were mated and GD 0 determined. A control group of 5 rats was given corn oil (2 mL/kg bw po) on GD 8 and 10. A group of 7 mated rats were treated with 20 mg/kg bw po *O,O,S*-trimethyl phosphorothioate on GD 8 and 10. The dose was selected because toxic effects without mortality were expected based on the oral LD_{50} was 85 mg/kg bw. The dosing times were chosen to maximise adverse effects on pregnancy and foetuses. Food and water were freely available to treated and control rats. The pair-fed controls (7) were dosed with corn oil at 2 mL/kg bw po on GD 8 and 10 and fed the restricted amounts of food which had been determined by the mean food consumption in treated rats on the corresponding d of gestation i.e. 3.6 g from GD 8-12, 7.0 g from GD 13-15, 17 g from GD 16-18 and *ad libitum* from GD 19-21. Severe emaciation was observed in the treated group (GD 12-15 or 21) and pair-fed groups from GD 12-21. The significant decreases in mean bw in treated group and pair-fed groups occurred in association with significant decreases in food consumption, amounting to

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an 88-66% decrement relative to controls over GD 8-15. Of the 7 treated dams, 4 resorbed all their foetuses, while the resorption rate was approximately doubled in the other 3 dams compared with controls. Resorption and foetal survival in the pair-fed control group were intermediate between the treated and control dams. The study authors considered that resorption in treated rats was not accounted for solely by a decrease in food consumption and was thus treatment-related. All foetuses were examined externally as were the soft tissues of 35% of foetuses from each group. No abnormalities were found. The foetal bw and crown-rump length of foetuses from treated dams were significantly smaller ($p \leq 0.01$) than that of controls or pair-fed rats. The incidence of delayed ossification in metacarpalis and metatarsalis was significantly higher ($p \leq 0.01$ or 0.05) than that of controls or pair-fed rats. Foetal growth was not affected in the pair-fed group.

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HAZARD ASSESSMENT

Discussion

Reasons for review

From a public health standpoint, methamidophos was selected as having a high priority for review under the CRP because of its high potential to cause toxicity following acute and chronic exposure. Concern was increased by reports of human poisonings, some of which involved the development of organophosphate-induced delayed neuropathy (OPIDN). These reports also led to methamidophos being classified as a chemical of high concern with regard to occupational health and safety. Furthermore, at the time of its nomination, the toxicology database on methamidophos contained a high proportion of studies that pre-dated good laboratory practice and internationally recognised testing guidelines, including a number of potentially invalid studies performed by the discredited International Bio-Test Laboratories.

Adequacy of the database

Nomination of methamidophos for assessment under the CRP has resulted in the submission of many studies that have not previously been evaluated in Australia; ie. dermal absorption, developmental, reproduction and neurotoxicity studies. The toxicological database on methamidophos is comprehensive. Several modern studies have examined the mechanism of methamidophos neurotoxicity and its potential to cause delayed neurotoxicity. Although delayed neurotoxicity has been demonstrated at supralethal doses, the mechanism of acute lethality, which was very high, has still not been elucidated. The toxicological database on methamidophos is considered to be adequate to support public health standards.

Metabolism and Toxicokinetics

Based on the observation that only small quantities of radioactivity are present in faeces (2-5%) after oral administration of radiolabelled methamidophos it is likely that absorption from the GI tract is almost complete. It is also rapidly absorbed and distributed to all tissues, including the placenta and foetuses in pregnant rats. Nearly all of an orally administered dose of methamidophos is metabolised within 24 h. Metabolites are excreted mainly via the urine; and a significant proportion is exhaled as carbon dioxide in expired air, although this pathway has not been fully elucidated. It is also excreted in the milk of lactating animals. The toxicokinetic and short-term toxicity studies indicated that there was a very low potential for methamidophos or its metabolites to bioaccumulate in tissues. This finding is consistent with its lipophobicity based on a consideration of the octanol/water partition coefficient (log K_{ow} = -0.8) of methamidaphos.

Dermal absorption of methamidophos in rats was ~40% after 10-24 h at application concentrations which ranged between 5 and 500 µg/cm^2. In humans the rate was much lower when methamidophos was applied as an EC formulation, ie. ~5% at 3 µg/cm^2/8h. It is possible that this low absorption rate in humans is an overestimate of the real methamidophos diffusion rate since *in vitro* studies with rat and human skin showed a diffusion rate difference of about 100-fold and the presence of excipients in the EC formulation appeared to increase the rate of absorption in both species.

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Acute Toxicity

Technical methamidophos (73-76% purity) is highly toxic by all routes of exposure, including the ocular route. The oral LD_{50}s in all species tested were in the range of 15-30 mg/kg bw. In general, the signs of acute methamidophos toxicity were typical of organophosphate poisoning and included severe tremors, salivation, dyspnoea and, rarely, clonic convulsions. Clinical signs occurred within 5-10 minutes and deaths occurred between 20-30 min and 72 h; these are consistent with toxicokinetic data which showed that T_{max} occurred after about 10 min and coincided with the maximum severity of clinical signs and inhibition of ChE activity in the CNS.

The dermal LD_{50}s ranged between 108 to 179 mg/kg bw in rats and between 69 and 122 mg/kg bw in rabbits. Deaths and clinical signs were seen in rabbits following dermal doses as low as 0.1 mL methamidophos (approx. 30 mg/kg bw), but no mortality or clinical signs were observed following dermal application of 0.003 mL to rabbits for 24 h (Hixson *et al*, 1980b). Methamidophos caused only slight eye irritation and it was not a skin sensitiser or irritant.

Modes of action and toxicological profile

In contrast to certain organophosphorus compounds such as parathion, chlorpyriphos and fenitrothion which require metabolic activation in order to generate a compound (ie. an oxon) capable of significantly inhibiting ChE activity methamidophos does not. Inhibition of ChE activity in rats appears to correlate with concentrations of methamidophos in the CNS and PNS and coincides with the severity of clinical signs and inhibition of ChE activity in the CNS. Clinical signs disappeared when ChE activity in the CNS was >50% of control activity, although plasma levels were still depressed (27% of controls). In repeat dose dietary studies in rats the plasma, RBC and brain ChE activities were significantly inhibited at 0.2 mg/kg bw/d and above, and the effects were generally greater in females than in males. The clinical signs (tremors, ungroomed coats, reduced mobility and weakness of the rear extremeties) occurred at doses which produced inhibition of ChE activities by 70-80% in plasma and 60-70% in brain. All rats that died after acute dosing with methamidophos did so within 15 min after treatment. In these rats the mean brain ChE activity was reduced to 12.4% of control activity. In those that survived the brain and plasma ChE was 50% of control activity by 24 hours after dosing.

With respect to lethality, clinical signs and degree of ChE inhibition there was no apparent differences between the technical grade methamidophos (~75% purity), individual enantiomers or the racemic mixture. Females were more sensitive than males to the effects of methamidophos; in general, clinical signs were more severe, mortality was greater and the degree of ChE inhibition was greater in females.

Studies on rat skeletal muscle suggested that the effect of methamidophos on neuromuscular transmission is most likely to be solely an inhibition of ChE activity. Following inhibition, ChE undergoes spontaneous reactivation Reversal of inhibition took twice as long as inhibition.

Carcinogenicity

Methamidophos was not carcinogenic in two-year (life-long) dietary studies at doses up to 25 ppm in mice or at up to 54 ppm in rats.

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Methamidophos has been assayed for genotoxicity in a wide variety of *in vitro* and *in vivo* test systems. Several reverse mutation assays in bacteria and forward mutation assays in mammalian cells were uniformly negative, with and without metabolic activation. Negative results were obtained in *in vitro* tests for DNA damage and repair, and *in vivo* tests for dominant lethal mutations and chromosomal effects. Negative, equivocal or weakly positive effects were obtained in *in vitro* tests for chromosomal aberrations. Some tests for chromosomal aberrations and sister chromatid exchange (SCE) were negative with metabolic activation and equivocal or weakly positive without metabolic activation, suggesting that the metabolites are not genotoxic. *In vivo* tests for tests for micronucleated marrow cells, chromosome aberrations and sister chromatid exchange were negative or weakly positive. The *in vivo* methylation of guanine (7- methylguanine) was demonstrated in mice and, in one assay for SCE, a cell-cycle delay was observed. Taken together, these results suggest that methamidophos can form DNA adducts and is weakly clastogenic, but is not mutagenic.

Reproduction and Development

Methamidophos does not cause reproductive toxicity or foetal developmental malformations, but was foetotoxic at maternally toxic doses. In 2-generation reproduction studies in rats the doses which produced parental toxicity (reduced food consumption and bodyweight gain) also caused a reduction in bodyweight and survival of the offspring. Inhibition of plasma, RBC and brain ChE activities were observed in both parental animals and neonates at equivalent doses.

In developmental studies in rats, foetal bodyweight was reduced and the development was delayed at maternally toxic doses but no malformations or variations were observed. In rabbits, maternal toxicity (clinical signs, reduced bodyweight gain) was evident at 0.1-2.5 mg/kg bw/d, but there was no effect of treatment on foetal development.

Neurotoxicity

Twenty-three neurotoxicity studies were reviewed. In acute studies, methamidophos produced signs of acute neurotoxicity in rats (ataxia, fasciculations, salivation and tremors), appearing soon after administration of methamidophos and resolving within a few days to a week. There was no histological evidence of injury to the central or peripheral nervous system following acute or chronic dosing.

In subchronic neurotoxicity studies, rats showed cholinergic signs and significant decreases in forelimb grip strength and motor activity, throughout the exposure period. Plasma, brain and RBC ChE activities were significantly decreased during the exposure period.

The inhibition of aged neurotoxic esterase (NTE) has been implicated in the development of organophosphate-induced delayed neuropathy (OPIDN), but little is known about the function of NTE or its specific role in OPIDN. Methamidophos technical (73% purity) and methamidophos analytical (>99% purity) showed a similar capacity to inhibit NTE *in vitro*; however, methamidophos technical produced a significantly higher proportion of aged-inhibited NTE than methamidophos analytical. This may be due to an impurity or to a metabolite, since the proportion of aged-inhibited NTE formed by methamidophos technical with metabolic activation was twice as high as that formed without activation. In most *in vivo* studies, neither methamidophos technical nor Tamaron™ Fl (52.4% methamidophos

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technical) produced signs of delayed neurotoxicity in treated animals and there were no histopathological findings of neuropathy in any study. OPIDN was produced in hens following high doses of (+)- methamidophos and, to a lesser extent, by the racemate, but there was no evidence of OPIDP following oral dosing with (-)-methamidophos. In another study, mild clinical signs of OPIDP were observed after a single dose of (+)- or (-)-methamidophos, but not with the racemate.

Developmental neurotoxicity

Two developmental neurotoxicity studies were reviewed, but neither was adequate to establish a NOEL. In one study, methamidophos produced evidence of cortical injury and behavioural toxicity in the offspring at the lowest dose (0.4 mg/kg bw/d), including delayed suspension reflex and auditory startle reflex, reduced swimming scores and elevated T-water maze scores. The other study was considered inadequate for regulatory purposes, due to the poorly documented methods, small group sizes (6 litters/group) and limited number of parameters examined. Pups at 1 mg/kg bw/d had delayed physical development parameters (pinnae detachment, eye opening, eruption of incisors, vaginal opening, testes descent) and an apparent increase in the amount of spontaneous movement in open field studies, but there was no effect of treatment on performance in the swimming test.

In humans, there is limited evidence that acute methamidophos poisoning in a pregnant female, sufficient to produce acute cholinergic signs, was not harmful to the unborn child.

Human Toxicity

There are a number of reports of accidental or intentional exposure to methamidophos. In general, typical signs of acute anticholinesterase poisoning (pallor, salivation, sweating, nausea or vomiting, headache and abdominal pain and/or swelling and arterial hypotension) appear soon after exposure, and patients may become unconscious for 1-3 d. the amounts ingested are usually in the range 30-80 mL but may be as much as 200 mL (600 mg/kg bw).
Following resolution of the acute cholinergic crisis, many patients develop proximal neurological signs known as the “intermediate syndrome” (parasthesia, muscle pain, muscle weakness), lasting a few weeks. Weeks or months after exposure, sensory and peripheral neuropathy, characteristic of OPIDN, may develop. The clinical manifestations of OPIDP were sensory disturbances (numbness and paresthesia in the distal limbs) followed by dyskinesia (weakness, difficulties in raising arms and legs, dysbasia, loss of capacity to stand and walk, and difficulty with fine movements of hands and fingers. Typically most recovered within about 2 years. All patients cited in these reports survived, and no reports of deaths have been found in the medical literature. Plasma or RBC ChE activities have generally not been measured in these reported cases. Sun, Zhou & Xue (1998) stated that, in all OPIDP cases, blood ChE activity was severely inhibited and the disappearance of cholinergic signs coincided with the restoration of ChE to "normal" levels, but no data were provided to substantiate this claim. In the young male who ingested 200 mL methamidophos (estimated dose 600 mg/kg bw), plasma and erythrocyte ChE activities were < 10% of unspecified normal values and began to recover from d 4. Lymphocytic neuropathy target esterase was decreased by up to 80% but showed some recovery at 4 d and by 9 d was restored to normal levels. This is consistent with the resolution of clinical signs, since the patient was fully recovered by d 11.

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DOSE LEVELS RELEVANT FOR RISK ASSESSMENT

To determine the lowest NOEL for the establishment of an ADI (health standards for methamidophos), a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is shown in the Table below.

Study	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d) and Toxic Effects
Rat 2-mo dietary Christenson *et al*, 1991	0.03	0.06; Plasma, RBC and brain ChE inhibition
Rat 2-y dietary Hayes *et al*, 1984b	0.1	0.3; Plasma, RBC and brain ChE inhibition
Dog 1-y dietary Hayes *et al*, 1984c	0.06	0.22; Plasma, RBC and brain ChE inhibition
Rat 2-generation reproduction Eigenberg *et al*, 1998; Astroff & Eigenberg, 1998; Moore, 1999	Parental toxicity: not established Reproductive toxicity: 0.2	Parental toxicity Reproductive toxicity 2; Reduced bw gain and survival of pups, plasma, RBC and brain ChE inhibition
Rat developmental Astroff *et al*, 1996b	Maternal and Developmental: 0.14	4; Maternal clinical signs and plasma, RBC and brain ChE inhibition; Reduced litter and placental weights bw gain and survival of pups;
Rabbit developmental Hoberman *et al*, 1996	Maternal: 0.2 Developmental: 2.5	0.65; Maternal clinical signs, reduced bw gain Developmental: no effect at highest dose
Rat acute neurotoxicity Sheets *et al*, 1994a	0.3	0.6; Plasma, RBC and brain ChE inhibition
Rat subchronic neurotoxicity Sheets *et al* 1994b, 1996	0.07	0.8; Plasma, RBC and brain ChE inhibition and neurobehavioural signs

HUMAN EXPOSURE

In Australia, two products containing methamidophos are registered for the control of thrips and insects. Over 95% of methamidophos use is on vegetables (principally brassicas, potatoes and tomatoes), with some additional minor uses on field-grown ornamentals, lupins, peaches and peanuts. Owing to its high oral toxicity methamidophos use is restricted to the professional agricultural sector. Consequently there are no registered home garden products.

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There are no objections on toxicological grounds to the ongoing approval of methamidophos active constituent.

Acceptable Daily Intake

The current ADI for methamidophos is 0.0006 mg/kg bw/d, set by the [then] PACC in February 1986. This ADI was derived from a NOEL of 0.06 mg/kg bw/d for inhibition of

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plasma, RBC and brain ChE activity seen in a dog 1-yr study, and using a 100-fold safety factor. This review has identified a new short term repeat-dose dietary study in rats, in which there was significant inhibition of plasma, RBC and brain ChE at 0.06 mg/kg bw/d with a NOEL of 0.03 mg/kg bw/d. Consequently, it is proposed that the ADI for methamidophos be revised. The revised ADI is 0.0003 mg/kg bw/d, based on a NOEL of 0.03 mg/kg bw/d for plasma, RBC and brain ChE inhibition in a rat 8-wk dietary study, and using a 100-fold safety factor.

Acute Reference Dose

At present, an ARfD has not been set for methamidophos. Acute neurotoxicity studies in rats (Sheets *et al*, 1993 & 1994a) showed that acute cholinergic clinical signs appeared on d 0 following administration of single oral gavage doses of 3 or 8 mg/kg bw. At 0.6 mg/kg bw and above, there were significant decreases in RBC and brain ChE activities. The NOEL was 0.3 mg/kg bw methamidophos, based on inhibition of RBC and brain ChE at the higher dose. Consequently, an ARfD of 0.003 mg/kg bw is established using a NOEL of 0.3 mg/kg bw for RBC and brain ChE inhibition in an acute neurotoxicity study in rats and the application of a 100-fold safety factor.

NDPSC Considerations

Methamidophos is currently classified in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons. There are no lower Schedule entries for dilute preparations. The acute toxicity of methamidophos is consistent with the S7 criteria and no change is proposed to this entry.

First-Aid Instructions

At present, the following standard statements for methamidophos are specified in the *Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary Chemicals* (see at http://www.health.gov.au/tga/docs/pdf/faisd.pdf).

Methamidophos	a, h	If poisoning occurs, contact a doctor or Poisons Information Centre. *Phone Australia 131126; New Zealand 03 4747000.* 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.

These instructions are considered appropriate for an OP of high acute toxicity, and no revisions are proposed.

Safety Directions

The current safety directions for products containing methamidophos are shown below.

Existing Safety Directions

AC EC LD all strengths

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100 101	Very dangerous, particularly the concentrate.
120 121 130 131 132 133	Product and spray are poisonous if absorbed by skin contact, inhaled or swallowed.
210 211 212	Avoid contact with eyes, skin and clothing.
220 223	Do not inhale spray mist.
190	Repeated minor exposure may have a cumulative poisoning effect.
373	Obtain an emergency supply of atropine tablets 0.6 mg.
279 280 281 282 290 291 294 298 301 303	When opening the container, preparing spray and using the prepared spray wear protective waterproof clothing, elbow-length PVC gloves, impervious footwear and full facepiece respirator with combined dust and gas cartridge.
330 331 332	If clothing becomes contaminated with product or wet with spray, remove clothing immediately.
340 341 342	If product or spray 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, respirator and if rubber wash with detergent and warm water and contaminated clothing.

Two methamidophos-based products are currently registered in Australia: **Nitofol Insecticide Spray** (a 580 g/L Emulsifiable Concentrate) and **Monitor Insecticide** (a 580 g/L Soluble Concentrate). The safety directions should be amended to include SC formulations.

Nitofol Insecticide Spray is applied by spray to brassicas, ornamentals, peaches and tomatoes at 50 – 200 mL product/100 L water, while capsicums and potatoes are treated at 50 – 190 mL/100 L or at a rate of 550 – 700 mL/ha. Volume rates are increased by up to 5-fold when semi-concentrate spray equipment is used. Peanuts and lupins are treated at 250 and 550 mL/ha, respectively. The required quantity of product is mixed with water in the spray tank under agitation. The use pattern of **Monitor Insecticide** is closely similar. The labels of both products warn against application in glasshouses or other enclosed areas.

NOTE: The NOHSC will advise of any changes that are necessary to personal protective equipment *before* amendments are included in the FAISD Handbook.

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RECOMMENDATIONS

1. Approval Status

The approval status of methamidophos active constituent remains appropriate.

2. Acceptable Daily Intake

The revised ADI for methamidophos is 0.0003 mg/kg bw/d, derived by application of a 100-fold safety factor to a NOEL of 0.03 mg/kg bw/d in a 2-month dietary study in rats, based on inhibition of plasma, RBC and brain ChE activity at the higher doses.

3. Acute Reference Dose

The ARfD for methamidophos is 0.003 mg/kg bw, using a NOEL of 0.3 mg/kg bw for plasma, RBC and brain ChE inhibition in an acute neurotoxicity study in rats and the application of a 100-fold safety factor.

4. Poisons Scheduling

Methamidophos is currently listed in Schedule 7 of the SUSDP. No change to the scheduling status is warranted.

5. First Aid and Safety Directions

The current first aid instructions remain appropriate. The safety directions to be provided by NOHSC and those provided here, together form the SDs which will be included in the FAISD Handbook, and which should be included on the product label.

Amended Entry

		Hazard statement codes
Methamidophos	AC EC SC LD all strengths	100 101 120 121 130 131 132 133 210 211 212 220 223 190 373 340 341 342 350

The hazard statement codes above refer to the following safety directions:
Very dangerous, particularly the concentrate; Product and spray are poisonous if absorbed by skin contact, inhaled or swallowed; Avoid contact with eyes, skin and clothing; Do not inhale spray mist; If product on skin, immediately wash area with soap and water; Repeated minor exposure may have a cumulative poisoning effect; Obtain an emergency supply of atropine tablets 0.6 mg; If product or spray on skin, immediately wash area with soap and water; After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.

NOTE: the NOHSC will advise of any changes that are necessary to personal protective equipment *before* amendments are included in the FAISD Handbook.

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6. Product Registration

There is no objection on public health grounds to the on-going registration of existing methamidophos products.

7. Additional Data

No additional data are required.

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MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Methamidophos is a S-substituted phosphorothioamidate organophosphorus (OP) pesticide having systemic and residual activity. It mediates toxicity through preventing the normal transmission of nervous impulses by inhibition of cholinesterase (ChE) enzymes.

In Australia, methamidophos-based products have been registered since the early 1970s. Currently, two products containing methamidophos are registered in Australia. They are intended for the control of thrips and insects in professional agricultural settings only. Over 95% of methamidophos use is on vegetables (principally brassicas, potatoes and tomatoes), with some additional minor uses on field-grown ornamentals, lupins, peaches and peanuts. Application is mainly by high volume boom spray equipment.

This review has been performed by the OCS to establish the potential for methamidophos to cause toxicity to humans (including the public and persons exposed in occupational settings), especially through acute or chronic injury to the nervous system. Utilising the expanded and modernised toxicology database now available, the review encompasses a hazard assessment of methamidophos and a risk assessment based on consideration of the toxicological hazards and the potential for human exposure to methamidophos. The review also includes risk management recommendations, including establishment of an Acute Reference Dose (ARfD) for methamidophos, and examination of the adequacy of label safety directions and existing public health standards including the Poisons Schedule and Acceptable Daily Intake (ADI).

1.1 History of Public Health Considerations In Australia

Australian public health standards for agricultural and veterinary chemicals that may enter the food chain include the Poisons Schedule, First Aid and Safety Directions, and the human acceptable daily intake (ADI). A further regulatory standard called the maximum residue level (MRL) is a measure of the residues present in unprocessed food (eg. grain, meat etc.) and hence is an indicator of good agricultural practice.

From the mid 1950s until 1992, Australian public health standards were set by committee process under the auspices of the NHMRC. “Pesticide Tolerances” in food were first set in 1956 by the Food Additives Committee. Between 1962 and 1966, the Food Additives Committee maintained a Sub-Committee on Pesticides and Agricultural Chemical Residues In Or On Foods (later re-named the Pesticide Residues in Food Sub-Committee), which adopted the then Canadian scheme as a basis for establishing tolerances. From 1967 onwards, Australian MRLs and ADIs for pesticides were established by the Pesticide and Agricultural Chemicals Committee (PACC), until the Department of Health and Ageing became directly responsible for setting ADIs in November 1992. Responsibility for pesticide and veterinary chemical MRLs in food was transferred to the NRA in June 1994, after which the PACC was removed from the control of the NHMRC and re-constituted as the Advisory Committee on Pesticides and Health (ACPH). The ACPH provides the Department of Health and Ageing, the OCS and the NRA with advice on issues of policy and practice having possible implications for public health and the proper use of chemicals in agriculture and elsewhere.

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Poisons Schedules for agricultural and veterinary chemicals, drugs and some other hazardous substances are set by the National Drugs and Poisons Schedule Committee (NDPSC). Originally known as the Committee on Poisons Scheduling, the NDPSC was established in 1955 as a sub-committee of the NHMRC Public Health Committee. The NDPSC publishes its decisions in the Standard for the Uniform Scheduling of Drugs and Poisons, which recommends controls on availability, labelling, packaging and advertising. These are incorporated into and enforced by the various Australian State and Territory legislative systems. In 1994, the NDPSC was transferred from the NHMRC to the Australian Health Ministers' Advisory Council, and was re-constituted again in 1999 as a Statutory Committee of the Therapeutic Goods Administration.

A third committee formerly involved in chemicals management was the NHMRC Standing Committee on Toxicity (SCOT), which was active between 1985 and 1994. SCOT was responsible for providing specialised advice on complex toxicological matters to all the NHMRC Public Health Committee subordinate committees, including the PACC and NDPSC. In response to referrals from these committees, SCOT undertook evaluation of some drugs, pesticides, food additives, poisons, consumer products, chemicals and other hazardous substances relevant to public health.

Methamidophos

A Poisons Schedule, Maximum Residue Limits and an ADI for methamidophos were first established in 1971. The ADI was set at 0.01 mg/kg bw/d based on a NOEL of 30 ppm in the diet of rats and dogs. In 1986, the ADI value was revised to 0.0006 mg/kg bw/d, based on a NOEL of 0.06 mg/kg bw/d in a chronic dog study. An acute RfD (ARfD) has not yet been recommended in Australia. A detailed history of the consideration of methamidophos by regulatory committees in Australia is presented below:

Date	Regulatory Activity
February 1971	NDPSC: Methamidophos placed in S7 following consideration of submission from Bayer. An explicit rationale for decision was not given but Committee noted the high acute oral and dermal toxicity of methamidophos.
March 1971	PACC: Evaluation report considered. Committee considered the pivotal NOEL to be 30 ppm in the diet of rats and dogs, and set an ADI of 0.01 mg/kg bw/d.
November 1984	PACC: Committee noted that database for methamidophos was almost entirely [potentially fraudulent] data from Industrial Biotest (IBT) Laboratories, including pivotal studies supporting the ADI. Some IBT replacement studies were considered. They indicated extreme dermal toxicity, possibly posing an occupational safety hazard, and Committee referred them to NDPSC. The [then] pivotal NOEL of 0.75 mg/kg bw/d was noted as exceeding those in the new 3-mo dog and rat studies (0.4 and 0.04 mg/kg bw/d, respectively). Further consideration of methamidophos deferred until foreshadowed submission of long term studies.
February 1985	PACC: Committee described most IBT studies as invalid, again noting heavy reliance on data from this source. Conclusions from the previous Meeting were reiterated.
February 1985	NDPSC: Committee considered additional data provided by Chevron, including percutaneous absorption in animals, delayed neurotoxicity in humans and an IBT Study Group review. Schedule status was not amended, but deaths of rabbits during skin and eye irritation studies were of concern and appropriate Safety Directions were recommended. Reported finding of delayed neurotoxicity in humans was of concern, but exposure levels required were

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Date	Regulatory Activity
	considered to be higher than expected if recommended precautions in handing OPs were adopted. It was noted that submission of IBT replacement studies was expected.
February 1986	PACC: A toxicological data set from Chevron, including chronic studies and IBT replacement data, was considered. Methamidophos lacked teratogenic, mutagenic or oncogenic activity. Based on a NOEL of 0.06 mg/kg bw/d in a chronic dog study, a revised ADI of 0.0006 mg/kg bw/d was agreed to. Committee requested provision of a delayed neurotoxicity study, due to unavailability of such data.
May 1986	NDPSC: Evaluation report from PACC considered. Committee found no reason to change S7 status of methamidophos, or amend Safety Directions.
November 1987	PACC: Committee noted the presence of methamidophos residues in commodities for which MRLs did not exist, and that methamidophos was a residue of acephate, resulting in unacceptable residues of methamidophos. MRLs for both acephate and methamidophos were therefore rationalised and harmonised with Codex.
February 1988	NDPSC: Following consideration of review of data submitted by Bayer, Committee noted the high acute toxicity of methamidophos. Concluded that new studies on potentiation and delayed neurotoxicity were similar to those seen previously. Studies also showed methamidophos lacked carcinogenic, mutagenic or teratogenic potential. Developmental studies were deemed satisfactory. Minutes record Committee as having considered that S7 was "***in***appropriate and that the existing Safety Directions and First Aid Instructions were adequate". In view of the lack of revision the Scheduling status or objection to clearance of the active constituent, it is probable that a typographical error caused the appearance of the word "inappropriate".
June 1988	PACC: Submissions from Bayer and Chevron were received, including acute dermal and 3-mo delayed neurotoxicity studies in hens. As previous recommendations were unaffected, no action was required.
February 1991	NDPSC: Committee noted acute and short-term repeat-dose inhalation toxicity studies in rats. The submission (from Bayer) was considered as additional toxicology for information and no decision resulted.

ADI

The current ADI for methamidophos is 0.0006 mg/kg bw/d, set by the [then] PACC in February 1986. This ADI was derived from a NOEL of 0.06 mg/kg bw/d for inhibition of plasma, RBC and brain ChE activity seen in a dog 1-yr study, and using a 100-fold safety factor.

Poisons Scheduling

Methamidophos is currently classified in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons. There are no lower Schedule entries for dilute preparations.

1.2 International Toxicology Assessments

JMPR

Toxicology evaluations of methamidophos have been conducted by Joint WHO/FAO Meetings on Pesticide Residues (JMPR) in 1976, 1982, 1985, 1990 and 2002.

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The 1976 review established an ADI for methamidophos of 0-0.002 mg/kg bw. The ADI was based on a NOEL for plasma and RBC ChE depression of 0.04 mg/kg bw/day in a 90-d dietary study in dogs (Loser & Lorke, 1970a). The choice of a 20-fold safety factor was supported by a short-term repeat dose study performed by Industrial Bio-Test Laboratories (IBT) in humans (Garofalo et al., 1973). Subjects received oral doses of methamidophos and acephate combined at a ratio 1:4 or 1:9. With the 1:4 mixture, there was a NOEL for plasma ChE depression of 0.1 mg/kg bw/d (ie, 0.02 mg methamidophos/kg bw/d). The NOEL with the 1:9 mixture was 0.2 mg/kg bw/d (also 0.02 mg methamidophos/kg bw/d).

The 1982 JMPR re-evaluated the toxicology of methamidophos, using data replacing invalid studies conducted by IBT. A temporary ADI (0-0.0004 mg/kg bw) was allocated, based on application of a 100-fold safety factor to a NOEL for plasma and RBC ChE depression of 0.04 mg/kg bw/day in the above 90-d dietary study in dogs. The Meeting requested further long term studies (or validation of an IBT long term study on rats), a carcinogenicity study and a reproduction study in mammals.

In the 1985 JMPR evaluation of supplementary data, the ADI for methamidophos was revised to 0-0.0006 mg/kg bw/d, based on a NOEL for ChE inhibition of 0.06 mg/kg bw/d in a 1-year dog study (Hayes et al., 1984c) and a 100-fold safety factor.

A further review of methamidophos was undertaken by the 1990 JMPR. Based on a re-evaluation of the [now validated] IBT study in humans (Garofalo et al., 1973), the ADI was revised to 0-0.004 mg/kg bw/d. However, the basis for the ADI was not the same as the rationale adopted by the 1976 Meeting. JMPR 1990 assigned NOAELs of 0.2 and 0.3 mg/kg bw/d for RBC ChE depression caused by mixtures of methamidophos and acephate at ratios of 1:4 and 1:9, respectively. In allocating the ADI, it was assumed that the effect of each mixture was attributable to methamidophos alone, and so the respective estimates of the NOAEL for methamidophos were 0.04 and 0.03 mg/kg bw/d. Therefore, it appears that a 10-fold safety factor was applied.

The most recent toxicology review of methamidophos by JMPR occurred in 2002. Although the ADI remained unchanged at 0-0.004 mg/kg bw/d, its basis was revised to application of a 25-fold safety factor to a NOAEL for RBC and brain ChE depression of 0.1 mg/kg bw/d in a 2-yr dietary study in rats (Hayes et al., 1984b). The choice of safety factor was supported by NOAELs for ChE depression of 0.03 mg/kg bw/d in humans (Garofalo et al., 1973), 0.03 mg/kg bw/d in a 90-d study in dogs (Loser & Lorke, 1970a) and < 0.1 mg/kg bw/d in a multi-generation reproduction study in rats (Eigenberg et al., 1998). The Meeting assigned an ARfD of 0.01 mg methamidophos/kg bw, based on a NOAEL of 0.3 mg/kg bw for inhibition of RBC and brain ChE activity in an acute neurotoxicity study in rats (Sheets, 1993). Again, a 25-fold safety factor was applied.

The WHO has also published a Health & Safety Guide for methamidophos (No. 79; 1993).

US EPA

The US EPA has published an Interim Reregistration Eligibility Decision (IRED) on methamidophos, dated April 7, 2002 (see http://www.epa.gov/pesticides/op/methamidophos

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.htm)[1]. The Agency has determined that methamidophos will be eligible for reregistration, subject to occupational health and environmental risk mitigation measures. Risks of concern were identified for workers mixing, loading and applying methamidophos to agricultural sites and workers re-entering treated areas. Consequently, use of methamidophos on cotton will be phased out in the USA over a 5-yr period and engineering controls introduced to reduce exposure of applicators and flaggers. Restricted entry intervals have been increased. Acute and chronic dietary intake estimates did not exceed the US EPA's level of concern.

Methamidophos was classified as Toxicity Category 1 for acute oral, dermal and inhalation toxicity. Although the chemical was considered to be only a mild skin and moderate eye irritant, methamidophos was classified as Toxicity Category 1 via these routes because deaths and clinical signs occurred in rabbits shortly after dermal and ocular application. Methamidophos was classified as not a skin sensitiser and as a Group E carcinogen (not likely to be carcinogenic in humans, based on no evidence of carcinogenic effects in mice and rats). The US EPA concluded that methamidophos caused delayed neurotoxicity in hens following oral administration of doses greatly exceeding the oral LD_{50}, but elicited only neurobehavioural effects in rats by acute and subchronic exposure.

The acute reference dose (ARfD) was set at 0.003 mg/kg, based on plasma, RBC and brain ChE inhibition in acute neurotoxicity studies in rats (Sheets & Hamilton, 1993 and Sheets, 1994), for which a NOAEL of 0.3 mg/kg bw was established. An uncertainty factor of 100 was chosen.

The US EPA set the chronic reference dose (RfD) at 0.0003 mg/kg bw/d on the basis of brain ChE inhibition in a subchronic study in rats (Christenson, 1991), for which the NOAEL was 0.03 mg/kg bw/d. A 100-fold uncertainty factor was applied.

EU

Methamidophos is included in the EU's List 1 of first priority substances - those most widely used on the market as well as those of clear concern.[2] With Italy as the rapporteur state, the EU is currently reviewing methamidophos under Directive 91/414/EEC (15 July 1991), which sets out a Community harmonised framework for the authorisation, use and control of plant protection products. The Italian review of methamidophos was considered by the ECCO 109 Peer Review Meeting (report 12084/ECCO/BBA/01, dated 14 May 2001). An ARfD and ADI of 0.004 mg/kg bw/d was proposed, based on application of a 10-fold safety factor to a proposed NOAEL of 0.04 mg/kg bw/d a 21-d human study. However, the decision is dependent on clarification on the relative sensitivity of humans and animals to ChE inhibition by methamidophos.

In November 2001, a final decision on methamidophos was postponed until after the submission of additional data, which was required by May 2003 (Commission Decision 2001/810/EC; Official Journal L305 of 22/11/2001, p 32). Methamidophos was due for

[1]In 2006, the US EPA finalised the Reregistration Eligibility Decision (RED) for methamidophos. In 2009, the sole methamidophos registrant, Bayer CropScience, voluntarily cancelled all remaining methamidophos product registrations. In 2010, the US EPA released the Methamidophos Registration Review Decision. Methamidophos is also registered for use in Canada. In 2007, a Preliminary Risk and Value Assessment of methamidophos was released requesting additional data to finalise the assessment of this active and to propose regulatory action. A final decision on methamidophos is yet to be made by the Canadian Pest Management Regulatory Agency (PMRA).

[2]Approvals for methamidophos expired in the EU on 30 June 2008.

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reconsideration during the Round 14 ECCO Peer Review Meetings scheduled for Feb – Sept 2003. Documents pertaining to the technical and administrative aspects of this review process can be selected at http://www.europa.eu.int/comm/food/fs/ph_ps/pro/index_en.htm.

IARC

Methamidophos has not been evaluated by the International Agency for Research on Cancer.

1.3 Chemistry - Active constituent

Approved common name: methamidophos (ISO)

Alternative names: SRA 5172, Bayer 71 628, RE 9006, Monitor, Tamaron, Nitofol

Chemical names: Thiophosphoramidic acid, O,S-dimethyl ester (IUPAC)

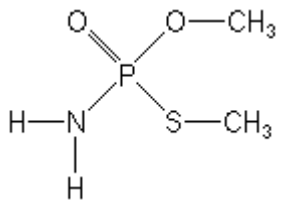
Phosphoramidothioic acid, O,S-dimethyl ester (CAS)

CAS Registry number: 10265-92-6

Empirical formula: $C_2H_8NO_2PS$

Molecular weight: 141.1

Chemical structure:



Chemical class: S-substituted phosphorothioamidate

Structural analogues: Acephate

Chemical and physical properties

Colour:	Colourless (pure) Colourless to yellowish (technical grade)
Odour:	Mercaptan-like
Physical state:	Crystals (pure) Slurry or liquid (technical grade)
Melting point	45 ^{0}C
Density (20^{0}C):	1.27 g/cm^3
Partition coefficient: (log K_{ow} @ 20^{0}C)	-0.80
Vapour pressure:	2.3 x 10^{-5} hPa @ 20^{0}C

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Solubility	
in water:	>200 g/L @ 20^0C
in organic solvents:	< 1 g/L in n-hexane, 2-5 g/L in toluene, > 200 g/L in dichloromethane, 2-propanol, acetone and dimethylformamide (all @ 20^0C)
Stability:	Stable at room temperature

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Impurities of Toxicological Concern

The APVMA Minimum Compositional Standards specifies a minimum content of 730 g/kg methamidophos in methamidophos active constituents. However, no maximum impurity levels are specified.

FAO Provisional Specification 355/TC/S/P (1992) specifies that methamidophos technical shall contain not less than 680 g/kg methamidophos, and lists the following impurities:

Impurity	Maximum content (g/kg)
O,O-dimethyl phosphoroamidothioate	90
N-methyl homologues	80
O,O,O-trimethyl phosphorothioate	70
O,O,S-trimethyl phosphorothioate	20
Water	1

FAO Provisional Specification 355/TK/S/P (1992) specifies that methamidophos technical concentrates shall contain not less than 600 g/kg methamidophos, and lists the following impurities:

Impurity	Maximum percentage of methamidophos content
O,O-dimethyl phosphoroamidothioate	13
N-methyl homologues	12
O,O,O-trimethyl phosphorothioate	10
O,O,S-trimethyl phosphorothioate	3
Water	2 g/kg

1.4 Products

There are 2 registrants for methamidophos in Australia and there are currently 2 registered products. Registered uses include control of thrips and insects. Over 95% of methamidophos use is on vegetables (principally brassicas, potatoes and tomatoes), with some additional minor uses on field-grown ornamentals, lupins, peaches and peanuts. Use is restricted to the professional agricultural sector, as no home garden products are registered.

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2. METABOLISM AND TOXICOKINETICS

2.1 Rats

Crossley J & Tutass HO (1969): Metabolism of Monitor^{R} insecticide by rats. Chevron Report No. 239-EX-60. Project No. not specified. File No. 721.14 (Monitor X File: 741.10). Lab: Research and Development, Ortho Division, Chevron Chemical Company, Richmond, California, USA. Sponsor: Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Project Duration: not specified. Report Date: October 1969. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos laboratory grade, ^{14}C or ^{32}P labelled. Purity: ≥ 99.5%. Source: not specified.
Test Species:	Rats: Sprague-Dawley, male and female; no other details provided.
Dose:	^{14}C methamidophos 0.16-0.19 mg (0.43-0.51 μCi) ig; ^{32}P methamidophos 0.210 mg (2.72 μCi) ig.

Method

The "several" female rats used in ^{14}C studies were kept in a glass metabolic cage. The ^{32}P rats (12/sex) were housed in pairs in standard metal metabolic cages. Food and water were supplied *ad libitum*. Each rat received a single dose of radiolabelled methamidophos po (intragastrically) as an aqueous solution. The ^{14}C rats were not pre- or post-conditioned with unlabelled test material but were narcotised with ether for dosing. The ^{32}P rats were pre-conditioned for 2 wk with non-radioactive methamidophos (0.5 mg/kg bw/d). This treatment resumed the day after dosing and continued until termination. Narcosis was not used for dosing ^{32}P rats.

The ^{14}C study proceeded first to establish methods of analysis and to estimate the rate and path of metabolism. Expired carbon dioxide, urine and faeces were collected twice daily. The urine and faecal samples were frozen until analysis. Rats were terminated 5 d after treatment in an unspecified manner and brain, lung, kidney, liver, heart, spleen, femur bone, blood and fat were removed. The organs and remaining carcasses were frozen in dry ice and lyophilised within 24 h. Another group of rats killed at 9 d after treatment were processed the same way. The distribution of ^{14}C in various tissue components was determined in these rats.

In the ^{32}P study, urine and faeces were collected daily. Samples for d 5-7, 8-14, 15-21 and 21-28 were combined. All urine samples were assayed immediately. Faecal samples were frozen. Two rats of each sex were terminated in an unspecified manner on d 1, 3, 7, 14, 21 and 28. Liver, kidney, heart, muscle and fat samples from each rat were frozen.

Each sample was assayed for total radioactivity using standard procedures. Each sample was then extracted and chromatographed for metabolite identification. Statistical evaluation of results was not performed.

Results and discussion

Body wt gains, food consumption and urine and faeces excretion patterns were not affected. At the time of termination, there were no gross pathological findings.

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^{14}C study: The excretion of ^{14}C in the first 24 h is tabulated below. No quantitatively valid conclusion is possible. However most excretion of ^{14}C occurred via exhaled CO_2. For urine and expired air, the bulk of excretion occurred within 6 h. Faecal excretion of ^{14}C was minimal.

The excretion of ^{14}C in 24 h*

Time	% dose excreted via each excretion route			
	Urine	Expired air	Faeces	Total
0-6 h	9.2	32.1	no sample	41.3
6-24 h	0.8	1.9	0.5	2.7
Total up to termination (5 d)	11.1	38.8*	1.5	50.4

* The study authors conceded that carbon dioxide extractor used absorbed carbon dioxide. They suggested that this figure was closer to 60%

In rats killed at 5 d after dosing the distribution of ^{14}C (% administered dose) in the carcass, liver, kidney, heart and lung was 21.9, 0.4, 0.1, 0.3 and 0.1 respectively. Radioactivity in muscle, fat or bone was below the limit of detectability (<0.1% administered dose). With 50.4% ^{14}C excreted and 22.6% detected as tissue residues, a 73% ^{14}C recovery rate was obtained.

The location of the highest proportion of ^{14}C within each tissue in a different group of rats killed 9 d after treatment was as follows. Within carcasses, 49.8% was located in polar lipids (cf liver 21.1, lung 22.2 and kidney 27.2). Within liver 52.3% was incorporated in proteins (cf carcass 12.6, lung 18.2 and kidney 23.8). Within lung, 33.9% was located in nucleic acids (cf carcass 4.8, liver 7.1 and kidney 31.3) and within kidney, 31.3% was located in nucleic acids.

^{32}P study: The urinary excretion of ^{32}P radiolabel is tabulated below. Urine was the major route of excretion in males (57.5-72.6%) and females (43.2-73.7%) with most of the excretion in urine occurring in the first 24 h after dosing (males 49.7-64.6%; females 40.8-73.7%).

The cumulative urinary excretion of ^{32}P labelled methamidophos

Period of urinary collection	^{32}P (% administered dose) in urine at times of termination (d)											
	Male						Female					
	1	3	7	14	21	28	1	3	7	14	21	28
d 1	64.4	51.4	55.1	64.6	49.7	49.7	73.7	40.8	55.4	54.6	60.8	60.8
d 2		3.1	2.5	2.7	3.2	3.2		1.9	3.0	2.7	2.5	2.5
d 3		3.0	1.8	1.1	1.2	1.2		0.5	0.7	0.5	0.9	0.9
d 4			1.1	1.0	1.0	1.0			0.6	0.7	0.9	0.9
d 5-7			1.5	1.8	2.0	2.0			2.0	1.8	2.0	2.0
d 8-14				1.4	2.1	2.1				2.3	2.0	2.0
d 15-21					1.0	1.0					1.0	1.0
d 22-28						1.1						1.1
Cumulative total	64.4	57.5	62.0	72.6	60.2	61.3	73.7	43.2	61.7	62.6	70.1	71.2

The cumulative faecal excretion of ^{32}P radiolabel is tabulated below. The excretion by the faecal route increased 7-fold in males and 10-fold in females from d 1-28 after dosing.

The cumulative faecal excretion of ^{32}P-labelled methamidophos

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Period of faecal collection	^{32}P (% administered dose) at times of termination (d)											
	Male						Female					
	1	3	7	14	21	28	1	3	7	14	21	28
d 1	2.8	4.6	2.2	4.4	11.0	11.0	1.7	12.0	3.2	3.9	8.0	8.0
d 2		2.5	2.6	1.8	3.9	3.9		2.5	3.7	1.7	4.4	4.4
d 3		0.7	0.5	0.4	1.1	1.1		0.1	2.4	1.7	1.1	1.1
d 4			0.2	0.4	0.4	0.4			0.7	0.9	0.7	0.7
d 5-7			1.2	1.2	1.8	1.8			1.4	1.6	1.1	1.1
d 8-14				1.3	1.6	1.6				2.3	1.9	1.9
d 15-21					0.7	0.7					0.8	0.8
d 22-28						0.1						0
Cumulative total	2.8	7.8	6.7	9.5	20.5	20.6	1.7	14.6	11.4	12.1	18.0	18.0

The % administered dose in tissues (tabulated below) decreased as faecal excretion increased. This was attributed to the gastro-intestinal excretion of ^{32}P as a component of proteins and nucleic acids which are normally excreted by that route. The rate of excretion of proteins and nucleic acids was believed to have progressively increased with continued feeding by rats killed later after dosing.

Tissue residue of ^{32}P-labelled methamidophos

Tissue	^{32}P (% administered dose) in tissues at times of termination (d)											
	Male						Female					
	1	3	7	14	21	28	1	3	7	14	21	28
Liver	6.9	3.0	1.3	0.4	0.4	0.1	5.6	2.8	1.3	0.5	0.3	0.1
Kidney	0.5	0.7	0.2	0.1	0.1	0	0.4	0.6	0.2	0.1	0	0
Heart	0.1	0.3	0.1	0	0	0	0.1	0.2	0.1	0	0	0
Carcass	9.9	12.8	12.8	8.9	8.8	6.7	11.3	9.7	10.3	11.5	7.8	4.4
Fat*	0.1	0.1	0	0	0	0	0.2	0.1	0.1	0	0	0
Muscle**	0.2	0.3	0.3	0.1	0.1	0.1	0.3	0.3	0.3	0.2	0.2	0.1
Total	17.4	16.8	14.4	9.4	9.3	6.9	17.4	13.1	11.9	12.1	8.1	4.6

* % administered dose in 4 g fat sample; ** % administered dose in 2 g muscle sample

Metabolites: The relative proportions of the four metabolites detected in urine are tabulated below. Methamidophos rapidly disappeared after the 1st day. O,S-dimethyl phosphorothioate (DMPT) appeared only on the 1st day. Both methyl dihydrogen phosphate (MDP) and phosphoric acid (PA) were present in all samples to a varying degree. At d 4, the majority of radioactivity was present as simple inorganic phosphate.

Distribution of ^{32}P in metabolites urine following dosing

Chromatographically identified metabolites	^{32}P as % of the total urinary radioactivity on d 1 and 2			
	Male		Female	
	d 1	d 2	d 1	d 2
Methamidophos	40	10	40	Trace
O,S-dimethyl phosphorothioate (DMPT)	5	-	5	-
Methyl dihydrogen phosphate (MDP)	40	25	40	30
Phosphoric acid (PA)	15	65	15	70

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The maximum liver concentrations of methamidophos, DMPT and MDP/PA were 0.098 ppm on d 1, 0.059 ppm on d 3, and 0.720 ppm on d 1 respectively. These decreased to 0.008, 0.008 and 0.029 ppm respectively on d 14. The maximum carcass concentrations of methamidophos, DMPT and MDP/PA were 0.004 ppm on d 3, 0.0076 ppm on d 3, and 0.047 ppm on d 1 respectively. These decreased to 0.0002, 0.0001 and 0.0047 ppm respectively on d 14. The half-lives of methamidophos in liver and carcass were estimated to be 3 and 5 d respectively. Faeces were not investigated for metabolites. Low level radioactivity was extracted only from the 1st d samples.

Conclusions

(1) Methamidophos administered po to rats was rapidly metabolised and excreted.

(2) The metabolites of methamidophos were carbon dioxide, O,S-dimethyl phosphorothioate, methyl dihydrogen phosphate and phosphoric acid.

(3) There were no differences between males and females in the rate of metabolism, excretion and nature of metabolites.

(4) Two phases of excretion were detected: a period of rapid excretion lasting 1-3 d during which more than 1/2 of the applied dose is excreted; and a second period during which 1-2% of the applied dose is excreted daily.

(5) The dose remaining in the body after the 1st phase consisted of traces of methamidophos, its P-containing metabolites and natural constituents of the body which incorporated the radiolabels. Residual levels of methamidophos and its P-containing metabolites decreased to a concentration of 0.029 ppm in liver and 0.005 ppm in carcass tissue within 14 d of dosing.

(6) The degradation of methamidophos was hydrolytic in nature. The first step was the rupture of the P-N bond to form O,S-dimethyl phosphorothioate and ammonia. Demethylation takes place at the P-S bond and at the P-O bond to form methyl dihydrogen phosphate and phosphoric acid respectively.

Comment

The study authors did not comment on the production of carbon dioxide in the degradation of methamidophos.

Fakhr IMI, Abdel-Hamid FM & Afifi LM (1982): In vivo metabolism of ^{32}P Tamaron in the rat. Isotope & Rad Res, 14: 49-55.

Test Substance:	^{32}P Methamidophos: radiochemically pure; specific activity of 3800 cpm/µg. Source: Prepared by study authors.
Test Species:	Rat: males and females, bw 200-250 g of an unspecified strain, age or source.
Dose:	15 mg/kg bw po

Method

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Six mg prepared ^{32}P methamidophos in 1 mL water was administered orally in a single dose to an unspecified number of rats. They were kept in metabolic cages which permitted the separate collection of urine during a 24 h period. Urine samples were extracted several times with chloroform and concentrated. Acidic ^{32}P-labelled metabolites in 4 mL fractions of concentrated urine were separated by ion exchange column. Eluted fractions containing the highest radioactivity were used for thin layer chromatographic analysis (TLC) on cellulose and silica gel thin layers on plates. ^{32}P activity eluted from column chromatographic analysis was determined in solution. All measurements were carried out in an end window Tracer-lab GM-tube. The data were corrected for decay and background and no allowance was made for self-absorption. Radiometric assay for thin layer plates was carried out with a similar device.

Results and discussion

In several experiments, the total radioactivity recovered in the urine after 24 h accounted for 77% of the administered dose. The chloroform extracts of urine showed radioactivity at approximately 2% of the administered dose.

Methamidophos was highly degradable and was excreted in urine unchanged or as five polar ionic metabolites. A highly non-polar unknown metabolite was also detected. These metabolites are products of cleavage of one or more bonds of the P-S, P-O and P-N bonds. The presence of unchanged methamidophos in urine was attributed to its simple structure and its solubility in water. The % of metabolites of methamidophos isolated from urine of treated rats are tabulated below.

Methamidophos and metabolites in urine of ^{32}P -methamidophos treated rats

Substance	**Percentage of methamidophos and metabolites#**
Methamidophos	1.4
Phosphoric acid	27.5
S-methyl thiophosphoric acid	11.2
O,S-dimethyl thiophosphoric acid	22.5
O-methyl phosphoric acid amide	18.0
S-methyl phosphoramidothioic acid	12.1
Unknown	7.3

Determined from the relative abundance of the ^{32}P-metabolites on the thin layer chromatograms; the total activity recovered in urine = 100%.

Conclusion

The study authors concluded that the toxicity of methamidophos was not attributable to an inability of rats to metabolise and eliminate the compound.

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Gray AJ, Thompson CM & Fukuto TR (1982): Distribution and excretion of [$^{14}CH_3S$]Methamidophos after intravenous administration of a toxic dose and the relationship with anticholinesterase activity. Pesticide Biochemistry and Physiology, 18: 28-37.

Test Substances:	[$^{14}CH_3S$]Methamidophos; unlabelled Methamidophos. Purity: > 99%. Source: Prepared by the study authors using materials supplied by Aldrich Chem. Co. Milwaukee, Wisc. ICN, Irvine Calif. and Analtech, Newark, Del.
Test Species:	Rats: Sprague-Dawley, female, bw 215-235 g, of an unspecified age obtained from Simonsen Laboratories, Gilroy, California.
Dose:	8 mg/kg bw iv.

Prelude

At issue for the study authors was the basis of the high mammalian toxicity of methamidophos. The paradox was that methamidophos is only a moderate inhibitor *in vitro* of AChE. It had been suggested (Khasawinah *et al.*, 1978) that either methamidophos persisted at sufficiently high concentrations *in vivo* to produce toxicity, or that it was metabolically activated to a more potent anticholinesterase, possibly to the S-oxide (Eto *et al.*, 1977; Suksayretrup & Plapp, 1977).

Method

The rats were given free access to Rat Chow 5012 pelleted diet (Lab Chows, Ralston Purina Company) and water. All unlabelled methamidophos dosing solutions were prepared in sterile normal saline immediately prior to use. The radiolabelled dosing solutions were prepared at 0.34-0.36 mCi/mmol by appropriate dilution with unlabelled methamidophos, stored at 4^{o}C and warmed to room temperature just prior to use. These solutions were periodically checked for radiochemical purity. If purity was < 99%, the solution was discarded and replaced. Doses were administered via the lateral tail vein. Four groups of 4 rats were given unspecified doses of unlabelled methamidophos to determine an iv LD_{50} (Weil, 1952). All distribution and excretion studies were carried out using a 16 mg/mL saline solution of radiolabelled methamidophos administered at 0.5 mL/kg - a dose of 8 mg/kg selected as just below the 10 mg/kg found to be the iv LD_{50} in rats. The clinical signs observed during the iv LD_{50} study were also recorded.

An unspecified number of rats were killed by decapitation between 1 min and 24 h after iv injection of labelled methamidophos and weighed portions of 20 tissues and fluids were taken. The tissues and fluids were solubilised for the determination of their radioactive content in a Beckman LS-230 scintillation counter. All results were corrected to disintegrations/min. Additional blood clearance curves were obtained from 4 rats cannulated in the tail artery and vein under ether anaesthesia. After injection of labelled methamidophos into the venous cannula, 20 x 50 µL blood samples of were taken from the arterial cannula between 15 s and 8 h later and weighed. These samples were solubilised and their radioactive content determined.

An unspecified number of rats were placed in all-glass metabolism cages at least 24 h prior to dosing for acclimatisation and collection of control samples. Immediately after iv dosing the

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rats were again placed in the cages and urine, faeces and expired CO_2 collected over 7 d and analysed for their radioactive content. At the end of each metabolism experiment, rats were killed with ether and their carcasses homogenised in water. Five 0.5 mL samples of each homogenate were combusted and their radioactive content determined. The cages were rinsed with water to collect residual radioactivity.

The *in vivo* pseudo and AChE activities of plasma, RBC, cerebellum and combined brainstem and spinal cord were determined (Ellman *et al.*, 1961). Thirty rats (3/time interval) given 8 mg/kg unlabelled methamidophos were killed by decapitation at 1, 10, 20, 30, 60, 120, 240, 360 min and 24 and 48 h after dosing. An additional 5 control rats were also used. Duplicate samples of heparinised blood were processed for plasma and RBC ChE determinations as soon as possible after sampling. The weighed central nervous samples (CNS) samples were stored up to 14 d at -20^{o}C and thawed prior to analysis. The CNS samples were then homogenised and ChE activity determined.

The *in vitro* rates of ChE activity inhibition (k_i values) of methamidophos with cerebrum, cerebellum, spinal cord and brain stem, RBC and plasma were also determined. Tissue samples from untreated rats were homogenised. Unlabelled methamidophos (10 µL of a 0.705 mg/mL solution) was added to the untreated homogenate samples and mixed for 0.5 min to give a final inhibitor concentration of 5 x 10^{-5} mol. ChE activity was determined at 1, 3, 5, 10, 15, 21 and 28 min after the addition of methamidophos. The *in vitro* rates of inhibition of RBC and plasma ChE were measured separately using the same final inhibitor concentration at the same time intervals.

Results and discussion

Toxicity: The iv LD_{50} of methamidophos was calculated as 10 mg/kg.

Clinical signs: The signs of toxicity observed during the LD_{50} study were fine muscular tremors, salivation and lacrymation. The time course and severity of the signs were dose-dependent, occurring earlier and with greater severity at higher doses. No deaths occurred at the 8 mg/kg dose chosen for the study. This dose induced mild tremors of the chin and nose within 5 min. These slowly increased in severity until whole-body tremors were noted at 10-12 min. Then profuse salivation also occurred in some rats. The tremors were most severe at 20-60 min when the rats had difficulty maintaining their balance and mostly remained prostrate. Most rats appeared to be showing some signs of recovery at 60 min but complete recovery took 6-8 h. All rats appeared normal at 24 h. Body wt loss reversed by 72 h.

Distribution and clearance: The distribution of radiolabelled methamidophos in various tissues and fluids at selected times after dosing is tabulated below. The data is presented in descending order of radiolabelled content concentration at 1 min. The measurements are the total of radiolabelled methamidophos and metabolites. The distribution of radiolabelled methamidophos was rapid with levels in most tissues peaking at 1 min. This suggests that as methamidophos has a high aqueous solubility it is distributed primarily in body water. Large amounts of radioactivity were detected in the kidney during the first 2 h indicating that labelled methamidophos and metabolites may be rapidly excreted in the urine. The radiolabelled content of brown fat was 3-5-fold higher than that of white fat - an observation consistent with the higher blood content and flow found in brown fat deposits. The increase in radiolabelled content of adrenals over 2-24 h presented a contrasting trend. Uniformity of distribution was less evident at 120 h and beyond. The differences between small intestinal

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and large intestinal contents were attributed to possible enterohepatic circulation of methamidophos.

The distribution of [$^{14}CH_3S$]methamidophos in various tissues

Tissue/fluid	Concentration of radiolabelled methamidophos (nmol/g) at various times (min) after dosing with 8 mg/kg iv*				
	1	**30**	**60**	**120**	**24 h**
Small intestine and contents	792.5	560.4	397.9	176.9	164.9
Large intestine and contents	226.3	242.0	242.1	144.3	45.4
Kidney	205.4	92.3	71.4	54.3	27.8
Plasma	131.1	70.1	51.1	37.9	8.5
Blood	114.0	65.7	58.2	30.7	10.7
Adrenals	112.5	71.1	52.5	65.4	96.2
Spleen	110.2	62.0	53.5	20.9	20.1
Salivary glands	102.0	65.1	44.6	28.7	22.4
Heart	101.1	57.5	46.0	20.7	11.4
Lung	97.1	60.2	57.2	47.5	28.8
Stomach and contents	84.6	153.1	143.3	48.9	24.0
Liver	77.6	53.7	56.0	42.0	42.1
Muscle	63.8	57.9	39.7	26.8	4.5
Brown fat	63.0	30.7	28.2	9.2	11.4
Thymus	45.1	57.3	42.6	23.3	21.4
RBC	38.0	25.0	28.5	8.7	5.7
White fat	17.8	6.2	6.2	2.3	4.1

* All values were from single rats except for those at 30 and 60 min which were the mean of 3 rats

The study authors presented the peripheral nervous system (PNS) and CNS radioactive levels as a curve against time which is not reproduced here. The level of radioactivity in the nervous system was generally lower than most other tissues with the exception of white and brown fat. The PNS (ie. sciatic nerve) and CNS (ie. cerebellum, cerebrum and combined spinal cord and brain stem) all contained similar amounts of labelled compound and reached maximum levels of 40 nmol/g between 20 and 60 min - coincident with an increasing severity of observed clinical signs. The even distribution of radiolabelled methamidophos in the CNS and PNS suggests that methamidophos is equally available to both systems.

A curve showing the clearance of radiolabelled content from the blood of 4 cannulated rats was presented by the study authors. The calculated dose for a 230 g rat was 13050 nmol. The blood volume was assumed to be "13 g". Approximately 80% of the dose had been cleared from the blood within 30-40 s. After 5 min, the rate of clearance was much slower. The initial phase of rapid clearance (0-5 min) was believed to correspond to the distribution of methamidophos to the tissues and the 2nd phase to its elimination from the body.

Excretion studies: Curves showing the mean cumulative excretion over 7 d of radiolabelled content via urine, faeces and expired CO_2 were presented by the study authors. The cumulative percentage of radioactivity excreted in urine was 52% with 47.5% being excreted in the first 24 h. Expired CO_2 accounted for 33.6% of radioactivity indicating loss of the labelled S-methyl moiety. Faeces contained trace amounts of radioactive content which never exceeded 5% over the period. A further 0.74% was recovered in cage washings and 6.4% remained in the carcass. The total recovery of radioactivity was 100.85 ± 5.2% of the administered dose.

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ChE determinations: The ChE activities in plasma, blood and 3 regions of the CNS at selected times after iv dosing with methamidophos are tabulated below. The highest ChE activity in the controls was measured in the cerebrum with those of the combined brain stem and spinal cord and cerebellum being 70 and 36% as active respectively. The relativity between the ChE activities of the 3 parts of the CNS was maintained over 48 h. The ChE activity of the CNS decreased to a minimum at 1 h - coincident with the increasing severity of clinical signs. Although the rats appeared normal at 24-48 h, their CNS ChE activity had not fully recovered by 48 h. The ChE activities in RBC showed a similar pattern of inhibition. However plasma ChE activity at 8 h was 26.8% of control activity cf CNS activity > 50% at the same time. At this time, the signs of toxicity had abated and rats were normal in appearance. Plasma ChE activity was not then a reliable guide to toxicity or to the degree of AChE inhibition in CNS.

ChE activities in plasma, RBC and CNS tissue at various times after dosing*

Tissue/fluid	**ChE activity (µmol acetylthiocholine/min/mL fluid or g tissue) and % of control activity at various times (h) after dosing with 8 mg/kg iv methamidophos#**					
	Control	**1 min**	**1**	**8**	**24**	**48 h**
Plasma	0.80 100.0	0.19 23.9	0.15 18.2	0.21 26.8	0.42 52.9	0.58 72.8
RBC	0.90 100.0	0.29 32.1	0.28 31.6	0.51 57.0	0.67 74.4	0.71 79.5
Combined brain stem and spinal cord	8.12 100.0	2.08 25.6	1.26 15.5	4.15 51.1	5.63 69.4	6.08 74.9
Cerebellum	4.17 100.0	1.48 35.5	0.80 19.2	2.36 56.4	3.09 74.0	3.64 87.1
Cerebrum	11.42 100.0	4.15 36.3	2.20 19.3	6.08 53.2	7.28 63.7	8.64 75.6

* It was not possible to determine ChE activity in PNS.
* Control group, n = 5; others, n = 3

ChE inhibition rate (k_i) determinations: The study authors presented rates of ChE inhibition curves for plasma, RBC and brain. The k_i values obtained for plasma ChE and RBC and cerebral AChE were 9.9×10^2, 8.2×10^2 and 0.2×10^3 mol/min respectively.

The inhibition constant for CNS tissue homogenates was low. This observation was similar to that of an earlier study (Robinson & Beiergrohslein, 1980). However, this value did not take into account the amount of methamidophos that may be available for AChE inhibition *in vivo*. The *in vitro* rate and extent of inhibition of AChE in the CNS homogenate was similar to that measured *in vivo* when the concentration of the labelled compound in the CNS was reproduced with methamidophos *in vitro* (approximately 50 nmol/g, 5×10^{-5} mol). Because methamidophos is stable *in vivo* (Khasawinah *et al.*, 1978) it was reasonable to assume that the majority of the radioactive material determined in the distribution experiments was present as the parent compound. This was confirmed by the study authors in work preliminary to this study which showed that brain samples from 3 rats killed 30 min after iv administration of [$^{14}CH_3S$]methamidophos contained only radiolabelled methamidophos. Methamidophos was the only radioactive compound isolated from liver homogenate and blood samples taken 30 min after dosing and also accounted for the majority of the radioactivity excreted in urine. It appeared therefore that unmetabolised methamidophos achieved sufficiently high levels in nervous tissue to account for the degree of AChE inhibition and toxicity observed.

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The ChE inhibition rates for plasma and RBC were of a low order and similar to previously published data (Robinson & Beiergrohslein, 1980). The degree and time course of *in vitro* inhibition was similar to that measured *in vivo* suggesting that unmetabolised methamidophos was probably responsible.

Conclusion

The study authors supported the suggestion that the high toxicity of methamidophos in mammals is due to large quantities of the inhibitor reaching the nervous system and to its *in vivo* stability rather than metabolic activation to the S-oxide.

Salama AKM (1990): Toxicological studies on some insecticides: pharmacokinetics, placental transfer, milk transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos and/or [^{14}C]-acephate in rat. Dissertation Alexandria University. Faculty of Agriculture. Bayer File No. M8399. Project No. not specified. Report No. not specified. Lab: Laboratory of Neurotoxicity, Department of Pharmacology, Duke University Medical Center, Durham, North Carolina, USA. Sponsor: not specified. Study Duration: not specified. Report Date: June 1990. Unpublished. (QA: Yes; GLP: No; Test Guidelines: not specified).

Test Substance:	Radiolabelled [$^{14}CH_3S$] methamidophos (SA 182 µci/mg) Source: Bayer Chemical Company, Leverkusen, Germany. Methamidophos laboratory grade. Purity: 99.8%. Source: Mobay Chemical Corporation, Kansas City, Missouri, USA.
Test Species:	Rat: Sprague-Dawley, female of average bw 330 ± 5.6 g, of an unspecified age obtained from Harlan Sprague-Dawley (Indianapolis, Indiana, USA).
Dose:	8 mg [$^{14}CH_3S$]-methamidophos/kg bw po (specific activity 8 µci/kg bw)

Method

Five studies were described in this report. Acephate is the *N*-acetyl derivative of methamidophos and is outside the scope of this review. Only aspects relating to methamidophos are evaluated.

Study 1: *Synthesis of acephate and some metabolites of acephate and methamidophos. Separation and identification by ^{1}H-NMRS and MS*: This study involved the synthesis, purification, separation and identification of radiolabel [^{14}C]-acephate and the metabolites of methamidophos and acephate. The metabolites synthetised were dimethyl phosphoramidate, methyl phosphoramidate, dihydroxy phosphoramidate, dimethyl phosphoroamidothionate, dihydroxy phosphoramidothionate, dimethyl phosphate and methyl phosphate. Acephate, methamidophos and their metabolites were then subjected to ^{1}H-NMRS, MS and ITLC.

Study 2: *Pharmacokinetics, tissue distribution, excretion, placental transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in pregnant rats*: Twenty four pregnant rats were given single oral doses of 0.5 mL/kg [$^{14}CH_3S$]-methamidophos at GD 18. Rats were terminated at 10 min, 0.5, 1, 3, 6, 12, 24 and 48 h after dosing. At each interval 3 rats were anaesthetised with diethyl ether and dissected. Blood samples were withdrawn from the heart by cardiac puncture with heparinised syringes and centrifuged. Brain, spinal cord, liver, lung, heart, kidney, spleen, muscle, small intestine and its contents, adipose tissue, skin and diaphragm

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were excised rinsed with saline, weighed and stored in glass vials at -60^{o}C. Uteruses were also dissected and placental/foetal units were removed carefully to avoid rupturing the amniotic sac. The cages were equipped to separate urine and faeces and permit the continuous drawing out of expired air. Traps were used to collect volatile organic compounds and CO_2. Urine, faeces and trapped materials were collected at each interval.

Samples of fresh tissues, body fluids, urine, dried ground faeces and homogenates of foetal rats were oxidised by combustion prior to determining total radioactivity trapped as $^{14}CO_2$, with a scintillation spectrometer. The radioactivity of trapped volatile organic compounds and CO_2 was also determined. Methamidophos and its metabolites were extracted from plasma, urine, faeces and homogenates of brain, liver, kidney, lung, heart, uterus, placenta and foetuses, faeces and urine. The identification of methamidophos and its metabolites was by GC-MS spectroscopic analysis and ITLC. Concentrations of metabolites were calculated from the radioactivity recovered from TLC sheets and specific activity of the dose.

The mean concentrations of methamidophos in plasma and other tissues as a function of time were determined and the pharmacokinetic profiles were fitted to a biexponential curve according to a two-compartment open model system (Gillette, 1974). The pharmacokinetics parameters were described by the exponential equation: $Ct = A_o e^{-\alpha t} + B_o e^{-\beta t}$ where Ct is the concentration of methamidophos in tissue at time t, A and B are the central and peripheral compartment of a two-compartment model, α, β are the rate constants for the 1st and 2nd phase of the biphasic model respectively

Study 3: *Pharmacokinetics, tissue distribution, milk transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in lactating rats*: The strain, source, bw, acclimatisation program and diet of the rats were the same as for the previous study. An unspecified number of pregnant rats were allowed to deliver and each dam and sucklings were caged together. Dams were dosed as in the previous study immediately after delivery. Sucklings were allowed to feed from their mothers for periods of 1, 3, 6, 12, 24, 36 and 48 h. At each interval sucklings were collected. At 12, 24 and 48 h, 3 dams were anaesthetised with diethyl ether and dissected. Blood and tissue samples were collected and stored and processed as before. Radioactivity counting and the identification and determination of methamidophos and its metabolites were carried out. Kinetic analysis was applied to methamidophos concentrations in the sucklings. Statistical analyses of results were not performed.

Results and discussion

Study 1: *Synthesis of acephate and some metabolites of acephate and methamidophos. Separation and identification by ^{1}H-NMR and MS*: This study was of a preliminary nature and established standards and criteria for the recognition of methamidophos and its metabolites. Various spectra showing retardation factors, retention times, relative abundance of the molecular ion and the prominent fragment ion for methamidophos and its metabolites were provided but are not reproduced here.

Study 2: *Pharmacokinetics, tissue distribution, excretion, placental transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in pregnant rats*:

Recovery rates: The % recovery of extractable radioactivity from tissues and fluids was plasma 72.1, brain 70.4, foetus 74.0, uterus 89.9, lung 93.1, kidney 75.4, placenta 78.4, liver 82.4, heart 82.3 and faeces 88.7.

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Clinical effects: No signs of acute cholinergic activity were observed. There were no effects on the number or viability of foetuses. At necropsy no differences between control and treated rats were observed.

Distribution of radioactivity: The mean concentrations of radioactivity in various tissues and plasma at each interval is tabulated below in descending order of radioactivity at 10 min. Within 10 min of dosing, radioactivity was observed in all tissues and fluids examined. Peak radioactivity occurred at 1 h except in plasma, RBC, heart and spleen. Periodic rises in mean concentration occurred in uterus and placenta at 1, 6 and 24 h and in foetus at 1 and 6 h. The low concentration of radioactivity in adipose tissue was consistent with the low lipophilicity of methamidophos and its metabolites.

Distribution of radioactivity in pregnant rats

Tissue or fluid	The mean concentration of radioactivity (ng methamidophos equivalents/g fresh tissue or mL of fluid) over time (h)							
	10 min	0.5	1	3	6	12	24	48
SI and c	784.11	1018.72	1406.15	251.32	234.24	118.86	117.86	71.73
Plasma	245.06	85.29	128.90	65.20	49.54	41.83	21.99	16.16
RBC	240.55	50.85	114.15	42.78	26.42	22.33	36.29	11.13
Kidney	223.28	136.02	280.87	113.92	184.01	91.03	112.22	66.94
Spleen	171.19	54.32	153.12	134.00	205.49	144.39	80.61	46.65
Lung	150.51	80.63	248.81	71.29	105.98	63.23	73.57	46.56
Liver	133.00	77.13	154.19	98.73	173.89	123.57	130.56	70.57
Heart	97.29	59.98	68.26	40.60	29.65	38.16	44.62	31.44
Placenta	96.84	53.90	124.66	58.32	74.30	58.96	75.67	32.45
D'gm	96.21	57.39	120.89	46.61	84.49	33.05	50.32	41.71
St and c	87.16	38.47	132.25	53.23	93.29	46.92	131.80	88.96
Muscle	85.02	63.64	103.18	31.37	51.04	19.42	15.89	14.26
Skin	82.62	57.85	112.32	42.14	57.66	37.74	15.09	50.99
Foetus	78.62	47.39	109.87	42.27	85.68	54.97	49.23	29.54
Uterus	64.46	76.60	223.61	82.22	136.79	90.12	159.15	62.45
Brain	56.12	36.51	100.98	55.17	53.13	18.69	18.86	13.06
Spinal c	37.88	26.08	61.42	33.41	45.09	12.96	19.26	9.23
AT	16.41	52.65	37.14	7.88	20.29	11.74	9.60	8.27

SI and c = small intestine and contents; D'gm = diaphragm; St and c = stomach and contents; Spinal c = spinal cord; AT = adipose tissue

Methamidophos in tissues: The concentrations of extractable radiolabelled methamidophos in tissues and plasma at each interval are tabulated below in order of descending concentrations at 10 min. The concentrations were corrected for 100% recovery and expressed on a wet wt basis. Within 10 min, methamidophos was found in all tissues examined and plasma. The rapid distribution of methamidophos is consistent with its high aqueous solubility. It is most likely distributed primarily in body water. The concentration of extractable methamidophos decreased with time. The study author suggested that its distribution became less uniform and that this was most likely the result of the selective retention of methamidophos.

Concentration of [^{14}C]-methamidophos in pregnant rats

Tissue or fluid	Mean concentration of [^{14}C]-methamidophos in tissues (ng methamidophos/g fresh tissue or mL of plasma) over time (h)							
	10 min	0.5	1	3	6	12	24	48

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Kidney	94.40	57.96	59.77	38.29	26.17	27.79	14.66	11.69
Plasma	69.95	23.43	26.75	24.31	20.74	25.62	18.53	16.55
Placenta	65.01	46.67	40.60	36.6	26.81	13.47	13.04	6.77
Foetus	62.49	39.84	39.47	21.97	11.17	9.80	6.77	1.23
Heart	62.84	56.30	48.76	36.88	25.05	27.23	19.93	10.49
Uterus	51.25	50.35	42.36	36.67	17.47	13.47	14.33	9.06
Lung	20.77	16.20	10.5	15.30	14.69	9.37	8.64	6.31
Brain	11.62	11.21	10.21	5.66	4.64	4.25	3.05	2.21
Liver	11.57	11.32	11.58	10.56	14.86	10.02	9.17	7.83

Excretion: The % cumulative excretion of [$^{14}CH_3S$]-methamidophos and metabolites in urine, faeces, organic volatiles and as expired CO_2 are tabulated below. Both urine and expired CO_2 were major routes of excretion, accounting for 52% of excretion at 48 h. The large proportion expired as $^{14}CO_2$ indicated loss of the labelled S-methyl moiety from methamidophos. Although large amounts of radiolabelled materials were detected in the small intestine, the amounts excreted in the faeces were very low. Some methamidophos may undergo enterohepatic circulation. The large amounts of radiolabelled material excreted in urine may also be related to the high aqueous solubility of methamidophos or its metabolites.

Cumulative excretion of [^{14}C]-methamidophos equivalents

Time after dosing (h)	**% cumulative excretion of [^{14}C]-methamidophos equivalents**			
	Urine	**Faeces**	**Organic volatiles**	**Carbon dioxide**
10 min	1.25	0.13	0.01	1.26
0.5	3.67	0.14	0.01	1.84
1	9.89	1.04	0.02	6.99
3	13.38	1.80	0.03	8.21
6	15.94	2.45	0.04	18.87
12	17.05	3.11	0.04	19.38
24	22.64	3.24	0.06	20.21
48	27.10	4.19	0.11	24.59

The cumulative % of unmetabolised [^{14}C]-methamidophos in urine is tabulated below. At 10 min 98.9% of the total radioactivity was excreted as [^{14}C]-methamidophos. At 48 h, 11.4% of excreted radioactivity was in the form of [^{14}C]-methamidophos.

Percentage of [^{14}C]-methamidophos in urine

[^{14}C]-methamidophos as a % of the total radioactivity excreted at each interval							
10 min	**0.5**	**1**	**3**	**6**	**12**	**24**	**48**
98.9	91.2	70.6	64.9	45.6	43.7	16.4	11.4

Placental transfer: The % placental transfer of radioactivity is tabulated below. At 48 h 1.73% of the administered dose was recovered from foetuses collected at that time. There were periodic increases in foetal concentration (at 10 min, and 1 and 6 h after dosing) followed by declines at 0.5, 3 and 12 h. This periodicity paralleled that shown by placental transfer for the same intervals. The study author did not explain the observed relationship between the concentration in foetuses and the total amount recovered at each interval.

Placental transfer of radiolabelled methamidophos in pregnant rats

Time (h)	**Placental transfer of [^{14}C]-methamidophos equivalents (total amount recovered from foetuses at each interval as % of the administered dose)**

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	ng/g foetus	Total amount recovered (ng)	% placental transfer
10 min	78.62	192.74	0.36
0.5	47.39	137.38	0.24
1	109.87	224.40	1.04
3	42.27	166.18	0.26
6	85.68	237.21	0.38
12	54.97	114.56	0.25
24	49.23	597.75	1.16
48	29.54	844.90	1.73

Metabolism studies: ITLC analysis identified the following metabolites of methamidophos (O,S-dimethyl phosphoroamidothioate) in all tissues and fluids examined: O,S-dimethyl phosphorothioate, S-methyl phosphoroamidothioate and methyl phosphoramidate. Methyl phosphate was found in all tissues and fluids except urine, while phosphoric acid was detected in plasma, liver, kidney, lung, uterus, foetus and urine. GC-MS analysis of plasma showed methamidophos, methyl phosphate, methyl phosphoramidate, phosphoric acid, O,S-dimethylphosphorothioate and S-methyl phosphoramidothioate.

The % of methamidophos, S-methyl phosphorothioate and S-methyl phosphoramidothioate in the tissues and fluids examined by ITLC are tabulated below. The *in vivo* biotransformation of methamidophos was considered to have been rapid with the range of unmetabolised methamidophos in tissues being 37.5% (liver) to 69.2% (uterus) at 48 h.

Methamidophos and two metabolites in tissues and fluids of pregnant rats

Tissue or fluid	Methamidophos and two metabolites in tissues and fluids at 48 h as a % of the total extractable radioactivity		
	Methamidophos	S-methyl phosphorothioate	S-methyl phosphoramidothioate
Faeces	93.4	5.6	1.0
Urine	77.8	20.3	1.9
Uterus	69.2	21.6	9.2
Foetus	65.7	30.5	3.8
Brain	57.4	23.2	19.4
Heart	51.2	26.2	22.5
Kidney	50.7	32.7	16.6
Placenta	49.4	31.1	19.5
Lung	46.4	35.5	18.1
Plasma	43.0	35.5	21.5
Liver	37.5	32.6	29.6

Pharmacokinetics of methamidophos: Pharmacokinetic parameter values for plasma and various tissues were provided but are not reproduced here.

Methamidophos concentration in plasma (Ct) declined biphasically according to the following equation: $Ct = 100.00e^{-0.06t} + 21.00e^{-0.05t}$. The elimination phase from plasma had a rate constant (β) of 0.005/h corresponding to a $t_{1/2}$ for β of 152.00 h for elimination of methamidophos. The distribution $t_{1/2}$ for α was 11.55 h.

Methamidophos concentration in foetuses also declined biphasically according to the equation: $Ct = 52.50e^{-0.69t} + 19.00e^{-0.051t}$. Elimination from the foetus had a rate constant (β) of 0.05/h corresponding to a $t_{1/2}$ for β of 13.86 h. The distribution $t_{1/2}$ for α was 1.40 h.

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Other elimination half lives were 124, 64, 39, 34.95, 23.10, 23.10 and 18 h in liver, lung, brain, uterus, kidney, heart and placenta respectively.

Study 3: *Pharmacokinetics, tissue distribution, milk transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in lactating rats*:

Recovery rates: The % recovery of extractable radioactivity from sucklings, plasma, brain, liver, kidney, lung, heart and uterus were 74.0, 72.1, 70.4, 82.4, 75.4, 93.1, 82.3 and 89.9 respectively.

Clinical effects: Clinical effects or necropsy observations were not reported.

Distribution of radioactivity: The distribution of radioactivity in tissues and fluids is tabulated below in descending order of concentration at 12 h. The liver showed a slight increase in the concentration of radioactivity from 12 to 24 h. The uterus showed a 197% increase in concentration between 12 and 24 h and a 58% decrease between 24 and 48 h. Otherwise the concentrations in other tissues decreased with time.

Distribution of radioactivity in lactating rats

Tissue or fluid	**Mean concentration of radioactivity (ng methamidophos equivalents/g fresh tissue or mL of fluid) over time (h)**		
	12	**24**	**48**
Small intestine and contents	203.83	92.37	46.00
Stomach and contents	106.98	78.59	34.57
Kidney	94.77	60.80	58.47
Lung	84.21	58.66	51.43
Liver	76.40	79.04	54.49
Spleen	53.33	40.71	36.28
Diaphragm	40.14	23.14	19.67
Plasma	34.67	22.32	12.46
Heart	31.61	21.92	19.76
Skin	31.31	20.48	22.80
Uterus	28.93	85.87	36.15
Muscle	21.16	10.09	11.68
Brain	13.54	9.60	8.79
RBC	13.50	7.25	9.47
Spinal cord	12.54	8.71	7.02
Adipose tissue	8.89	9.76	7.51

Methamidophos in tissues: The concentrations of extractable radiolabelled methamidophos in tissues and plasma at each interval are tabulated below in order of descending concentrations at 12 h. The concentrations were corrected for 100% recovery and expressed on a wet wt basis. For sucklings, the concentrations were 0.60, 0.49, 0.33, 0.29, 0.25, 0.24 and 0.14 ng/g at 1, 3, 6, 12, 24, 36, 48 h respectively.

Concentration of [^{14}C]-methamidophos in lactating rats

Tissue or fluid	**Mean concentration of [^{14}C]-methamidophos in tissues and plasma (ng methamidophos/g fresh tissue or mL of plasma) over time (h)**		
	12	**24**	**48**

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Liver	37.55	21.73	18.54
Kidney	35.92	21.73	8.66
Plasma	24.29	19.11	4.73
Uterus	19.09	11.97	5.68
Heart	10.85	10.71	7.71
Lung	8.96	9.44	7.56
Brain	3.26	3.00	2.30

Milk transfer: The % milk transfer of radioactivity is tabulated below. At 48 h 1.89% of the administered dose was recovered from sucklings collected at that time. There was a progressive increase in % radioactivity transferred in milk which paralleled a progressive increase in the concentration of radioactivity in litters.

Milk transfer of radiolabelled methamidophos in lactating rats

Time (h)	**Milk transfer of [^{14}C]-methamidophos equivalents (total amount recovered from sucklings at each interval as % of the administered dose)**		
	ng/g litter	**Total amount recovered (ng)**	**% milk transfer**
1	0.82	88.53	0.03
3	0.98	146.06	0.10
6	2.64	355.55	0.40
12	2.27	264.35	0.51
24	3.71	504.77	0.81
36	5.41	756.46	1.37
48	6.88	1067.43	1.89

Metabolism studies: ITLC analysis identified the following metabolites of methamidophos (O,S-dimethyl phosphoroamidothioate) in all tissues, fluids and sucklings examined: O,S-dimethyl phosphorothioate, S-methyl phosphoroamidothioate and methyl phosphoramidate and methyl phosphate. Phosphoric acid was detected in plasma, liver, kidney, lung and uterus. The radioactivity % of methamidophos, O,S-dimethyl phosphorothioate and S-methyl phosphorothioate in the suckling pups were 53.0, 30.3 and 16.7% respectively.

GC-MS analysis of plasma and suckling pups showed methamidophos, O,S-dimethyl phosphorothioate, S-methyl phosphoramidothioate, methyl phosphate and methyl phosphoramidate. Phosphoric acid was found in plasma.

Pharmacokinetics of methamidophos: Pharmacokinetic parameter values for sucklings were provided but are not reproduced here. The concentration of methamidophos in sucklings peaked at 0.60 ng/g at 1 h indicating a very rapid absorption. The decline in concentration (Ct) occurred biphasically according to the following equation: $Ct = 0.4e^{-0.06t} + 0.36e^{-0.016t}$. The elimination phase had a rate constant (β) of 0.016/h corresponding to a $t_{1/2}$ for β of 43.50 h for elimination of methamidophos. The distribution $t_{1/2}$ for α was 11.55 h.

Conclusions

Study 2: *Pharmacokinetics, tissue distribution, excretion, placental transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in pregnant rats*:

(1) Radiolabelled material was rapidly absorbed and distributed throughout the body. Within 10 min of administration it was observed in all tissues and fluids examined. From 10 min to 48 h, the mean concentration of radioactivity in the tissues and fluids had decreased by an

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average 74%. The greatest reduction in small intestine and contents (91%) and the least in uterus (3%). The largest concentration of radioactivity remaining at 48 h was in the liver and small intestine and contents.

(2) Methamidophos was rapidly absorbed and distributed throughout the body. Within 10 min of administration it was observed in all tissues and fluids examined. For all tissues and fluids except the liver, the maximum mean concentration was observed at 10 min. The concentration in the liver peaked at 6 h. By 48 h the concentration had progressively declined by an average of 84%. The greatest reduction occurred in foetus (99%) and the least in liver (32%). The largest concentration of methamidophos remaining at 48 h was in plasma followed by kidney.

(3) The metabolic transformations of [$^{14}CH_3S$]-methamidophos in pregnant rats occurred as one or more of the following pathways:-

a. to O,S-dimethyl phosphorothioate, methyl phosphate and finally phosphoric acid,
b. to methyl phosphoramidate and finally phosphoric acid,
c. to S-methyl phosporamidothiate, dihydroxy phosphoramidate and finally to phosphoric acid or,
d. transformation of methamidophos, O,S-dimethyl phosphorothioate and/or S-methyl phosporamidothiate to methyl mercaptan and finally to carbon dioxide.

(4) Approximately equal excretion of [$^{14}CH_3S$]-methamidophos equivalents via urine or via CO_2 in the expired air. At 48 h after dosing, 27 and 25% had been excreted in urine and expired air respectively, a total of 52%. Excretion via faeces increased with time but remained low (4% at 48 h). The [$^{14}CH_3S$]-methamidophos equivalents excreted in urine consisted of 99% unmetabolised methamidophos at 10 min after dosing. This proportion decreased to 11% at 48 h. Significant excretion of methamidophos metabolites occurred at and from 0.5 h after dosing.

(5) The placental transfer of [$^{14}CH_3S$]-methamidophos equivalents peaked initially at 1% at 1 h, declined, increased again to 1.2% at 24 h and then reached a maximum of 1.7% at 48 h.

(6) The disappearance of methamidophos was biphasic. The respective distribution half lives for plasma and foetus were 11.5 and 1.4 h. The respective elimination half times for plasma and foetus were 152 and 14 h. Elimination half lives for liver, lung, brain, uterus, kidney, heart and placenta were 124, 64, 39, 35, 23, 23 and 18 h respectively.

Study 3: *Pharmacokinetics, tissue distribution, milk transfer and metabolism studies of [$^{14}CH_3S$]-methamidophos in lactating rats*:

(1) There were no differences in the distribution and relative proportions of radiolabelled material or methamidophos in the tissues and fluids examined at 12, 24 and 48 h after dosing from that observed in pregnant rats that could be attributed to the different physiological states. As in pregnant rats the mean concentration of methamidophos in tissues and fluids progressively decreased from 12 to 48 h. The mean concentration of methamidophos in sucklings also progressively reduced from 0.60 to 0.14 ng/g over the interval 1 to 48 h.

(2) The transfer of [$^{14}CH_3S$]-methamidophos equivalents to milk progressively increased to a maximum of 1.9% of the administered dose at 48 h.

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(3) In sucklings, the distribution half life was 11.5 h. The elimination half time was 43.5 h.

Bagos AC, Beatty PW, Chen YS, Tucker BV & Wilkenfeld RM (1991a): The percutaneous absorption of methamidophos (SX-1757) in male rats. Mobay Report No 5331. Study No. S-2982, CEHC 2767. Reference No. 87-113. Lab: Chevron Environmental Health Centre, Chevron Chemical Company, Richmond, California, USA. Sponsor: Ortho Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 16 June 1987 - 8 January 1991. Report Date: 21 March 1991. Unpublished. (QA: Yes (with reservations); GLP: Yes: US EPA/FIFRA (40 CFR Part 160); Test Guidelines: Yes: US EPA Guideline No. 85-2).

This evaluation includes comment on a satellite study subsequently conducted by the same authors in order to improve the recovery rate of absorbed systemic ^{14}C with more rigorous collection conditions. The satellite study was the subject of a separate report which the study authors presented as an appendix to this main study:-

Bagos AC, Beatty PW, Chen YS, Tucker BV & Wilkenfeld RM (1991b): Satellite percutaneous absorption of [^{14}C]-S-methyl methamidophos in male rats. Study No. CEHC 3112. Reference No. 89-388. Lab: Chevron Environmental Health Centre, Chevron Chemical Company, Richmond, California, USA. Sponsor: Ortho Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 18 August 1989 - 8 January 1991. Report Date: 8 January 1991. Unpublished. (QA: Yes; GLP: Yes: US EPA/FIFRA (40 CFR Part 160); Test Guidelines: not specified).

Test Substance:	[S-Methyl-^{14}C]-methamidophos. Purity: 99.8% radiochemical purity Source: Mobay Chemical Corporation, Kansas City, MO, USA.
Test Species:	Rat: Sprague-Dawley Crl:CD (SD)BR, males, 49-51 d old, 241-317 g bw from Charles River Laboratories, Portage, Michigan, USA.
Dose:	0.05, 0.50 and 5.0 mg/rat applied dermally.

Method

A methanol solution of ^{14}C-methamidophos was evaporated to dryness under a stream of nitrogen. Appropriate amounts of unlabelled methamidophos (purity 81.4%) were weighed out and mixed with ^{14}C-methamidophos to form mixtures of approximately 40, 4.0 and 0.4 μCi/mg methamidophos. Deionised water was added to final concentrations of approximately 1, 10 and 100 mg methamidophos/mL dosing solution. Three 25 mg samples were weighed out and counted to determine the homogeneity of each solution before dosing. The specific activities of test materials were 84793, 8882 and 888 dpm/μg for the 0.05, 0.5 and 5.0 mg/rat dosing solutions respectively.

The application site was clipped on the day prior to dosing and 0.5 h before dosing was shaved, washed to remove sebaceous gland secretions and dried. A neoprene rubber template was glued to the back of each rat defining an area of 2.5 x 4 cm (10 cm^2). Total body surface area (A) was calculated by the equation $A = 9.64 \times (bw)^{0.66}$. The mean % of A treated was 2.7 for groups 1 and 2 and 2.5 for group 3. Group 1 was dosed on d 1, group 2 on d 3 and group 3 on d 8. The test material solution was vortexed immediately before dosing each rat. Aliquots were taken for analysis. Approximately 0.05 mL of the dosing solution was applied to the application site with a digital microdispenser. Each rat was placed individually into a

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Metrap™ restraining metabolism chamber equipped for the separate collection of urine and faeces and the charcoal trapping of volatile metabolites.

After exposure, rats were anaesthetised with sodium pentobarbital ip, removed from the restrainer and placed on a necropsy tray. The application site was scrubbed vigorously *in situ* with a soapy gauze pad. The procedure was repeated twice with the last scrub being with a deionised water-wet pad. The skin of the application site was excised by cutting around the template. The excised site was immersed twice in 20 mL acetone and agitated for 30 s. A 1 cm strip of skin around the perimeter of the site was also taken, frozen and retained by the testing laboratory. A blood sample was taken from the descending aorta of each rat with a heparinised syringe before the rat was exsanguinated. The urine was removed from the bladder and added to the urine from the cage collector. All faeces were removed from the colon and added to that collected from the cage. After removing the volatiles and CO_2 traps the Metrap™ caging was rinsed with 90 mL methanol. The carcasses was placed in specimen bags.

All of the soap/water and water scrubs were combined with 40 mL acetone and shaken frequently for 4 h. Aliquots of the acetone were counted directly for ^{14}C. The templates were rinsed with methanol which was then diluted in acetone and aliquots were counted directly for ^{14}C. The skin application site was combusted for ^{14}C determination. Frozen carcasses and charcoal samples were pulverised in dry ice. Aliquots were mixed with cellulose and combusted for ^{14}C determination. Aliquots of blood samples were also combusted for ^{14}C determination. Aliquots from the CO_2 traps and methanol cage rinses were counted directly for ^{14}C. Aliquots of faeces were combusted for ^{14}C determination and aliquots of urine were counted directly for ^{14}C. The term "skin surface compartment" was applied to the soap/water scrubs, the acetone skin rinses and the methanol template rinse. The "intradermal compartment" was the skin residue and the "systemic compartment" the carcass, blood, excreta, methanol cage rinse, CO_2 trap and volatiles trap. Statistical evaluation of the results was not provided.

Results and discussion

Administered doses: The mean dose administered is tabulated below. In all groups the administered dose was within 5% of the desired dose.

Mean dose administered to each group

Groups (n=12)	Exposure (h)	Mean bw (g)	Dose administered		
			mg/rat	mg/kg bw	mg/cm^2*
Group 1	2	251	0.048	0.189	0.005
(0.05 mg/rat)	10	247	0.049	0.199	0.005
	24	256	0.048	0.189	0.005
Group 2	2	255	0.484	1.902	0.048
(0.5 mg/rat)	10	248	0.491	1.978	0.049
	24	246	0.491	2.002	0.049
Group 3	2	284	4.80	16.96	0.48
(5.0 mg/rat)	10	297	4.91	16.58	0.49
	24	283	4.85	17.15	0.48

* concentration of test material at the 10 cm^2 application site

The mean distribution of ^{14}C in the 3 main compartments following the 3 exposure times is tabulated below. The greatest % administered dose of ^{14}C was recovered from skin surface at

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2 h. This recovery rate decreased with time. For each exposure period there was no substantial dose-related difference between the groups in the amount of ^{14}C recovered from skin surface. The amount of ^{14}C recovered from skin residue progressively increased with exposure time except at 10 h in group 1. The radiolabel not recovered was considered to have been absorbed through the skin, metabolised and excreted as one or more volatile metabolites which were not retained by the charcoal traps in the Metrap™ caging. This consideration was based on the results of a study previously evaluated in this section (Crossley & Tutass, 1969). The subsequent satellite study (Bagos *et al.*, 1991b) was conducted to explore aspects of this problem.

Mean distribution of ^{14}C-methamidophos equivalents

Groups	Exposure (h)	% administered dose		
		Skin surface	Intradermal	Systemic(*)
Group 1 (0.05 mg/rat)	2	71.1	13.7	15.2
	10	51.5	6.2	42.4
	24	46.1	14.1	39.8
Group 2 (0.5 mg/rat)	2	75.4	5.8	18.9
	10	51.0	13.4	35.6
	24	38.3	18.7	43.0
Group 3 (5.0 mg/rat)	2	76.3	3.4	20.3
	10	52.2	9.4	38.4
	24	43.7	12.2	44.1

* Assumed systemic absorption = measured systemic absorption plus % not recovered

The mean systemic absorption of ^{14}C is tabulated below. For each dose the measured absorption plateaued by 10 h, and the degree of absorption from 2-10 h was dose-related. The measured dermal absorption of methamidophos at 0.05 mg/rat (0.02 mg/kg bw) was 4.0, 6.0 or 5.5%, at 0.5 mg/rat (2.0 mg/kg bw) was 5.0, 9.0 or 10.0% and at 0.5 mg/rat (17.0 mg/kg bw) was 8.0, 20.0 or 17.0% of the administered dose at 2, 10 or 24 h respectively. For each exposure time there was a dose-related increase in the amount of ^{14}C recovered. The measured dermal absorption of methamidophos at 2 h was 4, 5 or 8%, at 10 h was 6.0, 9.0 or 20.0% and at 24 h was 5.5, 10.0 or 17% of the administered dose of 0.05, 0.5 or 5.0 mg/rat (or 0.2, 2.0 or 17.0 mg/kg bw) respectively.

Mean systemic absorption of ^{14}C-methamidophos equivalents

Groups	Exposure (h)	% administered dose		
		Measured absorption*	% not recovered	Assumed absorption#
Group 1 (0.05 mg/rat)	2	3.9	11.4	15.2
	10	6.0	36.4	42.4
	24	5.5	34.3	39.8
Group 2 (0.5 mg/rat)	2	4.7	14.2	18.9
	10	9.1	26.5	35.6
	24	10.2	32.8	43.0
Group 3 (5.0 mg/rat)	2	8.0	12.3	20.3
	10	19.7	18.7	38.4
	24	16.9	27.2	44.1

* Total ^{14}C in blood, urine, faeces, carcass, methanol cage wash, CO_2 and volatiles traps
# Measured absorption plus % not recovered.

The distribution of measured absorbed ^{14}C is tabulated below. The majority of the measured absorbed ^{14}C was in urine and carcass. The amount found plateaued by 10 h, the increase from 2-10 h being dose-related. For each exposure time the degree of absorption was dose-

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related except for group 2 urine at 2 h. The amount recovered from blood showed a dose-related increase at 2 h but it did not increase at 10 or 24 h.

Mean distribution of ^{14}C-methamidophos equivalents following systemic absorption

Groups	Exposure (h)	% administered dose					
		Blood	Urine	Faeces	Carcass	CO_2	Vols*
Group 1 (0.05 mg/rat)	2	< 0.1	0.5	< 0.1	1.9	1.1	0.1
	10	< 0.1	1.2	0.1	2.8	1.2	0.4
	24	0	1.2	0.2	2.5	0.5	0.8
Group 2 (0.5 mg/rat)	2	0.1	0.3	< 0.1	2.9	1.1	0.1
	10	0.2	2.0	0.2	4.6	1.5	0.4
	24	0.1	3.2	0.3	4.9	0.8	0.7
Group 3 (5.0 mg/rat)	2	0.2	1.8	< 0.1	4.5	1.3	0
	10	0.1	9.8	0.4	6.0	2.7	0.1
	24	0.1	8.4	0.5	5.8	1.0	0.5

*Vols: volatiles trapped in the charcoal filter in the metabolic caging

Conclusions

(1) At each exposure time the % administered dose of methamidophos absorbed was dose-related.

(2) The degree of absorption of methamidophos plateaued by 10 h and the degree of absorption from 2-10 h was dose-related.

Comment

Accurate estimation of transdermal uptake of methamidophos is hindered by the low recovery of administered radioactivity (approximately 64%). The conclusions were based on measured dermal absorption of methamidophos and not on the "assumed" dermal absorption, which included radioactivity that was not recovered. Hence, the above values may under-estimate the true extent of dermal absorption, but by an unknown. The study authors used the "assumed" absorbed methamidophos and concluded that the absorption of methamidophos was time-dependent and that at 24 h, 40.0, 43.0 or 44% of the administered dose of 0.05, 0.5 or 5.0 mg/rat (or 0.2, 2.0 or 17.0 mg/kg bw) respectively, was absorbed.

Satellite study: The satellite study was intended to duplicate the conditions of the main study except that changes were made in an attempt to identify possible sources of ^{14}C that were not identified in the main study. One test group of 4 male rats at 0.05 mg/rat was studied after a 10 h exposure. The exhaust air from each metabolism chamber was passed through a volatiles trap (glass bubblers in series containing approximately 150 mL ethanol or heptane and partially immersed in a dry ice/isopropanol bath), a CO_2 trap (glass bubbler containing approximately 150 mL of aqueous 0.5 NaOH) and final traps of activated charcoal.

The skin at the application site was scrubbed 3 times with soapy water scrubs and once with a distilled water scrub. During necropsy the skin of the application site, the skin adjacent to the application site, the template and each gauze scrub after use, were weighed. Samples from the ethanol or heptane cold traps were analysed by direct counting in scintillation fluid. Charcoal traps were analysed by combustion and scintillation counting of a portion of each trap. After extraction, the gauze pads used for the soapy water and water washes were cut into pieces and analysed by combustion and scintillation counting.

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Total recovery increased of ^{14}C increased from 63.6% in the main study to 75.3%. The largest increase was ^{14}C removable from the scrubs. An increase in systemic absorption from 6.0 to 9.0% administered dose was also observed.

The results suggested that the unrecovered ^{14}C in the main study was not lost as a volatile metabolite or as material unextractable from the scrubs. However the nature, location and distribution of the unrecovered ^{14}C was not determined.

Klein O (1997): Position paper on the metabolism of methamidophos in rats. Report on an incomplete study (Bayer AG, Study No. M 1820161-9) conducted in 1987. Bayer Document No. M 9942. Project No. not specified. Report No. not specified. Lab: not specified. Sponsor: Bayer AG, Crop Protection Development, D-51368 Leverkusen-Bayerwerk. Study Duration: not specified. Report Date: 19 February 1997. Unpublished. (QA: No; GLP: Yes; Test Guidelines: not specified).

Test Substance:	[S-methyl-^{14}C] methamidophos (SA 182 µCi/mg). Source: not specified.
Test Species:	Rat: male, bw 180-211 g, no further details provided.
Dose:	1 mg/kg bw po

Prelude

The objective of the 1987 study described in this position paper was to identify the highly volatile degradation products of methyl mercaptan (methanethiol) and elucidate the degradation pathway from methyl mercaptan to CO_2 in the biotranformation of methamidophos. The objective was not achieved. Three references (Canellakis & Tarver, 1953; Derr & Draves, 1983; Blom & Tangerman, 1988) are discussed briefly in Section 3.2.

Method

First part: No details of preliminary housing, environment and nutrition of the rats were provided. Eight male rats were given a single dose by gavage and placed singly in metabolic cages. Urine and faeces were collected. The rats were killed in an unspecified manner 24 h after dosing. The air drawn into the cage was CO_2 free. An ethanoline/methanol (1:1) solution was used to trap exhaled CO_2. Volatile radioactivity was trapped in an unspecified manner. The radioactivity in the carcass of each rat at termination was determined by combustion and liquid scintillation counting. The results were not subjected to statistical analysis.

Second part: Because of the variability in observed data and the non-recovery rate for radioactivity (30%), further work was pursued. It was assumed that non-recovered radioactivity was contained in volatile metabolites other than CO_2. A number of biotransformation products were considered possible beginning with methyl mercaptan as the primary hydrolysis product. They were methane, dimethyl disulfide, dimethyl sulfide and dimethyl sulfoxide. Additional methodology was employed to absorb and identify the volatile compounds. The absorbent material (Tenax TA) was placed to remove volatiles from extracted air. The identification of volatiles was attempted with GC using a FID-detector coupled to a radioactivity flow-through detector. Reference standards were subjected to the GC identification process. The limit of detection was 125 ng/peak. To ensure that the system was capable of detecting the metabolites, the author performed 4 h test runs involving

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extraction from an empty metabolic cage, a cage containing an untreated rat, a cage containing an untreated rat and 50 µg dimethyl sulfide and an empty cage with 50 µg dimethyl sulfide dissolved in 1,2-dichlorobenzene directly in the Tenax material. An unspecified number of rats were again dosed and the experiments repeated under identical conditions to those used in Part 1.

Results and discussion

Clinical signs and necropsy findings: None were reported.

First part; distribution of radioactivity: The mean body distribution of radioactivity at 24 h, expressed as % of the administered dose, was as follows: skin 5.2 (range 3.4-7.2), carcass 9.1 (6.7-13.9), "gut" 3.0 (2.4-3.4), "animal-gut" 17.0 (12.8-18.7), faeces 2.9 (1.2-4.2), urine 32.2 (16.1-51.7) and CO_2 15.1 (2.8-23.8). Thirty % (range 6.7-48.8) radioactivity was unrecovered. A mean 47.3% of radioactivity was excreted in urine and faeces over 24 h. The study author remarked on the high degree of variability in the results especially in the radioactivity excreted in urine and CO_2. There was also concern about the low level of recovery of radioactivity.

The distribution of radioactivity in tissues and fluids (equivalent concentration µg/g) was as follows: RBC (0-0.10), plasma (0.10-0.20), spleen (0.30-0.40), liver (0.60-0.70), kidney (0.40-0.50), testis (0.10-0.20), muscle (0-0.10), heart (0.10-0.20), lung (0.40-0.50), brain 0-0.10), skin (0.20), carcass (0.10-0.20) and renal fat (0-0.10). A range of values is given because data was presented in a histogram and except for skin the actual values were not interpretable.

Second part: The system capability test runs showed that the air which passed through the empty cage contained volatile compounds which were not markedly different from those detected when an untreated rat was kept in a cage. When dimethyl sulfide was placed in a cage with an untreated rat it was recovered at a rate of 40-50% in exhaust air. The test system design was considered suitable for the detection of the metabolites in question. The metabolites were not detected. These compounds were either not formed by the rat or the amounts were too low for detection.

Conclusion

The formation of quantifiable amounts of methyl mercaptan, methane, dimethyl sulfide, dimethyl disulfide or dimethyl sulfoxide could not be demonstrated even though the procedures used were proven to be capable of doing so.

Testman R (2000): Determination of the volatility of ^{14}C-methamidophos from rat skin. Bayer AG Report No. 109644. Bayer Toxicology Study No. 98C-992-UO, BTC Study No. P1098001. BTC Report No. not specified. Lab: Biological Test Centre (BTC), McGaw Avenue, Irvine, CA, USA. Sponsor: Bayer Corporation, 17745 South Metcalf, Stilwell, KS, USA. Study Duration: 6 October 1998 - 17 April 2000. Report Date: 8 August 2000. Unpublished. (QA: Yes; GLP: USA EPA 40 CFR Part 160; Test Guidelines: not specified).

Test Substance:	Radiolabelled ^{14}C-methamidophos. Lot number: 11659/4. Radiochemical purity: 97.3%. Specific activity 192.8 µCi/mg. Source: Bayer Corporation, Stilwell, KS, USA.

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	Non-radiolabelled methamidophos. Lot number: M00082. Purity: 99.3%.Source: Bayer Corporation, Stilwell, KS, USA.
Test Species:	Rat: Sprague-Dawley, female, bw 209-240 g, 10-11 wk old, obtained from Harlan Sprague-Dawley, San Diego, CA, USA.
Dose:	5 μg/cm^2, 50 μg/cm^2 applied dermally.

Method

Sixteen rats were housed individually and quarantined for 7 d prior to termination. They were of normal appearance and behaviour and free of disease. They received Teklad certified LM-485 Mouse/Rat Diet and tap water *ad libitum*. No contaminants were present in food or water.

Two dosing solutions were prepared using non-radiolabelled methamidophos in sterile water as diluent. The concentrations of the 2 solutions were 8.22 and 80.6 mg ^{14}C-methamidophos/g. In both solutions the amount of radioactivity in each 10 μL of ^{14}C-methamidophos was 0.8 μCi.

Sixteen rats were weighed and terminated using CO_2 inhalation. The back of each rat was shaved. The skin from the back was removed and divided into 2 pieces of 4 x 4 cm. ^{14}C-methamidophos was applied to the pieces of skin in 2 doses as follows: Groups A, B, C and D: 5 μg/cm^2, applied in a single dose in 10μL solution and processed at 0, 2, 8 and 24 h after application respectively and Groups E, F, G and H processed similarly but with dose 50 μg/cm^2. A total of 4 replicates were used for each dose and collection time. With the exception of those for immediate processing (0 h), skin samples were placed in a jar immediately following application of ^{14}C-methamidophos, which was then sealed with a non-occlusive lid containing an activated charcoal filter pad. The samples were then placed in a 37^{o}C chamber immediately after application where they were left for 2, 8 or 24 h. At the appropriate time, skin samples were transferred to a separate container, weighed and stored frozen until further processing. They were then homogenised in an amount of water equal to 10 times the wt of the skin. Triplicate aliquots of homogenate (containing 50 mg tissue) were pipetted into combustion cones, weighed and combusted in a Harvey sample oxidiser. The combusted samples were trapped in Carbon-14 cocktail in liquid scintillation counting (LSC) vials and the radioactivity determined by LSC.

The glass jars and carbon filters were processed for radioanalysis. All radioactivity measurements were performed using a Beckman liquid scintillation spectrometer. If radioactive measurements differed by > 20%, the sample was realiquoted and reanalysed. Any radioactive reading of < 100 dpm was considered too close to background and was not repeated.

Statistical analyses were not required.

Results and discussion

Dose and dosing solution analysis: The doses applied were 5.13 and 50.4 μg/cm^2. The total dose of radioactivity applied to each skin sample was 0.8 μCi. The means for radioactivity in the 2 dosing solutions were calculated to be 80.5 and 81.0 μCi/g for the low and high doses respectively. The radioactive purity of the 2 ^{14}C-methamidophos dosing solutions was determined to be 97.14 and 100% for the low and high doses respectively.

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Recovery of radioactivity: The recovery of radioactivity for each dose and interval of processing is tabulated below. The rate of total recovery was ≥ 90% applied dose. Virtually all radioactivity was found on skin samples. The levels of radioactivity did not decrease progressively with time.

Mean recovery of radioactivity

Sample	Mean recovery of radioactivity (% applied dose) of ^{14}C-methamidophos for each dose (µg/cm^2) and interval after application (h)							
	5				50			
	0	2	8	24	0	2	8	24
Skin	98.3	92.8	95.4	90.8	90.1	88.0	96.5	94.3
Jar	0	0.05	0.27	1.19	0.02	3.93	0.05	0.28
Filter	0.03	0.12	0.79	1.21	0.01	0.04	0.37	0.87
Total	98.3	93.0	96.5	93.2	90.1	92.0	96.9	95.5

Conclusion

When ^{14}C-methamidophos was applied to excised rat skin for up to 24 h at 37^{o}C, little variation in radioactivity on the skin surface was observed. Therefore methamidophos did not significantly volatilise from the skin surface.

2.3 Rabbits

Tutass HC (1968): Microsomal oxidation of Monitor^R. Project No. not specified. File No. 721.2. Lab: Research and Development, Ortho Division, Chevron Chemical Company, Richmond, California, USA. Sponsor: Ortho Division, Chevron Chemical Company, Richmond, California, USA. Project Duration: not specified. Report Date: 26 September 1968. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test substance: Methamidophos technical
No other details provided

Test Species: Rabbit: New Zealand White, female of an unspecified bw, age or source.

Dose: 50 mL of aqueous solution containing approximately 1 x 10^6 dpm S-methyl-^{14}C methamidophos.

Prelude

This study was intended to examine the value of microsomes derived from hepatic endoplasmic reticulum in *in vitro* determinations of metabolites of methamidophos. The method was believed to be rapid, quantitative and metabolites were easily recoverable. The basis of the hypothesis was the apparent strong correlation between metabolites produced by microsomal oxidation and those produced by the whole animal. NADPH was used here as cofactor as some metabolites are products of cofactor dependent enzyme systems. Microsomal deamination has been reported (Bray *et al.*, 1950) although the process is slow compared to ester hydrolysis. Both O- and S-dealkylation is known to occur for esters (Brodie *et al.*, 1958).

Method

The rabbit was killed and a 20% w/v liver homogenate in 0.25 M sucrose was prepared (Oonnithan & Casida, 1968). This was filtered and centrifuged at 15000g for 30 min at 0^{o}C in

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a Beckman Model L ultracentrifuge. The pellet was discarded and the supernatant centrifuged for 1 h at 95000g. The microsomal pellet was resuspended in fresh 0.25 M sucrose. To 1 mL of this suspension were added 0.5 mL of 0.1 M phosphate buffer (pH 7.4), 50 mL of aqueous solution containing approximately 1 x 10^6 dpm S-methyl-^{14}C methamidophos and 2 µmole reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the following combinations:-

	Blank	Minus NADPH	Complete
Microsomes	-	1.0 mL	1.0 mL
Phosphate buffer	0.5 mL	0.5 mL	0.5 mL
Methamidophos - ^{14}C	50 µL	50 µL	50 µL
NADPH	0.5 mL	-	0.5 mL
Water	1.0 mL	0.5 mL	-
Total	2.0 mL	2.0 mL	2.0 mL

Samples were incubated for 1 or 2 h. At the end of the incubation, the samples were extracted 3 times with equal volumes of ethyl acetate. The aqueous fraction was centrifuged for 10 min at 6000g after the addition of 10% trichloroacetic acid. Both the aqueous and ethyl acetate fractions were chromatographed on TLC plates using Silica Gel (Merck, Germany) and methanol-benzene (1:3) as solvent.

Results and discussion

Chromatographed extracts of the ethyl acetate fraction did not show the presence of metabolites. The aqueous phase of the extract showed two radioactive spots identified as two charged species of S-dimethyl phosphorothioate. Hydrolysis, described quantitatively as "considerable", also took place in the reagent blank but the degree of hydrolysis was "much less" than that occurring in the presence of microsomes. The method of determining the relative rates of hydrolysis was not described nor was the influence of NADPH.

Conclusion

The chromatographed extracts showed that microsomes accelerated the hydrolysis of methamidophos.

2.4 Goats

Lee H & Crossley J (1972): The fate of Monitor^R in a lactating ruminant (goat). Mobay Report No 32668. Project No. not specified. Chevron File No. 721.11. Lab: Research and Development, Ortho Division, Chevron Chemical Company, Richmond, California, USA. Sponsor: Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Project Duration: not specified. Report Date: 28 January 1972. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	S-methyl-^{14}C-methamidophos Purity: 99.8% Source: New England Nuclear (Boston, Massachusetts) under instructions from Mobay Chemical Corporation, Kansas City, USA.
Test Species:	Goat: miniature, female, 19 kg, of an unspecified age and source.
Dose:	3.75 mg/d for 7 d (95.0 µCi/d)

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Method

The test goat was in mid-lactation and was housed under standard conditions. Its diet consisted of 0.9 kg fresh hay and 0.45 kg of concentrate daily. The goat received 3.75 mg test substance/d po (by intubation of powdered test substance in a gelatine capsule) for 7 d. There was no pre- or post-radioactive dose conditioning with non-radioactive material. The level of dosing was calculated to represent 2 ppm in the diet. Total urine, faeces, milk and a 10 mL whole heparinised blood sample were collected 24 h before and just prior to the first radioactive dose. Total urine, faeces, milk and a 5 mL whole heparinised blood sample were then collected every 12 and 24 h after the first radioactive dose for the following 17 d. Six h samples of blood and milk were also taken after the first and last radioactive doses. Urine and faeces were also taken when available. The goat was terminated with sodium pentobarbital 11 d after the last dosing. Kidneys, liver, heart, brain, mammary gland (inner tissue), and representative samples of muscle and fat were removed from the carcass. All samples were frozen, including the remaining carcass.

Ethyl acetate extraction or hexane/acetonitrile partitioning were used to measure methamidophos in various excretion media and tissues. Under the conditions employed methamidophos is quantitatively extracted into ethyl acetate or acetonitrile and the amount of radioactivity in an extract as a percentage of the total in the tissue or media was described as the maximum concentration of methamidophos in the media or tissue. The concentration was also calculated as a ppm equivalent maximum concentration of methamidophos. Assays of extracts by TLC (ethyl acetate extraction) or GLC (hexane/acetonitrile partitioning) analyses were used to more precisely determine the concentration of methamidophos by determining the concentration (% radioactivity or ppm) of that substance in the extract with the same elution retardation factor (Rf) as standard methamidophos. Urine was also subjected to direct TLC in order to separate methamidophos from the metabolite O,S-dimethyl phosphorothiate (DMPT). Fat samples were extracted using hexane/acetonitrile partitioning. This method was also used in several tissues where the ethyl acetate extract indicated residues of methamidophos in excess of 0.01 ppm. The rationale for this activity or the choice of concentration was not provided. The ^{14}C in expired carbon dioxide was not measured.

Results and discussion

Distribution of ^{14}C: The distribution of ^{14}C in urine, faeces and milk is tabulated below. Approximately 18% of the administered dose was excreted in urine, 5% in faeces and 3% in milk. Ninety one % of the ^{14}C excreted via these 3 media was excreted during the dosing period; for urine the % excreted in this time was 96%, faeces 80% and milk 77%.

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The distribution of radioactivity (% dose) in excreta and milk

Period	% administered dose			
	Urine	**Faeces**	**Milk**	**Total**
Dosing period (d 0-8)	17.24	4.00	2.49	23.73
Recovery period (d 9-17)	0.63	0.98	0.74	2.35
Total excreted	17.87	4.98	3.23	26.08

The % dose of ^{14}C in tissues and blood at the end of the recovery period was blood 5.87, liver 3.93, kidney 0.61, heart 0.61, mammary gland 0.82, brain 0.21, fat 4.13 and muscle 33.10, total 49.28 (the assumptions made were that blood constituted 10% bw, fat 5% and muscle 40%). The total recovery of ^{14}C was 75% of the administered dose. It was assumed by the study authors that the remaining amount of ^{14}C was excreted via the lungs as CO_2. The assumption was based on data derived from a previous study (Crossley & Tutass, 1969).

The % dose of ^{14}C in tissues was also expressed as "ppm equivalent calculated as methamidophos" as follows: liver 0.22, kidney 0.16, heart 0.16, brain 0.08, mammary 0.10, fat 0.16 and muscle 0.16. No further clarification was provided by the study authors.

Urine: The amount of ethyl acetate-extractable ^{14}C present in urine expressed as a % of the total radioactivity at various times after dosing was as follows: evening after 4th dosing, 14.9; morning following 5th dosing, 14.9; evening after 7th dosing, 25.9; 4 d after the last dosing, 13.0 and 8 d after the last dosing, 6.6. At least 74 % of the radioactivity in urine was not methamidophos.

TLC of the urine sample taken 12 h after the 3rd dose showed that 7% of the radioactivity in the urine was methamidophos and 16% DMPT. This result suggests that methamidophos was readily metabolised by goats.

Faeces: The maximum amount of ethyl acetate-extractable ^{14}C in faeces, 9.7% of the total ^{14}C present in faeces, occurred 4.5 d after the last dose. This was equivalent to 0.008 ppm ethyl acetate-extractable ^{14}C. TLC findings were not provided.

Blood: The concentration of radioactivity in blood accumulated with each dose. At 25 h after the 1st dosing it was 2000 dpm/g and 8000 dpm/g 24 h after the 7th dosing. The maximum amount of ethyl acetate-extractable ^{14}C was 3.4% of the total ^{14}C present in blood. It occurred during the dosing period. During the recovery period it was 0.4%. TLC of the extracts indicated that none of the ethyl acetate-extractable ^{14}C contained methamidophos (0 ppm). The principle radioactivity in the non-extractable fraction was most likely due to the incorporation of ^{14}C into the synthesised natural constituents of the blood via the cleavage of the S-$^{14}CH_3$ bond.

Milk: Radioactivity was detected in the sample taken 6 h after the 1st dosing. Within the 1st 24-36 h it reached a peak level and then decreased gradually during the recovery period. The amount of ethyl acetate-extractable ^{14}C present in milk expressed as a % of the total radioactivity at various times after dosing was as follows: evening after 4th dosing, 10-12; 48 h after the last dosing, 11; 168 h after the last dosing, 19; 216 h after the last dosing, 0.

TLC studies showed that during the dosing period, 9.5% of the ethyl acetate-extractable ^{14}C (or approximately 1% of the total radioactivity in milk) was methamidophos (0.003 ppm).

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This was the maximum concentration of methamidophos detected. When TLC was applied to recovery period samples, no methamidophos was detected. This result was confirmed with GLC. A maximum of 0.002 ppm of methamidophos was detected in the dosing period and none was present during recovery. The principle radioactivity in the non-extractable fraction was most likely due to the incorporation of ^{14}C into the natural components of milk such as casein and lactose.

Tissues: The total ethyl acetate-extractable ^{14}C of each tissue, expressed as a % of the total radioactivity in the tissue and as ppm concentration of methamidophos, was liver 2.9 (0.007 ppm); kidney 4.1 (0.007 ppm); heart 16.1 (0.025 ppm); brain 5.7 (0.005 ppm); mammary 19.2 (0.02 ppm); muscle 5.0 (0.008 ppm).

Hexane/acetonitrile partitioning of fat and tissues with a total ethyl acetate-extractable ^{14}C concentration > 0.01 ppm (heart and mammary) showed in fat 2.0% (0 ppm methamidophos) of the total radioactivity as acetonitrile-extractable ^{14}C. Heart and mammary showed respectively 4.7 (0 ppm methamidophos) and 6.3% (0 ppm methamidophos) of the total radioactivity as acetonitrile-extractable ^{14}C.

The study authors claimed that on the basis of the findings of the combined extraction procedures, the maximum concentration of methamidophos in all tissues was < 0.01 ppm.

Conclusions

(1) At the end of the recovery period, 51% of the radioactivity administered had been excreted. Of amount excreted, 18% was excreted via urine, 5% via faeces and 3% via milk with the balance (25%) presumed to have been via expired CO_2.

(2) Of the amount of radioactivity excreted up to the end of the recovery period, 91% had been excreted during the dosing period. Of the amount excreted during the dosing period, 72.5% was excreted in urine, 17% in faeces and 10.5% in milk

(3) At the end of the recovery period, 49% of the radioactivity administered was distributed throughout the tissues and blood. The percentage distribution was as follows: muscle 33, blood 6, fat 4, liver 4, mammary 0.8, kidney 0.6, heart 0.6 and brain 0.2.

(4) The maximum concentration of methamidophos in tissues was 0.01 ppm.

Baker FC & Bautista AV (1997): The metabolism of [^{14}C]-methamidophos in the lactating goat. Analytical Phase. Bayer File No. M9971. Project No. 969E/565W. Report No. 1878. Lab: PTRL West Inc, Richmond, California, USA. Sponsor: Valent USA Corporation, Walnut Creek, California, USA. Study Duration: 16 October 1995 - 27 January 1997. Report Date: 27 January 1997. Unpublished. (QA: Yes; GLP: FIFRA Section 10(d), 1(A),(B),(C); Test Guidelines: not specified).

Note: There were two phases to this study, the Analytical Phase (above) conducted at the PTRL West Inc. facility at Richmond, California and the In-life phase (below) conducted at the PRTL East Inc. facility at Richmond, Kentucky but using the analytical facilities at PTRL West Inc, Richmond, California. The In-life Phase report was attached to the Analytical Phase report as "Appendix B". The In-life Phase report has separate authorship. The Analytical Phase described in detail the reference substances used, other reagents, apparatus,

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determination of total radioactive residues, extraction procedures for tissues and milk and the chromatographical methods used for the separation and identification of methamidophos and its metabolites. Methodological detail will not be reproduced here.

Krautter GR, Marsh JD & Downs JH (1997): The metabolism of [^{14}C]-methamidophos in the lactating goat. In-life Phase. Project No. 969E/565W. Report No. 1878. Lab: PTRL East Inc., Richmond, Kentucky, USA. Sponsor: Valent Corporation, Walnut Creek, California, USA. Study Duration: 16 October 1995 - 27 January 1997. Report Date: 27 January 1997. Unpublished. (QA: Yes; GLP: FIFRA Section 10(d), 1(A),(B),(C); Test Guidelines: not specified).

Test Substance:	[S-$^{14}CH_3$]-methamidophos (SA 80 mCi/g). Radiochemical Purity: 97.8%. Lot Number: 307-ID-A. Identity Number: PTRL Number 969-1. Source: Valent USA Corporation, Dublin, California, USA.
Test Species:	Goat: two adult lactating cross-breeds of 32 and 38 kg bw, 5 yr and 4 yr respectively obtained from Eldon Mattingly Farm (Lexington, Kentucky, USA).
Dose:	10.2 ppm methamidophos equivalents in the diet administered po via a capsule twice/d for 3 d (or 0.7 mg methamidophos/kg bw/d).

Method

Goats were administered [^{14}C]-methamidophos in gelatin capsules, equivalent to 10.2 ppm in the diet. A single dose capsule was administered orally by balling gun to one test goat twice/d after the morning and evening milk collections for 3 consecutive d. From 24 h prior to the 1st dose, milk was collected, identified and retained. The wt of milk produced by each goat at each milking was recorded. Daily milk composites consisting of milk produced during a 24 h period were prepared by combining the milk collected on a given evening with the milk collected the following morning. All milk samples were weighed and stored frozen. Urine and faeces samples were also collected each morning from 24 h prior to the 1st dose. The wt of urine and faeces produced during each collection interval was recorded and samples identified and stored frozen. Immediately before termination at 18 h after the last dose by captive bolt stunning and exsanguination, each goat was weighed and examined for vital signs and general appearance. The health status of each goat was recorded. After termination, necropsies were performed and liver, kidneys, composite muscle (round and loin) and composite fat (perirenal and omental) were collected. Paired tissue samples were stored frozen.

Milk and combusted control tissue background radioactivity values were used to calculate tissue-specific limits of quantitation (LOQ) and to adjust for background radioactivity during the radioanalysis of tissues and milk from the treated goat. HPLC and TLC identification of [^{14}C]-methamidophos was conducted using a reference standard of laboratory grade methamidophos (Lot No. AS-4k; batch VDL-258-19e) assigned an ID of PTRL No. 969-2. Its purity was reported as 99.4%. Mean and standard deviation calculations were the only statistical analyses used. In calculating mean residue levels, if an individual sample residue level was below the LOQ, the LOQ value was used in the calculation of the mean and the result was reported as "<" the mean value. Limits of quantitation for the tissues analysed were $\leq$ 0.001 ppm.

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The radiolabelled components of residues in extracts and post-extraction solids of tissues and milk were identified and quantitated. A proposed metabolic pathway for [S-$^{14}CH_3$]-methamidophos in the lactating goat was also presented.

Results and discussion

Radiochemical and chemical purity determinations: The chemical identity of the [^{14}C]-methamidophos used was confirmed by TLC. HPLC analysis found that the mean radiochemical purity of [^{14}C]-methamidophos to be 99.7% before capsule preparation, 99.1% in the pre-dose capsule extract and 99.2% in the post-dose capsule extract. HPLC analysis also showed that the chemical purity of the laboratory grade methamidophos reference standard was 96.0%.

Dose and dose verification: The treated goat received 2 dose capsules/d, a total of 3.4 mL treatment solution/d or 27.44 mg [^{14}C]-methamidophos/d for 3 consecutive d. On the basis of the mean food consumption of the goat during the 3 d prior to dose capsule preparation, this dose was equivalent to 10.2 ppm methamidophos equivalents in diet (or 11.5 ppm methamidophos equivalents in diet on a dry wt of food basis). This corresponded to a dose rate of 0.7 mg methamidophos/kg bw/d. Direct radioanalysis of pre and post-dose capsules accounted for 97.7% of the theoretical amount added. The treated goat received the targeted dose.

Animal observations: No signs of toxicity or gross organ or tissue abnormalities were observed. Mean daily food consumption, milk production and bw gain of each goat were unaffected by treatment.

Total radioactive residue (TRR) levels: TRR levels in tissue samples from treated goats were liver 1.744, kidney 0.645, muscle 0.206 and fat 0.033 ppm [^{14}C]-methamidophos equivalents. Total radioactivity recovered in the tissues accounted for 6.9% of the administered dose (liver 2.0, kidney 0.1, muscle 4.6 and fat 0.2%).

Radiolabelled components of tissue extracts: These components were natural products and were identified as glucose, galactose, phosphatidylcholine (lecithin) and other phospholipids, choline and methionine among other amino acids and proteins.

Concentration of identified radiocarbon in extracts of liver

Extract Component	**Component quantification**	
	Component ppm	**% of liver TRR**
Glucose, Galactose[a]	0.252	14.4
Methamidophos[a]	< 0.002	< 0.1
O,S-dimethylphosphorothioic acid[a]	0.008	0.5
S-methylphosphoramidothioic acid[a]	< 0.010	< 0.6
Phosphatidylcholine[b]	0.324	18.6
Other phospholipids[b]	0.058	3.3
Amino acids[c]	0.893	51.2

Extraction: a, methanol/water; b, chloroform; c, 6N HCl hydrolysate

Liver: The concentration of extracted and identified components is tabulated below. $^{14}CH_3$-S-phosphates were essentially absent. Methamidophos was present at < 0.002 ppm. S-methylphosphoramidothioic acid (SMPAA) was present at < 0.01 ppm. The maximum level

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of O,S-dimethylphosphorothioic acid (DMPTA) was 0.008 ppm. The total radiocarbon identified was 1.5 ppm; 88.7% of liver TRR.

Kidney: The concentration of extracted and identified components is tabulated below. $^{14}CH_3$-S-phosphates were essentially absent. Methamidophos was present at < 0.003 ppm. SMPAA was present at 0.027 ppm. The maximum level of DMPTA was 0.002 ppm. The total radiocarbon identified was 0.5 ppm; 79.3% of kidney TRR

Concentration of identified radiocarbon in extracts of kidney

Extract Component	Component quantification	
	Component ppm	% of kidney TRR
Phosphatidylcholine[a]	0.027	4.2
Other phospholipids[a]	0.028	4.3
Choline[a]	0.075	11.6
Methamidophos[a]	< 0.003	< 0.5
O,S-dimethylphosphorothioic acid[a]	0.002	0.3
S-methylphosphoramidothioic acid[a]	0.027	4.2
Polar-immobile	0.054	8.4
Phosphatidylcholine[b]	0.033	5.1
Other phospholipids[b]	0.010	1.6
Amino acids[c]	0.251	39.1

Extraction: a, acetonitrile/water; b, methylene chloride; c, 6N HCl hydrolysate

Muscle: No radiolabelled methamidophos, DMPTA or SMPAA, were observed. Ninety two per cent of the radiolabel extracted with acetonitrile/water consisted of low MW compounds and thus were not muscle structural compounds. HCl hydrolysis suggested that amino acids were the predominant radiolabelled components.

Fat: Saponification of a portion of the chloroform extract resulted in distribution of the radiolabel throughout the non-saponifiables (eg. cholesterol), free fatty acids and glycerol (aqueous) fractions.

Concentration of identified radiocarbon in extracts of milk

Extract Component	Component quantification	
	Component ppm	% of milk TRR
Lactose[a]	0.374	48.6
Methamidophos[a]	0.020	2.6
O,S-dimethylphosphorothioic acid[a]	-	-
S-methylphosphoramidothioic acid[a]	-	-
Triglycerides[b]	0.083	10.8
High MW protein[d]	0.120	15.6
Low MW protein[d]	0.024	3.1

Extraction: a, acetonitrile/water; b, methylene chloride; c, 6N HCl hydrolysate

Milk radioactive residue levels: TRR levels in daily milk samples ranged from 0.571 to 0.769 ppm [^{14}C]-methamidophos equivalents. Total radioactivity recovered in the milk accounted for 7.6% of the administered dose (milk (0-24 h) 2.0, milk (24-48 h) 2.9 and milk (48 h+) 2.7%). The components of milk extract are tabulated below. Lactose was the major radiolabelled component of milk. Triglycerides were also radiolabelled with most of the radiolabel associated with the glycerol skeleton following saponification, with lesser amounts

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of ^{14}C in the free fatty acid fraction. Radiolabel was also found in high MW protein - probably caseinogen. The total radiocarbon identified was 0.6 ppm; 80.7% of kidney TRR.

The results of radioanalysis or extraction of urine and faeces were not reported.

Conclusion

(1) Within 18 h of dosing with [S-$^{14}CH_3$] methamidophos at 0.7 mg/kg bw/d for 3 consecutive d, methamidophos was extensively metabolised by the goat. Radiolabelled residues containing the organophosphate moiety were minor components (< 0.03 ppm) of the total radioactivity residue of any of the tissues or milk.

(2) [S-$^{14}CH_3$] methamidophos is metabolised via intermediary metabolism into numerous endogenous natural products: sugars (lactose, glucose and galactose), triglycerides (fatty acids, glycerol), phospholipids (choline, lecithin) and proteins.

(3) Approximately 7.6% of the administered radioactivity was excreted in milk. [S-$^{14}CH_3$] methamidophos showed only low potential for accumulation in the body tissues examined.

2.5 Monkeys

Fuller B, Wills M, Testman R, Selim S & Nelson J (2000): A dermal/intravenous crossover study to determine the dermal absorption of [^{14}C]-methamidophos in male rhesus monkeys. Study Nos. Bayer AG 99C-B29-DX & Sierra Biomedical 0694-90 Report No. not specified. Lab: Sierra Biomedical, Dunn Circle, Sparks, Nevada, USA & Biological Test Centre, McGaw Avenue, Irvine, CA, USA. Sponsor: Bayer AG, 17745 South Metcalf, Stilwell, KS, USA. Study Duration: 9 October 1998 - 15 August 2000. Report Date: 15 August 2000. Unpublished. (QA: Yes; GLP and Test Guidelines: US EPA (40 CFR, Part 160), OECD C(97)186,,Yes; Japan 59 NohSan No 3850 & Guideline No 24 PAB MH&W Japan, Nevada Radioactive Material License Standards).

Test Substance:	[^{14}C]-methamidophos in 0.9% saline for iv administration. ID Number: ECW 11652-A. Radiochemical purity: >99%. Specific activity: 116 µCi/mg. [^{14}C]-Tamaron 600 SL aqueous solution for dermal administration. Source: Bayer AG, Stilwell, KS, USA. ID Number: ECW 11654-A.
Test Species:	Monkey: Rhesus, male, 4.1-4.4 yr old, 4.6-5.5 kg bw, obtained from Scientific Resources International, Reno, Nevada.
Dose:	240 µg/monkey

Method

The objective of this study was to determine the dermal absorption of ^{14}C-labelled methamidophos from Tamaron 600 SL formulation ([^{14}C]-Tamaron 600 SL) in monkeys. The dermal route was chosen because it is the main route of human occupational exposure. The iv route, providing 100% absorption, was selected to provide a reference by which to calculate the % of dermally applied dose of [^{14}C]-Tamaron 600 SL absorbed. The rhesus monkey was chosen because it is an accepted animal model for predicting dermal absorption in man.

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The single group of 4 rhesus monkeys each of which had passed a comprehensive health screen, was acclimatised to their assigned study rooms for 2 wk and adapted to the primate restraint chairs for 1 h on 2 occasions prior to the 1st d of dosing. They were housed individually in stainless-steel cages equipped for urine and faeces collection. The room was well ventilated. A 12 h light/12 h dark photoperiod was maintained. Room temperature was kept at 64-84^{o}F. When room radioactivity levels exceeded background levels, radioactive decontamination procedures were used. Harian Teklad Primate Diet^{TM} (Certified) was provided daily in amounts appropriate for the size and age of the monkeys. The diet was supplemented with fruit or vegetables 2-3 times weekly. Other treats such as biscuits were also provided. Tap water was available *ad libitum* via an automatic watering device. The monkeys were in the restraint chairs for pre-dose sampling, dosing and post-dose sampling up to 8 h post dosing. Their time in the chairs was 8-10 h for each dosing. Food was withheld for 4 h prior to dosing. While in the chairs the secure ties on the monkeys were periodically released and the limbs massaged. They were offered water *ad libitum* and fruit, vegetables and treats were offered at 4 h post-dose. The diet and drinking water contained no contaminants likely to have interfered with the results.

All monkeys were observed for changes in general appearance and behaviour at least twice daily from 3 d prior to the 1st dose and continuing until the radioactivity in the urine aliquot samples was less than twice background. While in the restraint chairs they were examined at 4 h after dosing and every 2 h until they were returned to the cages. Body wts were measured within 48 h of each dosing - on d 2 and d 13.

The monkeys received a single iv dose of [^{14}C]-methamidophos and a single dermal dose of [^{14}C]-Tamaron 600 SL on d 1 and d 15 respectively. The dermal application was an 8 h exposure. The target dose for each route was 240 µg methamidophos/monkey (28 µCi/monkey).

For iv administration, the monkeys were anaesthetised with ketamine HCl. Catheters were placed in the urinary bladder and saphenous vein and secured to the body. The venous catheter was capped with an injection port. The monkeys were then placed in primate restraint chairs and secured. Once the monkeys had recovered from anaesthesia, the pre-dose samples were collected and the test material was administered iv (1 mL as supplied by the sponsor) as a bolus injection over a 10 s period through the injection port and immediately flushed with 2 mL of sterile 0.9% saline. The dose syringe and iv catheter were stored in polypropylene containers for radioanalysis. A tray was placed to capture any faecal material during the time spent in the chairs. The procedure for returning the monkeys to cages after iv dosing was not described but it was apparent that the urinary catheter was removed before they were returned.

On the day prior to dermal administration, the monkeys were anaesthetised with ketamine HCl. An area of the back large enough to accommodate a Duoderm^{TM} patch and protective dome (6 x 4 cm) was shaved and cleansed. On the morning of dosing monkeys were again anaesthetised with ketamine HCl and catheterised for urine and blood collection. The catheters were secured and the monkeys were placed in restraint chairs and secured. Full recovery from anaesthesia occurred before dosing began. A Duoderm^{TM} patch was placed over the dosing site and after pre-dose sample collection the chairs were rotated until the monkeys were in a prone position. A 100 mL aliquot of the test material was spread evenly over the site with a calibrated glass micropipette. This task was performed by the Sponsor's consultant who performed the same task in the human dermal absorption study evaluated in Section 2.7 (Selim *et al.*, 2000). The site was covered with an aluminium dome with air holes.

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The chairs were then returned to the upright position. After the collection of blood and urine at 8 h after dermal dosing, the monkeys were anaesthetised with ketamine HCl and removed from the restraining chairs. The protective dome and Duoderm^{TM} patch were removed and the test site swabbed with a series of 16 soapy water soaked cotton-tipped swabs followed by 2 washes with isopropyl alcohol soaked swabs. At 24 and 48 h following dosing (d 16 and 17) the monkeys were lightly anaesthetised with ketamine HCl and removed from their cages. The dose site was swabbed with 2 isopropyl alcohol swabs. The dose site was then tape-stripped 4 times each time using 4 cellophane-tape strips to cover the whole site. On d 19 the monkeys were again anaesthetised with ketamine HCl, removed from their cages and swabbed with 2 isopropyl alcohol swabs. Preliminary radioactivity counts showed values < 50000 cpm and no further swabs were required. The monkeys were released from the study when urine radioactivity levels were at or below twice background. They were returned to the Sierra Biomedical colony.

One mL volumes of venous blood were collected into heparin tubes prior to dosing and at 5, 15 and 30 min, and at 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, and 120 h post-dosing. Venous sampling was also conducted prior to and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h after dermal dosing. Blood samples were collected from the saphenous catheters for the 1st 8 h after dosing. Once the monkeys were back in their cages blood samples were obtained by venipuncture.

Urine and faeces collection intervals were 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96 and 96-120 h after dosing. For the 1st 2 periods, samples were collected from the monkeys while they were in the restraint chairs - urine into polypropylene vials via the catheter and faeces into trays. After returning to their cages, the excreta was collected into cage pans designed to separate urine and faeces. Biscuits were also removed from the collection screen and placed into collection bags because they may have come into contact with urine and faeces. At the end of the collection period, the collection pan was rinsed with 100 mL water and the rinse was added to the urine collected.

Urine bottles, faecal collection bags and biscuit collection bags were frozen and shipped for analysis. Dose solutions and swab washings were sent on ice packs for analysis of radioactivity. Radioanalysis was also conducted on syringes with needles, catheters with ports, dermal application pipettes, skin wash swabs, tape strips and the Duoderm^{TM} patches and domes and on plasma and RBCs.

The observed plasma and RBC levels of radioactivity were used to calculate $AUC_{0\text{-}120\ h}$ and % absorption for the dermal route. The method of calculating the $AUC_{0\text{-}120}$ was not specified. The formula used for % dermal absorption was:-

$$\%\ \text{dermal absorption} = \frac{AUC_{0\text{-}120\ h\ (dermal)}}{AUC_{0\text{-}120\ h\ (iv)}} \times \frac{Dose_{iv}}{Dose_{(dermal)}} \times 100$$

The mean % of absorbed radioactivity was also calculated as the sum of total radioactivity excreted following dermal dosing divided by the total radioactivity excreted following iv dosing.

Statistical analyses were not required.

Results and discussion

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Dose and analysis of dosing solutions: The mean dose administered to each monkey was for iv injection 239 µg (27.7 µCi) and for dermal administration 239 µg (27.7 µCi) or 10 µg/cm^2. The concentrations of radioactivity in the solutions prepared for the iv and dermal doses were determined to be 27.3 µCi/g and 280.1 µCi/mL respectively. The radiochemical purity of the dosing solutions, before and after dosing was reported as 100%.

Clinical signs: No treatment-related clinical signs were observed in any monkey at any stage of the study.

Plasma and RBC radioactivity: The mean radioactivity in plasma and RBC for each route of administration is tabulated below. Only data for the 1st 5 intervals, 12 and 36 h and the last 3 intervals are shown. For the dermal route the level of radioactivity in either plasma or RBC was < 1 ppb except for a value of 1.38 at 72 h.

Mean radioactivity in plasma and RBC for each route of administration

Time (h)	Radioactivity (ppb methamidophos equivalents) at selected intervals			
	Plasma		RBC	
	iv	dermal	iv	dermal
Pre-dose	0.03	0.03	0	0.03
5 min	69.6	-	44.7	-
0.25	60.8	-	36.2	-
0.5	49.8	0.15	29.9	0.13
1	39.8	0.17	23.3	0.22
2	30.0	0.09	15.9	0.74
12	14.2	0.50	1.3	0.24
36	10.9	0.71	3.25	0.59
72	6.27	0.56	4.75	1.38
96	5.52	0.56	4.53	0.61
120	4.71	0.43	4.57	0.68

The mean $AUC_{0\text{-}120}$, maximum concentration (C_{max}) and % absorption of ^{14}C-methamidophos derived activity following iv or dermal administration is tabulated below. The $AUC_{0\text{-}120\ h}$ in plasma was approximately twice that of RBC. The study authors indicated that the elimination of the iv dose was biphasic with a long β half life.

Mean $AUC_{0\text{-}120\ h}$, C_{max} and % absorption for each route of administration

Parameter	$AUC_{0\text{-}120}$ (ng eq x h/mL), C_{max} (ng x eq/mL) and % absorption			
	Plasma		RBC	
	iv	Dermal	iv	dermal
$AUC_{0\text{-}120\ h}$	1021.5	65.5	504.6	84.1
C_{max}	69.6	0.71	44.7	1.38
% absorption	-	6.41	-	-

Radioactivity in urine: The radioactivity present in urine at the specified collection times and the cumulative excretion is tabulated below. A mean of 11.3 and 1.2% of the dose was excreted via urine following iv and dermal administration respectively. For iv administration 72% of that excreted in 120 h via urine was excreted in the 1st 4 h. For dermal administration the same % excretion was observed at 48 h. The β half life for ^{14}C-methamidophos in urine was indicated by the study authors to be 51 h for iv and 47 h for dermal administration.

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Mean radioactivity in urine for each route of administration

Time interval (h)	Radioactivity (% dose) at various intervals			
	iv		dermal	
	% dose	Cum % dose	% dose	Cum % dose
0-4	8.22	8.22	0.04	0.04
4-8	1.84	10.06	0.08	0.11
8-12	0.28	10.34	0.09	0.20
12-24	0.38	10.71	0.33	0.53
24-48	0.22	10.94	0.29	0.82
48-72	0.19	11.13	0.17	0.99
72-96	0.12	11.25	0.11	1.09
96-120	0.10	11.35	0.09	1.18

Radioactivity in faeces: The radioactivity present in faeces at the specified collection times and the cumulative excretion is tabulated below. A mean of 0.6% and 0.07% of the radioactivity dose was excreted following iv and dermal administration respectively.

Mean radioactivity in faeces for each route of administration

Time interval (h)	Radioactivity (% dose) at various intervals			
	iv		dermal	
	% dose	Cum % dose	% dose	Cum % dose
0-4	0.02	0.02	0	0
4-8	0.05	0.07	0	0
8-12	0.02	0.09	0	0
12-24	0.07	0.16	0.01	0.01
24-48	0.2	0.36	0.02	0.03
48-72	0.1	0.46	0.02	0.05
72-96	0.06	0.52	0.01	0.06
96-120	0.05	0.57	0.01	0.07

Radioactivity in biscuits: A mean of 0.08 and 0.1% of the dose was found in biscuits for the iv and dermal routes of administration respectively

Total recovery of radioactivity over 0-120 h: Following iv administration, the total recovery of radioactivity was 12% of the administered dose (urine 11.3, faeces 0.6, biscuits 0.08%). Following dermal administration the total recovery of radioactivity was 67% of the administered dose (urine 1.2, faeces 0.07, biscuits 0.1, tape strips 0.15, dermal dome 1.33, soap and water swabs 57.3, alcohol swabs 4.1 and Duoderm^{TM} 2.8). The low mean recovery from the subjects resulting from the use of the ^{14}C label was attributed to the loss of volatile metabolites discussed in Section 2.2 (Canellakis & Tarver, 1953).

Calculation of dermal absorption: Using the $AUC_{0\text{-}120\ (dermal)}/AUC_{0\text{-}120\ (iv)}$ ratio formula, the % applied dose absorbed was calculated as 6.4%. Dermally absorbed radioactivity was also calculated as the sum of radioactivity excreted following dermal dosing (urine 1.18, faeces 0.07 and biscuits 0.1 = 1.35% dose) divided by the sum of radioactivity excreted following iv dosing (urine 11.3, faeces 0.57 and biscuits 0.08 = 11.95% dose) was 1.35/11.95 x 100 = 11.3%. The study authors did not comment on the 1st result and presented only the 2nd as a conclusion.

Conclusion

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The amount of methamidophos absorbed through monkey skin following a single 8 h exposure of the skin to a dose of 240 µg methamidophos/monkey applied as Tamaron 600 SL, was 11.3% of the applied dose.

2.6 Humans

Van de Sandt JJM, Groten JP, Leeman WR & Penninks AH (1998): In vitro percutaneous absorption of methamidophos through human and rat skin. Study No. 1952, Project No. 470861. TNO Report No. V97.1035. Lab: Toxicology Division, TNO Nutrition and Food Research Institute, Utrechtseweg 48, Zeist, the Netherlands. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 25 August 1997 - 31 October 1997. Report Date: 8 January 1998. Unpublished. (QA: Yes; GLP and Test Guidelines: OECD, OECD draft guideline (1996)).

Test Substance:	*Non-radiolabelled*: Methamidophos, Batch Number: 50809ELB01, Purity: 98.9%. *Radiolabelled*: [^{14}C-methyl-S-]methamidophos, Specific activity: 7.13 MBq/mg, Batch Number: ECW 11182, Purity: >99%. *Formulated test substance*: Methamidophos - SL600 formulation, Concentration of methamidophos: 48.40%, Radio-label: [^{14}C-methyl-S-]methamidophos, Specific activity: 7.13 MBq/mg, Batch Number: ECW 11195. *Formulated test substance (diluted)*: Methamidophos - SL600 formulation, Concentration of methamidophos: 48.08%, Radio-label: [^{14}C-methyl-S-]methamidophos, Specific activity: 0.0185 MBq/mg, Radio-dilution 333-fold with water, Batch Number: ECW 11196.
Reference substances:	*Radiolabelled water*: Tritium water [^{3}H]H_2O, Specific activity: 37.0 MBq/g, Purity: not determined, Source: DuPont NEN. *Non-Radiolabelled*: Testosterone (4-androsten-17β-ol-3-one), Batch Number: G469, Source: Steraloids Inc (Wilton, NH). *Radiolabelled*: [4-^{14}C]testosterone, Specific activity: 2.1 GBq/mmol, Purity: 98.2%, Source: DuPont NEN.
Test Species:	Rat: Wistar outbred (Crl:[WI]WU BR)), no further details provided.

[Prelude: *The study authors described their use of methamidophos as "methamidophos as technical substance" or Tamaron™ (98.9% purity) and methamidophos as a commercial formulation (Tamaron SL 600) with methamidophos at 48% concentration . Their use of these terms is to be distinguished from the commercially supplied "Tamaron™" (or methamidophos technical) which has methamidophos at approximately 75% purity]*.

Method

Human skin was obtained from a 36-yr old Caucasian woman after abdominal surgery. The skin was transported on ice within 1 h of dissection and was prepared and cultured immediately after arrival at the laboratory. Skin was also obtained from 2 young rats. The dorsal and flank skin of 4 young rats was clipped free of fur before the selection of 2 with even skin was made. The next day the skin was isolated and cultured.

Skin was cultured in a 2-compartment system (Van de Sandt *et al.*, 1993). Circular membranes of 2 cm^2 were punched out from isolated skin after removal of sc fat. Sterile glass rings of internal area 0.6 cm^2 were glued to the dry epidermis using cyanoacrylate-based glue.

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The skin membranes were washed 3 times for 15 min in medium supplemented with bactericides and fungicides to prevent contamination. Skin membranes were cultured in 6-well plates on a Netwell insert (200 μm mesh), which allows transport of nutrients present in the receptor fluid via the dermis into the epidermis, while the epidermal side of the skin remains free of contact with the receptor fluid. The skin membranes were incubated in a humidified incubator gassed with 5% CO_2 and 40% O_2 at 32°C. The plates were rocked on a platform at 9 times/min. The thickness of the skin membranes was assessed at the end of the experiment.

The doses applied to viable skin membranes are tabulated below. The study authors stated that "the doses of technical methamidophos were selected based on the dose range of the formulated test substance". Testosterone was used as a reference or positive control substance.

Doses of methamidophos applied to human and rat skin membranes

Group	Test substance	Vehicle/formulation	Dose (μg/cm^2)[a]	
			Human skin	Rat skin
A	Methamidophos	ethanol[b]	9375	9383
B	Methamidophos	ethanol[b]	935	936
C	Methamidophos	ethanol[b]	30.2	30.3
D	Methamidophos	SL 600 concentrate[c]	7659	9070
E	Methamidophos	SL 600 diluted[d]	16.7	16.6
F	Testosterone	ethanol[b]	14.8	16.0

a: 10 μL test solution was applied to a surface of 0.6 cm^2
b: Ethanol was evaporated using pressurised air immediately after administration
c: Methamidophos 48%
d: 333-fold aqueous dilution of the concentrate formulation

The integrity of the skin membranes was assessed by determining the permeability coefficient (Kp) of tritium water ($[H^3]H_2O$). The Kp value (cm/h) was calculated as follows: Kp = flux constant [μg/cm^2/h]/applied concentration [μg/cm^3]. The flux constant was calculated from the linear portion of the cumulative penetration curve. Only skin membranes with a tritium water permeability coefficient (Kp) of less than 2.5 x 10^{-3} cm/h (human skin) or 3.5 x 10^{-3} cm/h (rat skin) were used to determine the percutaneous absorption of methamidophos and testosterone. The absorption of methamidophos was assessed 12-15 h after removal of tritium water. Ten μL of test substances were applied topically to 0.6 cm^2 surface area of skin. Samples of receptor fluid (200 μL) were collected at 1, 2, 4, 6, 8, 10, 20, 22 and 24 h after application. Total radioactivity of the dosing solutions was determined before they were applied to skin. The recovery of radioactivity was determined 24 h after application of the test substance. The remaining test substance was removed from the application site with cotton swabs soaked in ethanol (6 times) and the skin membranes were solubilised in 5 mL 1.5M KOH in 20% ethanol. The remaining receptor fluid was collected and the wells washed twice with 1 mL ethanol. Total radioactivity was determined in all compartments separately (cell wash, skin membrane and skin rinse). The radioactivity in the samples was determined using a LKB/Wallac S1409 scintillation counter.

Skin toxicity was evaluated 24 h after application of the test substances. Cytotoxicity caused by the test substances was evaluated by lactate dehydrogenase (LDH)-leakage using a Boehringer reagent kit. One skin membrane of each experimental group was fixed in 4% buffered formaldehyde and processed for embedding in paraffin, sectioned and stained with haematoxylin. General histomorphology of the skin membranes was compared to freshly isolated skin. The statistical evaluation of the LDH-leakage data was performed using analysis

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of covariance followed by Dunnett's control group comparison test (in case of homogeneous variances), or t-test with α-Bonferroni correction (in case of heterogeneous variances). The thickness of the skin membranes was assessed using a microscopic raster at 3 levels in the cross sections.

The cumulative penetration of the applied test substances was calculated from the 200 µL receptor fluid samples using the following equation: Cumulative DPM_T = (6 x DPM_T(200 µL) + Σ(DPM_{T-1}(200 µL)...DPM_1(200 µL)) where DMP_T = radioactivity at sampling time T; DPM_{T-1} = radioactivity at the sampling time preceding T and DMP_1 = radioactivity at the 1st sampling time.

Results and discussion

The mean thicknesses of the human and rat skin membranes were 1.9 and 1.3 mm respectively. The study authors did not comment on the difference and its possible effect on permeability differences between the species or between the test substances.

Percutaneous absorption of methamidophos is tabulated below. The experimental groups listed were as described in the method section. The data for 2 and 8 h is not shown since they represented progressive steps in the cumulative data. Twenty four h after application the % applied dose of methamidophos absorbed through human skin was 0.3, 0.8 and 1.0% for Groups A, B and C respectively. For rat skin the values were 1.2, 9.8 and 2.3%. For Groups D and E the % applied dose absorbed at 24 h was 0.2 and 0.6% respectively in human skin and 29.7 and 7.2% in rat skin. The amount of methamidophos technical absorbed through human skin increased with dose and at each of the two higher doses increased with time. The absorption of methamidophos from the formulation concentrate across human skin increased with time. The data obtained for testosterone was consistent with that previously found at the study author's laboratory. The permeability coefficients for testosterone were not provided.

Mean cumulative penetration of methamidophos

Species/ Group	Mean cumulative penetration of human and rat skin by methamidophos (µg/cm^2) or testosterone at selected intervals (h), the % applied dose penetrating over 24 h and the permeability coefficient (Kp) for each group (x 10^{-3} cm/h)								
	1	**4**	**6**	**10**	**20**	**22**	**24**	**% 24 h**	**Kp**
Human A	11.8	17.3	19.3	21.5	25.3	26.2	27.4	0.3	0.02
B	0.6	1.6	2.3	3.8	6.3	6.9	7.6	0.8	0.006
C	0.01	0.04	0.06	0.1	0.3	0.3	0.3	1.0	0.007
Rat A	1.9	9.8	17.4	36.1	87.5	102	116	1.2	0.007
B	0.3	5.7	13.5	31.7	72.2	82	91	9.8	0.072
C	0.01	0.07	0.1	0.2	0.6	0.6	0.7	2.3	0.017
Human D	2.5	3.0	3.4	4.3	9.2	11.2	13.7	0.2	0.002
E	0.04	0.06	0.07	0.08	0.1	0.1	0.1	0.6	0.005
Rat D	2.4	30.2	177	765	2144	2467	2700	29.7	0.242
E	0	0.03	0.08	0.2	0.9	1.1	1.2	7.2	0.066
Human F*	0	0.01	0.06	0.2	0.9	1.0	1.2	8.0	-
Rat F*	0.01	0.2	0.4	0.8	1.5	1.7	1.9	12.0	-

% 24 h = penetration within 24 h as % of the applied dose
* F - Reference substance testosterone

On the basis of the Kp, the permeability of human skin to methamidophos technical in Group A was 3 times that of rat skin. For Group B and Group C, rat skin was 12 and 2 times more permeable than that of human skin respectively. The permeability of rat skin to

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methamidophos in the formulation concentrate was 121 times that of human skin and 13 times more permeable than human skin in the case of the diluted concentrate.

No epidermal damage was observed in human skin following exposure to methamidophos. Pycnosis was observed in rat skin exposed to methamidophos technical at the highest dose (A) or to the diluted SL 600 concentrate (E). The measurements of LDH-leakage into the receptor fluid of human and rat skin membranes showed no statistically significant differences between test groups. The mean LDH concentration in the receptor fluid in human skin was 119.0, 119.6, 122.8, 116.8, 115.2 and 112.4 U/L for Groups A, B, C, D, E and F respectively. The mean values for rat skin were 636.8, 985.5, 1166.5, 1968.8, 2032.0* and 599.3 (*statistically significantly different ($p \leq 0.05$) from the reference value 599.3).

Conclusions

(1) The cumulative *in vitro* penetration of human skin by methamidophos technical during 24 h continuous exposure to each of 3 applied doses, 0.03, 0.9 and 9.4 mg/cm^2, did not exceed 1% of the applied dose.

(2) The cumulative *in vitro* penetration of human skin by methamidophos formulated as Tamaron SL 600 concentrate (applied dose 7.7 mg methamidophos/cm^2) or its 333-dilution (applied dose 0.02 mg methamidophos/cm^2) during 24 h continuous exposure, did not exceed 1% of the applied dose and was comparable to the absorption of unformulated methamidophos.

(3) The cumulative *in vitro* penetration of rat skin by methamidophos technical during 24 h continuous exposure to each of 3 applied doses, 0.03, 0.9 and 9.4 mg/cm^2 exceeded 1% of the applied dose at each dose. The maximum was 9.8% applied dose at 0.9 mg/cm^2.

(4) The cumulative *in vitro* penetration of rat skin by methamidophos formulated as Tamaron SL 600 concentrate (applied dose 9.0 mg methamidophos/cm^2) or its 333-dilution (applied dose 0.02 mg methamidophos/cm^2) during 24 h continuous exposure, exceeded 1% of the applied dose. The maximum was 29.7% applied dose for the formulation concentrate.

(5) With the exception of the penetration through human skin of methamidophos technical at 9.4 mg/cm^2, the permeability of rat skin to methamidophos was higher than that of human skin. The maximum factor was 121 times in the case of methamidophos in the formulation concentrate.

(6) Under the conditions of this study and the doses used, exposure to methamidophos did not damage human skin membranes.

(7) In rat skin epidermal pycnosis was observed following incubation with methamidophos technical at 9.4 mg/cm^2 and also with the diluted methamidophos formulation. Increased leakage of LDH from rat membranes following exposure to the undiluted and diluted methamidophos formulation was also observed. The study authors indicated that these observations support the opinion (ECETOC, 1990) that rats are generally more sensitive than humans to chemically induced skin irritation.

(8) The enhanced penetration of methamidophos across rat skin when formulated as Tamaron SL 600 may arise from the presence of excipients in the formulation.

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Selim S, Van Lier JJ & Verheggen FTM (2000): Absorption, excretion, balance and pharmacokinetics of ^{14}C radioactivity after single dose dermal application of one dose level of ^{14}C labelled methamidophos from a Tamaron 600 SL formulation administered to healthy volunteers. Study No. Bayer: 99C-B29-BX; XenoBiotic Laboratories: 98053; Pharma Bio-Research: PBR-981661. Bayer AG Report No. 109805. Lab: Pharma Bio-Research (PBR) Clinics, Beilerstraat 16, 9401PK Assen, The Netherlands; XenoBiotic Laboratories Inc (XBL), Morgan Lane, Plainsboro, NJ & Bayer AG, PH-PDT Toxicology, Elberfeld, Wuppertal, Germany. Sponsor: Bayer Corporation, 17745 South Metcalf, Stilwell, KS. Study Duration: 12 January 1998 - 11 July 2000. Report Date: 11 July 2000. Unpublished. (QA: Yes; GLP and Test Guidelines: OECD GLP, Declaration of Helsinki (1989), WHO Technical Report Series Nos. 403 and 563, FDA USA (1977), CFR Title 21 Parts 50, 56, 58, 312, 320; FIFRA 40 CFR Part 160).

[Prelude: *Appendices 3-6 inclusive of this submission were not attached]*

Test Substance:	^{14}C-methamidophos as a.i. in Tamaron 600 SL Formulation Radiochemical purity: >99% Specific activity: 192.8 μCi/mg Formulation composition: not provided; considered proprietary. Source: Bayer Corporation, Stilwell, Kansas, USA.
Test Subjects:	Human, male volunteers
Dose:	3 μg a.i./cm^2/8 h applied dermally

Method

The study protocol was reviewed and approved by Bayer Corporation's Internal Medical Committee and approved by the Pharma Bio-Research (PBR) Medical Ethics Committee. An adhesive template (Duoderm^{TM}, Squibb, The Netherlands) outlined a 6 x 4 cm application site on the volar aspect of the non-dominant forearm of each subject. ^{14}C-methamidophos was administered in a single dermal dose of Tamaron 600 SL formulation as 100 μL of dosing solution spread evenly over the application site with a calibrated glass micropipette. An aluminium dome with air holes was used to protect the site. The dosing solution contained 72 μg ^{14}C-methamidophos/100 μL. The dose was approximately 3 μg a.i/cm^2. This dose was chosen after discussion with the Californian EPA. It represented the low end of exposure typically encountered by agriculture workers.

Indwelling venous catheters was placed in each arm for simultaneous collection of blood. After an 8 h exposure period, the application templates and domes were removed and the sites were cleaned 16 times with cotton swabs dipped in 2% solution of liquid soap to remove surface radioactivity. The application site was then thoroughly rinsed with a steady stream of soapy water. At 18 h, 45 h and on d 5, 6 and 7 after application, the sites were swabbed with isopropyl alcohol (IPA). At d 7 the radioactivity on the application site was less than 5000 dpm (disintegrations/min) and the subjects were released from the Centre

Blood samples were collected from each arm pre-dose and at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h after application. Blood samples were drawn into a heparinised tube from both arms simultaneously. One mL aliquots of the blood samples taken from the non-test arm were processed for the determination of ChE at the Bayer AG laboratory in Germany using the modified Ellman method (Ellman *et al.*, 1961). The rest of the blood samples were

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centrifuged and plasma and RBC separated and placed in polypropylene tubes, frozen and forwarded to XenoBiotic Laboratories (XBL), USA for radioanalysis.

All urine and faeces produced over a 120 h period were collected for analysis. The total volume of excreted urine was collected during the following intervals: pre-dose, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 and every 24 h after application of the dose for the 1st 120 h. A 40 mL aliquot of each collection was also forwarded to XBL for radioanalysis. The remaining urine was forwarded to Bayer AG for metabolic profile analysis. One pre-dose faecal sample was collected from each volunteer. All post-dose faecal samples were collected for the 1st 120 h. If radioactivity was less than 75 dpm in 400 mg homogenised faeces at 120 h, samples were sent frozen to XBL for radioanalysis.

Radioanalysis of the dosing solutions, the applicators, application site protective enclosures, swabs, application site rinsates, protective gauze and tape strippings was also conducted by XBL.

The formulae used in calculations were as follows:-

Dose applied = Dose present in glass applicator* - Dose remaining on the glass applicator
(*derived from dosing solution sent to XBL)

$$\% \text{ of dose in each sample} = \frac{\text{Total radioactivity in sample (dpm)}}{\text{Total radioactivity administered (dpm)}} \times 100$$

% of dose absorbed = % dose in urine + % dose in faeces

Statistical analyses were not performed.

Results and discussion

Mean applied dose: Mean volunteer bw, total methamidophos applied and the total radioactivity applied is tabulated below. The mean dose of methamidophos applied was 0.88 µg/kg bw.

Mean applied dose

Volunteer mean bw (kg)	**Total µg applied**	**Total methamidophos applied (µg/kg bw)**	**Total radioactivity applied**	
			dpm	**µCi**
80.9	71.05	0.88	30409887	13.7

Clinical report: The full clinical report contained in Appendix 6 of the study report was not available. All 6 volunteers enrolled in the study completed the study. No abnormalities were observed in clinical parameters measured pre-dose and at the end of the study. The dose used was well tolerated and no application site abnormalities were observed. Five/6 volunteers reported 9 mild adverse effects and 2 moderate ones. The 2 moderate effects headache and faintness after the 1st blood sampling. Seven mild effects were listed. They were pain or haematoma at the site of the iv cannula (4/5), myalgia (1/5), headache (1/5), rhinitis (1/5), abdominal cramps (1/5) and nausea (1/5). The attending physician did not consider these effects to be treatment-related.

Cholinesterase: There was no effect on ChE activity in any subject at any time.

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Plasma radioactivity: The concentration of radioactivity found in plasma taken from the test arm and the non-test arm is tabulated below. The study authors indicated that the concentration of radioactivity in plasma collected from the non-test arm is representative of the systemically circulating radioactivity. That found in plasma collected from the test arm is representative of systemically circulating radioactivity plus the amount of radioactivity being absorbed through the skin. Radioactivity in plasma from the test arm was higher than the corresponding value for the non-test arm up to 72 h indicating a slow but continuous absorption of radioactivity from the test site. Radioactivity in plasma from the non-test arm increased slowly and then peaked at 24 h. The data suggested that radioactivity was not completely removed from the skin with soapy water swabs and the remaining radioactivity was slowly absorbed. RBC were not tested for radioactivity because the plasma concentrations were at detection limit.

Mean plasma radioactivity*

Arm	Mean radioactivity (dpm/mL) in plasma collected from each arm at each interval (h)													
	0#	2	4	6	8	10	12	16	24	36	48	72	96	120
TA	1	7	15	13	15	16	24	14	31	24	19	21	14	14
NTA	2	3	6	4	4	8	5	8	12	12	14	15	15	13

* Centre background radioactivity: 24 dpm; # Pre-dose; TA = Test Arm; NTA = Non-test Arm

Urine radioactivity: The mean radioactivity excreted in urine is tabulated below. Urinary excretion of radioactivity was slow with the highest % of dosed radioactivity excreted between 48 and 72 h. A mean of 0.55% of the applied radioactivity was excreted in the urine over 120 h.

Mean urine radioactivity

Mean radioactivity (% applied dose) in urine collected over each time interval (h) shown									
0#	0-4	4-8	8-12	12-24	24-36	36-48	48-72	72-96	96-120
0	0.01	0.03	0.03	0.08	0.09	0.06	0.11	0.07	0.06

Pre-dose

Faecal radioactivity: In 2 subjects, 0.01% applied dose was recorded for the 72-96 h period. The mean radioactivity excreted in faeces was < 0.01% of the applied dose.

Skin surface radioactivity: The mean % applied dose recovered from swabs, template, skin rinsate, dome, gauze and tape strippings was 60.72, 3.39, 2.67, 2.29, 1.48 and 0.89 respectively - a total of 71.4% of the applied radioactivity. The soapy water swabs removed a mean of 58.47% of dosed radioactivity within 18 h of dosing. The 1st and 2nd IPA swabs contained higher mean radioactivity (159903 and 253947 dpm respectively) than the last (16th) soapy water swab (44602 dpm). It was apparent that a significant amount of radioactivity remained in the epidermal layer of the skin after the soapy water swabbing. The study authors stated that this persistence of radioactivity in the skin could not be attributed to its presence in a water soluble compound (methamidophos) and concluded that the radioactivity remaining on the skin surface was probably not methamidophos but a bound metabolite. This observation was consistent with those made in other studies performed in monkey (Fuller *et al.*, 2000) evaluated in Section 2.6, and rat (Bagos *et al.*, 1991a) evaluated in Section 2.2. The collective data supports the conclusion that methamidophos is metabolised in the skin surface. It was shown (Canellakis & Tarver, 1953) that the metabolite methyl mercaptan is lost or bound to proteins on the skin.

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Total recovery of radioactivity: The total recovery of radioactivity from urine, faeces and application and cleaning material was 72.0%.

Dermal absorption: The study authors estimated that the dermal absorption was 4.84% of the applied dose. This parameter is usually determined by dividing the sum of the radioactivity excreted following dermal dosing by the sum of radioactivity excreted following iv dosing and expressing the result as a %. Since the determination of the sum of radioactivity excreted following iv dosing in humans was not possible, the study authors used the ratio (8.8) of iv to dermal excretion obtained for monkeys (Fuller *et al.*, 2000), evaluated in Section 2.6. The total mean radioactivity excreted following dermal dosing in humans was 0.55% applied dose and 8.8 x 0.55 = 4.84%.

Conclusion

The amount of methamidophos absorbed through human skin following a single 8 h exposure of the skin to a dose of 3 µg a.i./cm^2/8 h (0.88 µg/kg bw) methamidophos applied as Tamaron 600 SL, was 4.8% of the applied dose.

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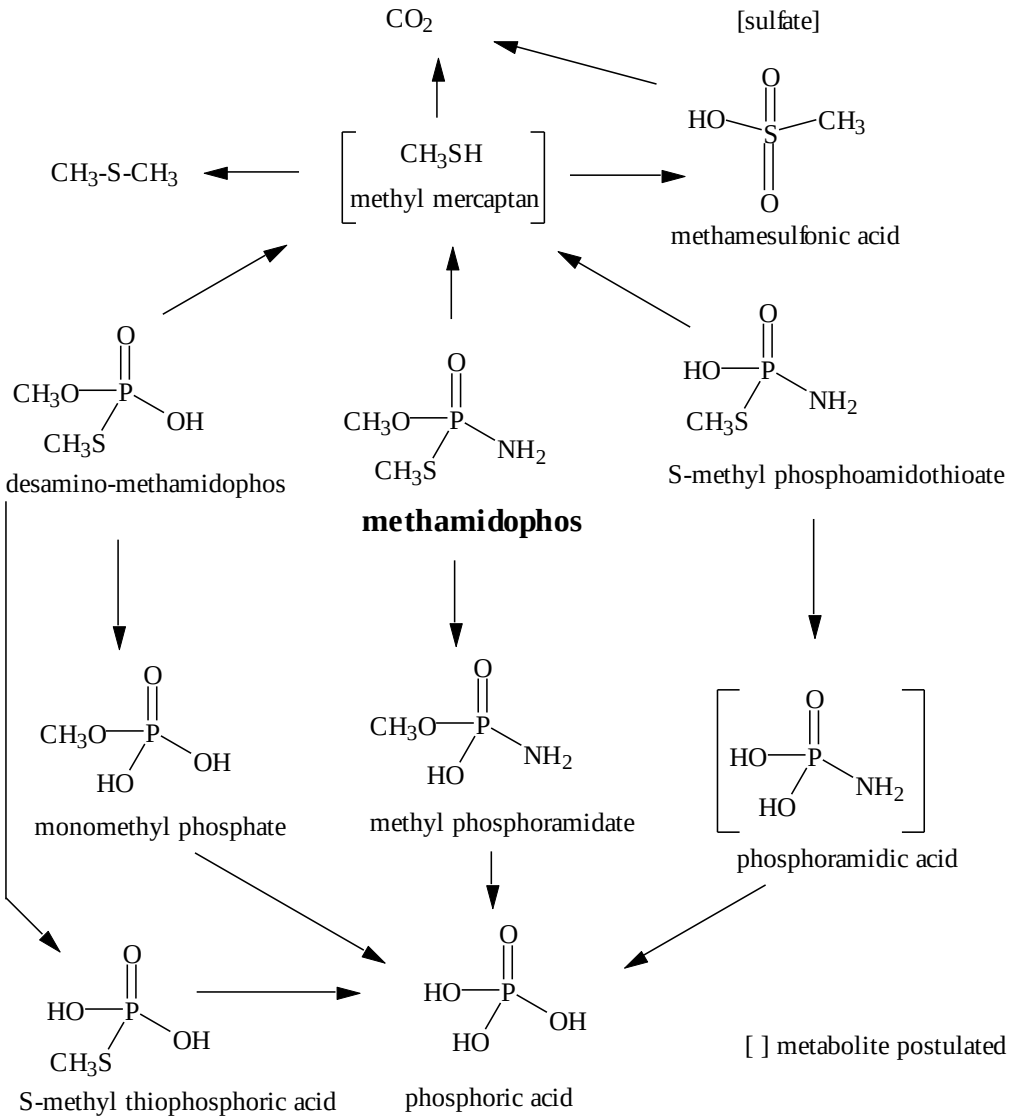
2.7 Proposed metabolic pathway of methamidophos in the rat



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3. ACUTE STUDIES

3.1 Active Constituent

3.1.1 Median Lethal Dose Studies

A summary of submitted and published findings of acute median lethal dose studies with technical methamidophos is shown in the tables below. The oral LD_{50}s were similar in rats and mice and ranged between 9 and 33 mg/kg bw. In general, the signs of acute methamidophos toxicity were similar in rats and mice and are typical of organophosphate poisoning. Clinical signs occurred within 5-10 minutes and included severe tremors, salivation, dyspnoea and, rarely, clonic convulsions. Deaths occurred between 20-30 min and 72 h. Animals that died had pulmonary congestion and oedema, and distension of the stomach and intestines with gas. Surviving animals recovered completely within 7 d. (Cavalli, Hallesy and Spence, 1968d).

Oral

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Mouse [ns]	ns	15	Glycol monoethyl ether	ns	1.0, 2.5, 5.0, 12, 25, 30, 38, 50	30	(Kimmerle & Lorke, 1967a)
Mouse [SW]	F	6	De-ionised Water	75	7.0, 10, 15, 19, 22, 34	18	(Cavalli *et al.*, 1968a)
Mouse [SW]	F	6	De-ionised Water	95	7.5, 11, 14, 16, 17, 25	16	(Cavalli *et al.*, 1968b)
Mouse [Swiss White]	F	5	Corn oil	ns	ns	14	(Kao & Fukuto, 1977)
Mouse [ns]	M	10	Distilled Water	ns ns ns ns	10, 13, 15, 17, 18 [f] 15, 18, 19, 22 [nf] 14, 15, 20 [f] 15, 18, 20, 25, 30 [nf] 15, 17, 20, 23 [f] 15, 18, 20, 25, 30, 35 [nf] 12, 14, 15 [f] 10, 12, 15, 16, 18, 20 [nf]	13 18 16 27 18 28 14 18	(Mihail, 1981c)
Mouse [Out-bred CD-1]	M/F	5/sex	Distilled water	74	9.0, 14, 20, 30	F 15 M 1/5 deaths at 14, 5/5 at ≥ 20	(Esber & Carver, 1983) [GLP]
Mouse [K]	M/F	ns	Distilled Water	90	ns	M 12 F 10	(Guo *et al.*, 1986a)
Rat [SD]	M/F	5M 5F 10F	Water	75	10, 15, 22, 34 7.0, 10, 34 15, 19, 22	M 21 F 19	(Cavalli *et al.*, 1968c)

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Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rat [ns]	M/F	5M 5F	De-ionised Water	95	5.0, 7.5, 11, 14, 17, 25 11, 14, 17, 25	M 16 F 13	(Cavalli *et al.*, 1968d)
Rat [ns]	M	15 30	Glycol monoethyl ether	ns	1.0, 2.5, 5.0, 25, 30, 50 38	30	(Kimmerle & Lorke, 1967a)
Rat [Wistar II albino]	M	10 20	Distilled water and Cremophor EL	ns	15, 25, 30, 35, 45 20	32	(Thyssen & Kimmerle, 1976)
Rat [Wistar albino]	M	15	Distilled water and Cremophor EL	64	25, 30, 35, 50	30	(Flucke *et al.*, 1977)
Rat [ns]	M	10	Distilled Water	ns	10, 25, 30, 50, 75	33	(Thyssen & Lorke, 1977a)
Rat [Wistar albino]	M M	15 30	Cremophor EL and Distilled Water	64	25, 30, 35, 50	30	(Thyssen & Kimmerle, 1977b)
Rat [Wistar albino]	M	ns	Cremophor EL and Distilled Water	64	ns	24	(Thyssen *et al.*, 1977c)
Rat [Wistar albino]	M	15	Cremophor EL and Distilled Water	87.7	20, 22.5, 25, 35, 50	29	(Flucke & Lorke, 1978)
Rat [ns]	M/F	10M 10F 20F	Distilled Water	64.5	10, 15, 20, 22.5, 25, 27.5, 30, 35, 50 [f] 17.5, 25, 30, 35, 40 [f] 20 [f]	M 18 F 26	(Mihail, 1981a)
Rat [ns]	M	10 10 10 10	Distilled Water	ns ns ns ns	7, 8, 10, 15 [f] 10, 12.5, 15, 20, 25 [nf] 12, 15, 20 [f] 15, 25, 30 [nf] 12, 15, 18, 20 [f] 10, 15, 20, 25 [nf] 7, 8, 10, 11, 12 [f] 10, 12, 18, 20, 22, 25 [nf]	9 16 16 22 15 19 9 16	(Mihail, 1981b)
Rat [Bor: WISW (SPF-Cpb)	M	5	Distilled water and Cremophor EL	73	1.0, 10, 14, 16, 20, 25	16	(Heimann & Lorke, 1983)
Rat [S]	M/F	10/sex	Peanut oil	ns	4 unspecified doses	M 25 F 27	(Gaines & Linder, 1986)

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Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rat [Wistar albino]	M/F	ns	Distilled Water	90	ns	M 23 F 13	(Guo *et al.*, 1986a)
Rat [Wistar Bor:WISW (SPF-Cpb)	M	5	Demineral-ised water	R(±) 95.3 E1(+) 98.5 E2(-) 97.8	R(±) 10, 14, 16, 20, 32 E1(+) 10, 12, 13, 14, 16, 20, 32 E2(-) 10, 12, 14, 16, 20, 32	R(±) 16 E1(+) 14 E2(-) 16	(Flucke *et al.*, 1990a) [GLP]
Rat [Wistar HSD/ WIN: WU]	M	5	2% v/v Cremophor EL in deionised water	73.8%	10, 13, 16, 18, 25	15	(Krotlinger *et al.*, 1994) [GLP]
Guinea pig [ns]	ns	5	Glycol monoethyl ether	ns	30, 50	0/5 deaths at 30, 5/5 at 50	(Kimmerle & Lorke, 1967a)
Rabbit [ns]	ns	3	Glycol monoethyl ether	ns	5.0, 10, 30	1/3 deaths at 10, 3/3 at 30	(Kimmerle & Lorke, 1967a)
Cat [ns]	ns	2 1	Glycol monoethyl ether	ns	10 30	0/2 deaths at 10, 1/1 at 30	(Kimmerle & Lorke, 1967a)
Dog [ns]	ns	2 1	Glycol monoethyl ether	ns	10 30	0/2 deaths at 10, 1/1 at 30	(Kimmerle & Lorke, 1967a)

Abbreviations: ns=not specified, SD=Sprague-Dawley, SW=Swiss-Webster, S=Sherman, K=Kunming, R(±)=(±)-methamidophos:racemic, E1(+)=(+)-methamidophos, E2(-)=(-)-methamidophos, M=Male, F=Female, f=fasted, nf=non-fasted.

Dermal

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rat [NS]	M M	5 5	Oil (nature ns) Test material applied as 10% solution in oil	ns	5.0, 50, 100, 200, 500 (4 h exp non-occ/7 d obs) 5.0, 10, 40, 50, 100, 500, 1000 (7 d non-occ exp/7 d obs)	110 50	(Kimmerle & Lorke, 1967d)
Rat [Wistar albino WISW (SPF-Cpb)	M/F	5M 10M 5F 10F	Cellulose thickener or none	64.5	10, 50, 1000 75, 100, 150, 200, 250, 350 (all 24 h occ exp/14 d obs) 10, 1000 50, 75, 100, 150,200 (all 24 h occ exp/14 d obs)	M 162 F 108	(Heimann & Lorke, 1981a)
Rat [S]	M/F	10/sex	Ethanol	ns	4 unspecified doses	M 179 F 151	(Gaines & Linder, 1986)
Rat [K]	M/F	ns	Distilled Water	90	ns	M 355 F 380	(Guo *et al.*, 1986a)

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Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rabbit [NZW]	M	4	None	75	100, 125, 156, 222 (24 h occ exp/14 d obs)	118	(Cavalli *et al.*, 1968e)
Rabbit [NZW]	M/F 4M	4/sex	None	73.2	30, 45, 68, 101 (24 h occ exp/14 d obs) M Higher dosing: 152, 342 (24 h occ exp/14 d obs)	F 69 M 0/4 deaths at ≤ 45, 1/4 at ≥ 68 M 122	(Hixson *et al.*, 1980a)

Abbreviations: NZW=New Zealand White, M=Male, F=Female, ns=not specified, occ=occlusive, exp=exposure, obs=observation

Inhalational

Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%)	Concentrations Tested (mg/m^3)	LC50 (mg/m^3)	Reference
Mouse [ns]	NS	20 20	Alcohol and Lutrol (1:1)/static inh apparatus/ wb exp	ns	16, 30, 102 (1 x 1 h /14 d obs) ds ns 3.0, 11, 30 (1 x 4 h /14 d obs) ds ns	0/20 deaths at ≤ 30, 1/20 at 102 0/20 deaths at ≤ 11, 3/20 at 30	(Kimmerle & Lorke, 1967e)
Mouse [ns]	F	8	Distilled Water/wb exp	ns	single concentration 650 mg/m^3 at various unspecified times to a 5 h maximum/ds ns	19* (mg/kg bw)	(Berteau & Chiles, 1978)
Rat [ns]	M	20 20	Alcohol and Lutrol (1:1)/dyn-amic inh apparatus	ns	69, 171, 265, 680, 785 (1 x 1 h exp pbe ns/14 d obs) ds ns 33, 83, 158, 317 (1 x 4 h exp pbe ns/14 d obs) ds ns	525 (1 h exp) 162 (4 h exp)	(Kimmerle & Lorke, 1967e)
Rat [ns]	ns	10 10	Alcohol and Lutrol (1:1)/static inh apparatus	ns	16, 30, 102 (1 x 1 h wb exp/14 d obs) ds ns 3.0, 11, 30 (1 x 4 h wb exp/14 d obs) ds ns	0/10 deaths at ≤ 30, 1/10 death at 102 0/10 deaths at ≤ 30	(Kimmerle & Lorke, 1967e)
Rat [ns]	F	8	Distilled Water/wb exp	ns	single concentration 650 mg/m^3 at various unspecified times to a 5 h maximum/ds ns	9* (mg/kg bw)	(Berteau & Chiles, 1978)

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Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%)	Concentrations Tested (mg/m^3)	LC50 (mg/m^3)	Reference
Rat [ns]	M/F	10/sex	Water/ dynamic inh apparatus	75	60, 168, 259, 163, 196, 160, 319, 253 (1 x 1 h ho exp/14 d obs) (MMD 90% < 5 μm)	M 377 F 241	(Sangha *et al.*, 1983b)
Rat [ns]	M/F	10/sex 10F	Water/ dynamic inh apparatus	70.5	19, 33, 57, 56, 82 (1 x 4 h ho exp/14 d obs) (MMD ≈ 0.5 μm) 62, 172 (1 x 4 h ho exp/14 d obs) (MMD ≈ 0.6 μm)	M 63 F 76	(Sangha *et al.*, 1984a) [GLP]
Rat [Wistar WISW (SPF-Cpb)	M/F	5/sex	Polyethylene glycol E 400-ethanol mixture (1:1)/dyn-amic inh apparatus	75.7	11, 24, 45, 196, 242, 350 (1 x 4 h ho exp /14 d obs) (MMAD=1.5μm)	213 (no sex-specific variations given)	(Pauluhn & Machemer, 1987a)
Guinea pig [ns]	ns	5 5	Alcohol and Lutrol (1:1)/static inh apparatus	ns	16, 30, 102 (1 x 1 h wb exp/14 d obs) ds ns 3.0, 11, 30 (1 x 4 h wb exp/14 d obs) ds ns	0/5 deaths at ≤ 102 0/5 deaths at ≤ 30	(Kimmerle & Lorke, 1967e)
Rabbit [ns]	ns	3 3	Alcohol and Lutrol (1:1)/static inh apparatus	ns	16, 30, 102 (1 x 1 h wb exp/14 d obs) ds ns 3.0, 11, 30 (1 x 4 h wb exp/14 d obs) ds ns	0/3 deaths at ≤ 102 0/3 deaths at ≤ 11, 1/3 at 30	(Kimmerle & Lorke, 1967e)

Abbreviations: SD=Sprague-Dawley, MMD=Mass Median Diameter (of droplet), MMAD=Mass Median Aerodynamic Diameter (of droplet), GLP=Good Laboratory Practice, M=Male, F=Female, ns=not specified, ds=droplet size, exp=exposure, obs=observation, ho=head only, wb=whole body, pbe=part body exposed
* mg/kg bw dose based on aerosol concentrations, duration of exposure, minute volumes and information on whole body deposition

3.1.2 Skin Irritation Studies

Kimmerle G & Lorke D (1967f): Toxicological studies on the active ingredient Bayer 71 628. Skin function tests. Study No. not specified. Report No. not specified. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: not specified. Report Date: 23 February 1967. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance: A mixture containing 90% methamidophos and 10% glycol monoethyl ether. Source: not specified.
Test Species: Rabbits: unspecified breed, strain, source, age, sex or body weight.
Dose: Not specified.

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Method

Small compresses of cottonwool impregnated with the test mixture were placed inside the ears of 2 rabbits. The compresses were fixed in position by means of adhesive bandage for 24 h. No other details of the experimental protocol were provided.

Results and discussion

It was reported that exposure resulted in moderate reddening and swelling of the treated areas of the skin. Clarification as to whether or not both ears of each rabbit were test sites was not provided. These effects were reported to have persisted for "about 24 h". It is not known whether or not the test mixture was washed from the test sites. No effects were observed at 8 h after the exposure. The method of progressive observation of the effects was not discussed.

Conclusions

The test mixture used was demonstrated to have caused irritation of the inner ear of the rabbit. Glycol monoethyl ether is a slight skin irritant (Union Carbide Corp, 1966). It is not possible to unequivocally attribute the irritation observed to methamidophos. It is also not possible to grade the severity of the irritancy observed. Given that the protocol did not yield an interpretable result, this study cannot be used in the hazard assessment of methamidophos.

Rittenhouse JR & MacGregor JA (1977a): The skin irritation potential of MONITOR™ Technical. Study No. S-1144. Report No. SOCAL 1109/30:111. Lab: Environmental Health & Toxicology, Standard Oil Company, San Francisco, California. Sponsor: Ortho Division, Chevron Chemical Company, Richmond, California. Study Duration: not specified. Report Date: 28 October 1977. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Technical grade methamidophos. Identification Code: SX 887. Physical state: clear semi-solid.
Test Species:	New Zealand White Rabbits: male, of an unspecified source, age or body weight.
Dose:	0.5 mL/test site.

Method

The rabbits used in this study were individually housed in wire-bottom cages in an air-conditioned environment maintained at a temperature of 70^{o}F. The rabbits were given a daily ration (approximately 4 ounces) of commercial laboratory food. They had free access to water. The fur on the backs of 6 rabbits was clipped away on the day prior to testing. The test material was applied to an intact and an abraded site (of an unspecified size) on the back of each rabbit. The epidermal abrasions were made with a hypodermic needle. Each treated area was covered with a gauze patch secured by adhesive tape. The trunk of each rabbit was loosely wrapped in a plastic sheet. Paper towels were wrapped around the plastic sheet to prevent tearing. A collar was also placed on each rabbit to protect the wrappings during the exposure period. After a 24 h exposure, the wrappings and patches were removed. The report did not indicate whether or not the treated areas were washed at this time. Irritation was scored at 24, 48 and 72 h and at 7 d. A modification of a previously established scoring system (Draize *et al.*, 1944) was used for this purpose.

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Results and discussion

The results of the study are tabulated below. Shortly after treatment, all of the rabbits salivated, became ataxic and developed tremors. Three died overnight. The surviving rabbits had slight to well defined (Grade 1-2) erythema at 24 h at both the intact skin and abraded skin sites. A fourth rabbit died between the 24 and 48 h reading. One rabbit had slight (Grade 1) erythema at both test sites at 48 h. The 2 surviving rabbits had normal skin at each test site at 72 h. Oedema was not observed. The primary skin irritation score could not be calculated because of the high mortality among the test rabbits. The authors did not offer any explanation for the high mortality.

Mean skin irritation grades in rabbits following the application of methamidophos

Time after Treatment (h)	Number of Rabbits	Intact Skin		Abraded Skin	
		Erythema	Oedema	Erythema	Oedema
24	3#	1.7	0	1.7	0
48	2##	0.5	0	0.5	0
72	2	0	0	0	0

Three rabbits died within 24 h
One rabbit died after 24 h but before 48 h

Conclusions

The results of this study indicate that the slight to well defined erythema caused by the test substance reverses completely between 48 and 72 h after treatment. The test substance can be classified according to OCS guidelines as not irritant to the skin.

Levy JE, Wong ZA & MacGregor JA (1979): The skin irritation potential of Monitor™ technical. Study No. SOCAL 1445/39:24. Report No. not specified. Lab: Environmental Health & Toxicology, Standard Oil Company, San Francisco, California. Sponsor: Ortho Division, Chevron Chemical Company, Richmond, California. Study Duration: 12 June 1979 - 19 June 1979. Report Date: 22 August 1979. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Technical grade methamidophos. Identification Code: SX-1128. Physical state: clear viscous liquid. Source:
Test Species:	New Zealand White Rabbits: male, aged 8-12 w of an unspecified bw, obtained from the Nitabell Rabbitry, Hayward, California.
Dose:	100 μL/test site.

Method

Nine rabbits were clipped free of fur and the test material was applied to an intact and abraded site on the back of each rabbit. The epidermal abrasions (cross hatching) were made with a hypodermic needle penetrating the stratum corneum over areas of 1 square inch. Each treated area was covered with a gauze patch secured by adhesive tape. The trunk of each rabbit was loosely wrapped in a plastic sheet and paper towels were wrapped around the plastic sheet to prevent tearing. A collar was also placed on each rabbit to protect the wrappings during the exposure period. The collars were removed soon after application because of the appearance

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of clinical signs described below. After a 24 h exposure, the wrappings and patches were removed and the skin wiped to remove any remaining test material. Irritation was scored at 24, 48, 72 and 96 h and at 7 d. A previously established scoring system (Draize *et al.*, 1944) was used for this purpose.

Results and discussion

The results of the study are tabulated below. Clinical signs were observed after application of the test material. They were reported as increased respiration, ataxia, salivation, miosis, tremors, diarrhoea, collapse and death. Five rabbits died within 24 h of application of the test material. All of the 4 surviving rabbits had well-defined (Grade 2) erythema at their abraded skin sites at 24 h. One had very slight (Grade 1) erythema at the intact skin site and 3 had well-defined erythema at their intact skin sites. Two of the surviving rabbits had very slight (Grade 1) oedema at their intact skin sites. One showed the same response at the abraded skin site.

At 48 h, very slight erythema at all skin sites was noted. Oedema was not observed at that time. At 72 h, one rabbit exhibited very slight erythema on its abraded skin. At 96 h, all skin responses had reversed completely. The primary irritation score was estimated to be 0.6. There were no significant differences in the severity of effect between the intact or abraded sites. The authors did not offer any explanation for the high mortality among the test rabbits.

Mean skin irritation grades in rabbits following the application of methamidophos

Time after Treatment (h)	Number of Rabbits	Intact Skin		Abraded Skin	
		Erythema	Oedema	Erythema	Oedema
24	4[#]	1.75	0.5	2	0.25
48	4	1	0	1	0
72	4	0	0	0.25	0
96	4	0	0	0	0

Five rabbits died within 24 h

Conclusions

Under the conditions of the study, methamidophos technical caused very slight to well defined erythema of the skin. At the 72 h assessment, one very slight oedema reaction at an abraded skin site remained but irritation of intact skin had reversed. However because of the low dose applied, the skin irritancy potential of the test substance may have been under estimated.

Hixson EJ, English TD, Mallicoat DR & Lamb DW (1980b): Eye and Dermal Irritation of Methamidophos (MONITOR™). Mobay Report No. 68792. Study Nos. 80-023-06 & 80-033-10. Toxicology Report No. 121. Lab: Stanley Research Centre, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 11 August 1980 - 5 September 1980. Report Date: 29 September 1980. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance: Technical grade methamidophos. Formulation number: 605500. Batch number: 9030005 Purity: 73.2 % w/v.

Test Species: New Zealand White Rabbits of an unspecified age, sex and bw, obtained from Small Stock Inc, Pea Ridge, Arkansas.

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Dose: 0.003 mL /test site.

Method

The backs and sides of 6 rabbits were closely shaved. Four test sites were used on each animal. Two sites were abraded by crosshatching through the stratum corneum with a sterile 19-gauge needle. The other 2 sites were left intact. Plastic collars were placed on the rabbits. The test substance was applied to each test site on each rabbit under a 1-inch square gauze patch. The trunks of the rabbits were wrapped with a plastic sheet and secured with adhesive tape. After 24 h the patches were removed. The test material was removed by wiping the backs of the rabbits with a damp cloth. The test areas were evaluated at 24 and 72 h after the test material was removed. A previously established method (Draize, 1959) was used for this purpose.

Results and discussion

The dermal responses to the test material are tabulated below. At 24 h slight (Grade 1) erythema was observed on the abraded skin sites of 4/6 rabbits; however the authors suggested that the erythema appeared to be due to the abrasions more than the application of the test material. No other responses were observed at 24 or 72 h. The authors did not report any mortality or other clinical signs in the test animals.

Mean skin irritation grades in rabbits following the application of methamidophos

Number of Rabbits	Time of Appraisal (h)	Intact Skin		Abraded Skin	
		Erythema	Oedema	Erythema	Oedema
		Test Site		Test Site	
		1 2	1 2	1 2	1 2
6	24	0 0	0 0	0.7# 0.5#	0 0
	72	0 0	0 0	0 0	0 0

The slight (Grade 1) erythema at these sites was attributed by the authors to the abrasion process

Conclusions

The results of this study indicated that the test substance did not irritate the intact skin sites nor apparently the abraded skin sites under the conditions of the study. However any dermal reaction to the test substance may have been reduced by the low dose applied.

Martins T, Machemer L & Pauluhn J (1990): SRA 5172 (methamidophos). Study for skin and eye irritation/corrosion in rabbits. Study No. T 0036010. Report No. 19610. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 24 April 1990 - 2 May 1990. Report Date: 10 October 1990. Unpublished. (QA: Yes; GLP: US EPA/FIFRA (40 CFR Part 160) & OECD (Bundesanzeiger 35, No 42a); Test Guidelines: OECD Guidelines Nos 404 and 405, EEC Directive 84/449/EEC Part B No B4 and B5, Guideline 83/467/EC & US EPA/TSCA (798.4470 & 798.4500).

Test Substance: Technical grade methamidophos. Product number: 783390
Batch number: 233090326. Purity: 74.2%.
Source: Bayer AG, Leverkusen, Germany.

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Test Species: New Zealand White Rabbits: Strain HC:NZW (albino), approximately 3.0 to 3.5 kg. bw, obtained from Interfauna UK Ltd., Wyton, Huntingdon, England.
Dose: 500 μL /test site.

Method

Approximately 24 h before the test, fur was shorn from a 6 x 6 cm area on the dorso-lateral aspect of the body of each of 3 female rabbits. The test substance was applied to a hypoallergenic patch. A further patch was moistened with water. The patches prepared in this way were placed on the opposite dorso-lateral areas of the trunk of each rabbit and were loosely held in place with a semiocclusive dressing for the duration of the exposure period. After an exposure period of 4 h, the dressing and patches were removed. The exposed skin areas were washed with water without altering the existing response, or the integrity of the epidermis. The contralateral skin areas not treated with test substance served as control areas. Dermal irritation was scored according to a previously established method (Draize, 1959) at 1, 24, 48, 72 h and 8 and 14 d after the termination of exposure. The rabbits were also monitored closely for mortality or other signs of toxicity

Results and discussion

No skin reactions were observed. No other adverse events were reported.

Conclusion

The test substance showed no capacity to irritate rabbit skin. It can be classified according to OCS guidelines as not irritant to skin.

Comment

Note the disparity in toxicity observed in this study compared with that of Rittenhouse & MacGregor (1977a), even though the dose applied in each study was the same (ie. total of 1 mL/rabbit). This may be due to enhanced dermal absorption of methamidophos across abraded skin.

3.1.3 Eye Irritation Studies

Rittenhouse JR & MacGregor JA (1977b): The eye irritation potential of MONITOR™ Technical. Study No. S-1143. Report No. SOCAL 1108/30:110. Lab: Environmental Health & Toxicology, Standard Oil Company, San Francisco, California. Sponsor: Ortho Division, Chevron Chemical Company, Richmond, California. Study Duration: not specified. Report Date: 28 October 1977. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance: Methamidophos technical. Identification Code: SX 887
Physical state: clear semi-solid. No other details provided.
Test Species: New Zealand White Rabbits: of an unspecified source, age, sex or bw.
Dose: 0.1 mL/test site.

Method

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The test material was placed in the conjunctival sac of 1 eye in each of 6 rabbits. The untreated eye served as the control. The eyes were examined and graded for ocular reaction at 1 h and at 1, 2, 3, 7, 10 and 14 d using a modification of a previously established system (Draize *et al.*, 1944).

Results and discussion

The results are tabulated below.

One rabbit died 30 min after treatment. An explanation for this mortality was not provided. Slight (Grade 1) and moderate (Grade 2) corneal opacity were observed 1 h after treatment in 1 and 4 rabbits respectively. The slight corneal opacity in the single rabbit had progressed at 2 d to moderate which was still present at 3 d. It had reversed to slight at 7 d. This slight corneal opacity was still present at 10 d. At 7 and 10 d the cornea was also noted to have slight pannus. All eye lesions in this rabbit had completely reversed by 14 d. The moderate corneal opacity in the other 4 rabbits had reversed to slight corneal opacity at 1 d. It had completely reversed in 3 of them by 7 d. In the remaining rabbit, slight corneal opacity together with slight pannus was present at 7 and 10 d. Corneal opacity was not present in any rabbit at 14 d.

At 1 h, 3 rabbits showed mild (Grade 1) iritis which was still present in 2 of them at 1 d. Iritis was not observed in any rabbit at or after 2 d. At 1 h, all rabbits showed moderate (Grade 2) erythema of the conjunctiva, severe (Grade 3) ocular discharge and moderate (Grade 2) chemosis. At 1 d after treatment the erythema in 3 rabbits had progressed to severe, the ocular discharge in 3 rabbits had reversed to moderate and 4/5 rabbits showed a reversal in chemosis to slight. All of these conjunctival effects had reversed to slight at 2 d. The only remaining signs were seen in 1 rabbit at 7 d. It had slight erythema and slight ocular discharge. Conjunctivitis was not seen in any rabbit at or after 10 d.

The effects (mean grades) of Monitor™ technical on the eyes of rabbits

Time (d)	Number of Rabbits	Corneal Opacity	Iritis	Conjunctivitis		
				Erythema	Discharge	Chemosis
1 (h)	5[#]	1.8	0.6	2	3	2
1	5	0.8	0.4	2.6	2.4	1.2
2	5	1.2	0	1	1	1
3	5	1.0	0	1.2	1	0.8
7	5[##]	0.4	0	0.2	0.2	0
10	5[##]	0.4	0	0	0	0
14	5	0	0	0	0	0

One rabbit died 30 min after treatment
Slight pannus noted in 2 rabbits

Conclusions

The findings indicate that the test substance can be classified according to OCS guidelines as a severe irritant to the rabbit eye.

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Hixson EJ, English TD, Mallicoat DR & Lamb DW (1980b): Eye and Dermal Irritation of Methamidophos (MONITOR™). Mobay Report No. 68792. Study Nos. 80-023-06 & 80-033-10. Toxicology Report No. 121. Lab: Stanley Research Centre, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 11 August 1980 - 5 September 1980. Report Date: 29 September 1980. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Technical grade methamidophos. Formulation number: 605500. Batch number: 9030005. Purity: 73.2 % w/v.
Test Species:	New Zealand White Rabbits: male, of an unspecified age and bw, obtained from Small Stock Inc, Pea Ridge, Arkansas.
Dose:	0.01 mL/test site.

Method

The eyes of 9 rabbits were examined using fluorescein dye and an ultraviolet dye to ensure that the eyes were free of defects or irritation. After 3 d the rabbits were treated by placing 0.01 mL of the test substance in the left eye. The study authors indicated that the volume applied was 0.1 of the volume specified in the protocol because the dermal LD_{50} of this compound is 122 mg/kg bw in male rabbits. They did not elaborate further except to say that "this amount approximated 0.1 x LD_{50}". The treated eyes of 3 rabbits were washed 45 s after the test material was administered, with ≈ 200 mL of lukewarm water. The treated eyes of the remaining 6 rabbits were not washed. On 1, 2, 3, 4 and 7 d, the eyes were examined for lesions of the cornea, iris and conjunctivae. The eye reaction evaluations were conducted using a previously established method (Draize, 1959).

Results and discussion

The study authors did not describe any other clinical signs or mortality in the rabbits.

Washed Group: No corneal lesions were seen in the 3 rabbits of the washed group. Slight (Grade 1) iritis was observed in all 3 rabbits at 1 d and 2 d. This had completely reversed in all rabbits by 7 d. Slight (Grade 1) erythema and moderate (Grade 2) chemosis was present in all 3 rabbits at 7 d. At 7 d, 1 rabbit showed no ocular discharge, another showed moderate (Grade 2) discharge and the third showed slight (Grade 1) discharge. Slight to moderate conjunctival effects were present at 13 d in 2 of the rabbits and in 1 of those 2, the responses were deemed by the study authors to have been irreversible.

Unwashed Group: No corneal lesions were seen in the 6 rabbits of the unwashed group. Slight iritis was observed at 1 d in five rabbits. This had reversed at 4 d. Slight erythema appeared in 6 rabbits and was still present in 3 at 7 d. Moderate chemosis was noted in 6 rabbits at 1 d. At 7 d, 1 rabbit had moderate chemosis and 4 had slight chemosis. At 13 d, 2 rabbits had slight erythema, 3 had slight chemosis and 1 had slight discharge. At 10 and 13 d the eye responses of 1/6 rabbits was not recorded.

The results are tabulated below:-

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The effects (mean grades and scores) of Monitor™ technical on the eyes of rabbits*

Parameter Examined	Examination Time (d)						
	1	**2**	**3**	**4**	**7**	**10**	**13**
Mean grades:							
C Iritis w	1	1	0.3	0.3	0	0	0
uw	0.8	0.7	0.2	0	0	0	0
D Erythema w	1	0	1	1	1	0.4	0.4
uw	1	1	0.7	0.5	0.5	0.6	0.4
E Chemosis w	1	0.7	0.7	0.3	2	1.3	1.0
uw	2	1.7	1.3	1.3	1	1.2	0.6
F Discharge w	1	1.3	1	1	1	0.7	0.7
uw	1.7	1	0.5	0.3	0.3	0.2	0.2
Mean total scores:							
Iris** w	5	5	1.7	1.7	0	0	0
uw	4.2	3.3	0.8	0	0	0	0
Conjunctivae*** w	6	4	5.3	4.7	8	5.3	4.7
uw	8.7	6.5	5	4.3	3.6	4	2.4

* the negative corneal findings are not presented here
w 3 rabbits - eyes washed with water 45 s after treatment
uw 6 rabbits - eyes not washed after treatment: 5 rabbits only recorded at 10 and 13 d
**means of score C x 5: maximum = 10
***means of score (D + E + F) x 2: maximum = 20

Conclusions

The test substance did not cause corneal lesions in the rabbit eye. According to OCS guidelines the test substance would be classified as a slight irritant to the rabbit eye under the study conditions, but its irritancy potential would be underestimated because of the low dose used. The ocular reactions observed were persistent and washing did not reduce the severity or duration of effects on the iris or conjunctiva.

Martins T, Machemer L & Pauluhn J (1990): SRA 5172 (methamidophos). Study for skin and eye irritation/corrosion in rabbits. Study No. T 0036010. Report No. 19610. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 24 April 1990 - 2 May 1990. Report Date: 10 October 1990. Unpublished. (QA: Yes; GLP: US EPA/FIFRA (40 CFR Part 160) & OECD (Bundesanzeiger 35, No 42a); Test Guidelines: OECD Guidelines Nos 404 and 405, EEC Directive 84/449/EEC Part B No B4 and B5, Guideline 83/467/EC & US EPA/TSCA (798.4470 & 798.4500).

Test Substance: Methamidophos.
Batch number: 233090326.
Purity: 74.2%.
Physical state: transparent brown liquid.

Test Species: New Zealand White Rabbits: Strain HC:NZW (albino), of an unspecified age, female, and approximately 3.0 to 3.5 kg. bw, obtained from Interfauna UK Ltd., Wyton, Huntingdon, England.

Dose: 100 µL /test site.

Method

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The test substance was instilled into the conjunctival sac of 1 eye of each of 3 female rabbits. The other eye remained untreated and served as a control. Twenty four h after instillation of the test substance, 1 drop of 1% fluorescein solution was applied to the corneal surface to assist in the definition of epithelial damage. Where positive effects were recorded this was repeated at the later observation times. The eye was then rinsed with saline to remove non-absorbed excess fluorescein. The eyes were examined with optical instruments under ultraviolet illumination and diffuse white illumination in a darkened room (McDonald & Shadduck, 1987). They were examined and scored at 1 h, 24 h, 48 h, 8d, 14 d and 21 d. Only effects persisting for more than 24 h were included in the evaluation. Previously established scoring methods (Draize, 1959) were used. Serious lesions or systemic toxic effects were also recorded.

Results and discussion

At 1 h after the administration of the test substance, 2 rabbits experienced diarrhoea, tremor, salivation and tachypnoea. At 24 h these signs had completely reversed. In 1/3 rabbits no irritation of the eye occurred. None of the 3 rabbits experienced corneal or iridial damage. One rabbit showed slight (Grade 1) conjunctival erythema and slight ocular discharge (Grade 1) at 24 h. At 48 h the slight erythema was still present but had completely reversed at 72 h. At and after 48 h the discharge was not observed. At 1 h, 24 h, 48 h and 72 h, the third rabbit showed slight erythema which had completely reversed by 8 d. At 1 h and 24 h, it had shown moderate (Grade 2) and slight (Grade 1) chemosis respectively. This had completely reversed by 48 h. At 1 h, 24 h and 48 h, the third rabbit also showed slight, moderate (Grade 2) and slight discharge respectively. The discharge had completely reversed by 72 h.

At 24 h, the total ocular irritation severity score for the 2 rabbits that showed irritation, was 6 and 8 respectively - giving a mean total score of 4.6 for the 3 rabbits. At 72 h, 1 of the rabbits showed a total score of 2 - giving a mean score of 0.6.

Conclusions

The test substance did not cause corneal lesions in the rabbit eye. According to OCS guidelines the test substance can be classified as a slight irritant to the rabbit eye.

Comment

Despite the application of a 100 µL volume of the test substance, the ocular reactions recorded in this study were less severe and of a shorter duration than those reported by Rittenhouse & MacGregor (1977b) and Hixson *et al.*, (1980b).

3.1.4 Skin Sensitisation Studies

Korenaga GL, Cushman JR & Wong ZA (1984): Modified Buehler test for the skin sensitisation potential of methamidophos technical (SX-1490). Study No. SOCAL 2135. Toxicology Report No. 596. Lab: Chevron Environmental Health Centre, Richmond, California, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 25 April 1984 - 11 June 1984. Report Date: 21 December 1984. Unpublished. (QA: Yes; GLP: Yes: US EPA/FIFRA & USA/FDA; Test Guidelines: not specified).

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Test Substance: Methamidophos technical.
Identification codes: SX-1456 & SX-1490
Purity: SX-1456: 71.3% methamidophos used for pretest screening.
Purity: SX-1490: 73.8% methamidophos used at 25% w/w in distilled water for the study.
Physical state: both SX-1456 and SX-1490 were a brown liquid.

Test Species: Hartley Guinea Pigs: male, albino, 31 d old on the day of induction, 251-326 g at assignation to treatment groups. They were supplied by the Stoneridge Division of Charles River Breeding Laboratory, Wilmington, Massachusetts.

Dose: 0.3 mL/test site for the first induction; 0.4 mL/test site for the next 9 induction applications & 0.3 mL/test site for each of 2 challenges.

Method

A modification of a previously described procedure (Buehler, 1965) was used in the design of this study. All doses were administered using a Pipetman 1000^{R}. The scoring of dermal reactions was based on a previously established system (Draize *et al.*, 1944). The design of this study is specified in the following table:-

The design and test materials used

Group	**Number of Guinea Pigs**	**Test Materials**		
		Induction	**First Challenge**	**Second Challenge**
Methamidophos Technical (MT)	15	25% w/w MT (in distilled water)	25% w/w MT (in distilled water)	25% w/w MT (in distilled water)
MT Irritation Control	10	Distilled water	25% w/w MT (in distilled water)	Distilled water
MT Vehicle Control	10	Distilled water	Distilled water	25% w/w MT (in distilled water)
1-chloro-2, 4-dinitrobenzene (DNCB)	10	0.1% DNCB (in 80% v/v ethanol)	0.1% DNCB (in acetone)	0.1% DNCB (in acetone)
DNCB Irritation Control	10	80% v/v Ethanol	0.1% DNCB (in acetone)	Acetone

The procedures used are described as follows:-

Induction: On d -1 and weekly thereafter, the right flank of each guinea pig was clipped free of fur. From d 0 onwards, a dose of the appropriate test or control material was applied to the skin on alternate days over 22 d for a total of 10 doses. A 6 h exposure period was used. Skin irritation reactions were blindly scored at 24 h and 48 h after the 1st dose. Skin irritation reactions were also blindly scored at 24 h after the 5th and 10th induction dose. The readings following the 5th and 10th dose were taken to assess the effects of repeated dosing.

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For the 1st induction dose the test material(s) were placed in a Hill Top Chamber™ (Hill Top Research Inc, Cincinnati, Ohio) applied to the right flank of each guinea pig. The chamber was held in place by a PEG^{R} cohesive bandage encircling the trunk of the body. The wrap was secured at each end by a strip of porous tape. After removal of the wrap, the sites treated with 1-chloro-2, 4-dinitrobenzene (DNCB) or the DNCB vehicle were wiped with dry gauze pads. All other sites were wiped with gauze pads moistened with distilled water. The remaining 9 doses were administered to the skin of the right flank. They were kept in contact with the skin with a 1-inch square of Webril^{R} gauze covered by a 2-inch square of polythene.

First Challenge: 1 dose of test or control material was applied to the skin 14 d after the 10th induction dose. The test sites were depilated at 24 h after dosing with Neet Cream Hair Remover^{R} (Whitehall Laboratories Inc, New York). The depilatory was placed on the skin for not more than 20 min then wiped off with gauze pads under a stream of warm tap water. The guinea pigs were dried and returned to their cages. Skin irritation reactions were blindly scored 2 h later and at 48 h and 72 h after dosing.

Second Challenge: 1 dose of test or control material was applied 7 d after the first challenge application. The test sites were depilated as before and the skin reactions were scored.

A Hill Top Chamber™ was used to apply the test materials to the upper left flank in the 1st challenge and to the lower left flank in the 2nd challenge. These sites had been clipped free of fur on the day prior to the challenge. Wrapping was as described above for induction.

Incidence of sensitisation: This was presented as the fraction: number of guinea pigs sensitised/number of guinea pigs tested.

Mean irritation score: For any particular scoring episode, this was calculated as the sum of the combined Draize scores for erythema and oedema of each individual guinea pig, divided by the number of guinea pigs in the group. All calculated mean scores were rounded to one decimal place.

Interpretation: A reaction was considered to be a sensitisation reaction when two conditions were met:-

1. the skin irritation scores observed following the challenge of a methamidophos-or DNCB-induced guinea pig were greater than the scores observed in those guinea pigs following the first induction dose of methamidophos or DNCB,

2. the challenge scores of a methamidophos-or DNCB-induced guinea pig were greater than any scores observed in the corresponding vehicle and/or irritation control group(s) following either the 1st induction or challenge doses.

Results and discussion

The incidences of response and mean irritation scores following the 1st, 5th and 10th induction dose and the 1st and 2nd challenges are tabulated below.

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Incidence of sensitisation and mean irritation scores obtained in guinea pigs

Event & Time (h)	Group				
	MT	**MT Irritation Control**	**MT Vehicle Control**	**DNCB**	**DNCB Irritation Control**
	[#]n = 15 (14)	N = 10	n = 10	n = 10	n = 10
FID					
24	0.1*	0	0	0	0
48	0.1	0	0	0	0
5ID					
24	0.4	0	0	3.6	0
10ID					
24	0.9	0	0	6.0	0.3
FCI	0/14**	na[##]	na	10/10	na
24	0.4	0.2	0.3	4.8	1.4
48	0.3	0.1	0.3	4.2	0.5
72	0.6	0.2	0.2	2.9	0.5
SCI	0/14	na	na	1/10	na
24	0.5	0.1	0	2.5	0
48	0.4	0.2	0.1	2.4	0
72	0.2	0.2	0.1	1.8	0

FID = 1st induction dose, **5ID** = 5th induction dose, **10ID** = 10th induction dose
FCI = First Challenge Incidence, **SCI** = Second Challenge Incidence
MT = Methamidophos Technical; **DNCB** = 1-chloro-2, 4-dinitrobenzene
n = number of guinea pigs tested; 1 guinea pig found dead on d 6 of the study
* = mean score at the interval specified
** = incidence of sensitisation (number guinea pigs sensitised/number tested)
na = not applicable

The guinea pig found dead had intussusception of the anterior jejunum. This was attributed to the wrapping process and not to the test material.

One-way analysis of variance in the mean bw of the various groups revealed no significant differences between the means throughout the study.

In the DNCB group, none of the 10 guinea pigs treated with 0.1% DNCB (in 80% ethanol) showed skin irritation at 24 and 48 h after the 1st induction dose. At 24 h after the 5th induction dose, very slight (Grade 1) to severe (Grade 4) erythema was observed in all 10 guinea pigs. Seven of the 10 also showed very slight (Grade 1) or slight (Grade 2) oedema. At 24 h after the 10th induction dose, moderate (Grade 3) to severe erythema and very slight to moderate (Grade 3) oedema were observed in all 10 guinea pigs. After the first challenge, all animals showed sensitisation reactions of very slight to severe erythema and very slight to moderate oedema. Following the 2nd challenge, 10/10 guinea pigs showed very slight to severe erythema. In 5/10 guinea pigs very slight to slight oedema was also observed.

The study authors suggested that only 1 guinea pig in this group gave a definite sensitisation reaction following the 2nd challenge. However they also indicated that 7/10 DNCB-induced guinea pigs showed combined irritation scores of 2 or more after the 2nd challenge, "indicative of a sensitisation response".

In the DNCB irritation control group, none of the 10 guinea pigs treated with 80 % ethanol showed skin irritation after the 1st or 5th induction doses. After the 10th induction dose one guinea pig showed very slight erythema and another showed well defined (Grade 2) erythema.

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Oedema was not observed. After the 1st challenge 9/10 guinea pigs showed very slight to well-defined erythema. Very slight to slight oedema was also noted in 2/10 guinea pigs. There were no skin reactions following the 2nd challenge. None of the DNCB controls were considered to have been sensitised to the vehicles used.

In the methamidophos technical group, very slight erythema and no oedema were observed in 2/15 guinea pigs after the first induction dose. At 24 h after the 5th induction dose very slight erythema without oedema was observed in 5/14 guinea pigs. At 24 h after the 10th induction dose, very slight to moderate erythema was observed in 6/14 guinea pigs. Two of the 6 also had very slight oedema and slight oedema respectively. Responses following the 1st and 2nd challenges were similar to those seen during induction and none of the animals in this group were considered to have been sensitised.

In both the methamidophos technical irritation control group and the methamidophos technical vehicle control group, there were no signs of skin irritation during induction. Following both challenges, 2 or 3/10 guinea pigs in both groups showed very slight to well-defined erythema but no oedema.

Conclusion

Given that none of the test guinea pigs showed sensitisation reactions and that a sensitisation response was obtained in the positive control group, the study is considered to have demonstrated that methamidophos technical did not sensitise the skin of the guinea pig.

Porter MC, Craigo RE & Hartnagel RE (1987): Dermal sensitisation evaluation of MONITOR-4LC (M-4) in guinea pigs. Chevron Report No. 94982. Study No. MTD0038. Toxicology Report No. 963. Lab: Toxicology Department, Miles Inc, Elkhart, IN, USA. Sponsor: Chevron Chemical Company, Richmond, California, USA. Study Duration: not specified. Report Date: 23 November 1987. Unpublished. (QA: Yes: GLP and Test Guidelines: FIFRA 40 CFR Part 160; TSCA-792).

Test Substances:	Methamidophos-4LC (M-4) formulation. Formulation composition: Methamidophos technical 42.7%, Propylene glycol 57.3%: Batch No. 9030152. Purity: 40.2% methamidophos technical as active ingredient. Source: Mobay Corporation, Stilwell, California, USA. Positive control: 1-chloro-2,4-dinitrobenzene (DNCB). Lot No. 5204 CX1010. Source: Matheson, Coleman and Bell, East Rutherford, NJ, USA
Test Species:	Guinea pig: Hartley outbred albino, male, 5-6 wk of age, 295-362 g, obtained from Harlan Sprague Dawley, Indianapolis, IN, USA.
Dose:	

Method

A preliminary primary irritation study was conducted to determine the highest non-irritating concentration of the test substance. Four concentrations (1, 10, 25 or 100%) of test substance in distilled water were used, none of which were irritating to the skin. Death or ataxia was

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observed at 25% and so a 10% solution of test substance in distilled water was used. DNCB was applied as a 0.05% solution in 50% ethanol/distilled water.

Thirty guinea pigs were randomly assigned to test and negative control groups as follows: 15 to the test substance group and 5 to each of test substance negative controls, DNCB test group and DNCB negative controls respectively. Body wts were measured for all guinea pigs at initiation (d 0) and termination (d 31). The Buehler method (Buehler, 1965) was used to assess the potential for delayed contact hypersensitisation of the skin.

On d 1, hair was clipped from the dorsolateral aspect of the thoracic region on the left side of each guinea pig in the test groups. On d 0, 0.4 mL of test substance was applied under an adhesive patch - a 2 x 2 cm webril pad on hypoallergenic backing (Professional Medical Products, Greenwood, SC, USA), to the left side of each test guinea pig near the scapula. DCNB was similarly applied to 5 guinea pigs. Each patch was secured by an elastic adhesive bandage and adhesive tape. After 6 h, the patches and test substances were removed. The procedure was repeated on d 7 and d 14.

On d 27 hair was clipped from the dorsolateral aspect of the lumbar region on the left side of each test substance and negative control guinea pigs and from both sides of each DNCB test and DNCB control guinea pig. On d 28, the test substance or DNCB was applied near the left pelvic girdle on the individuals in those groups and DNCB vehicle was applied to the right side of DNCB test and DNCB control guinea pigs using the same technique as used for induction. After 24 h the wrapping and patches were removed and each test site was depilated by application of Neet lotion (Whitehall Laboratories Inc, New York.) for 30 min. This was then washed off with warm water. Guinea pigs were dried and the test substance, DNCB and DNCB vehicle sites for induced and naive guinea pigs were scored at 24 and 48 h for erythema.

Induction and challenge sites were scored for erythema at 24 and 48 h after each induction application according to the following scale: 0 = no erythema, 1 = slight, barely perceptible erythema, 2 = moderate, clearly perceptible erythema and 3 = severe, marked erythema which extends beyond the treated area. Statistical analyses of results were not performed.

Results and discussion

Clinical signs and body wt: No clinical signs of systemic illness were observed. The mean bw gains (bw at d 31 less bw at d 0) for each group over the study were 235, 218, 200 and 199 g for the test substance, test substance negative controls, DNCB and DNCB negative controls respectively.

Induction applications: Erythema was not observed following any of the 3 induction exposures to the test substance. Following the 1st, 2nd and 3rd DNCB induction exposures erythema was observed in 0/5 guinea pigs at both intervals, 4/5 at both intervals, and 5/5 at 24 h and 1/5 at 48 h respectively. The respective mean erythema severity score at the 2nd and 3rd DNCB induction exposures were 1.2 for both at 24 h and 1.2 and 0.2 at 48 h. The highest erythema score observed was 2.

Challenge applications: Erythema was not observed in the test substance induced or non-induced negative control guinea pigs. Erythema was observed in 4/5 DNCB induced guinea pigs at 24 and 48 h with a mean score of 1.8 and 1.6 respectively. The highest erythema score

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was 3. Erythema was not seen in DNCB induced guinea pigs treated with DNCB vehicle. Erythema was not seen following the application of DNCB or DNCB vehicle to DNCB non-induced control guinea pigs.

Conclusion

The methamidophos formulation M-4 did not cause skin sensitisation in guinea pigs.

3.2 Metabolites/Degradation Products

Canellakis ES & Tarver H (1953): The metabolism of methyl mercaptan in the intact animal. Arch Biochem Biophys, 42:446-455.

This work was concerned with some of the qualitative aspects of the metabolism of methyl mercaptan in rats. In experiments in which methyl mercaptan-C^{14} or S^{35} had been administered to rats both the carbon and the sulfur of mercaptan were rapidly oxidised to CO_2 and sulfate respectively. The methyl carbon of mercaptan was converted to β-carbon of serine and methyl groups of methionine, choline, and creatine. Sulfur from methyl mercaptan did not appear to any significant extent in the methionine or cystine of liver protein.

Derr RF & Draves K (1983): Methanethiol metabolism in the rat. Research Communications in Chemical Pathology and Pharmacology, 39(3):503-506.

This work was stimulated by a previous implication (Challenger & Walshe, 1955) of methanethiol in a synergistic relationship with ammonia and fatty acids, in the induction of hepatic coma in humans and by the fact that methanethiol inhibits the urea cycle at the carbamyl phosphate synthetase step. In this work, the terminal oxidation products of C^{14} or S^{35}-methanethiol in the rat were CO_2 and sulfate. During the first 2 h after dosing these oxidation products were excreted at an equal rate. After 2 h, oxidation of the methyl group to CO_2 slowed and the methyl group was then metabolised via the 1-Carbon fragment pool. Ninety four % of ^{35}S-sulfate was removed from the body within 21 h.

Blom HJ & Tangerman A (1988): Methanethiol metabolism in whole blood. J Lab Clin Med, 111:606-610.

These authors indicated that methanethiol was an "extremely toxic compound" and that it caused reversible coma in rats in small amounts (Zieve *et al.*, 1974). Their work considered the formation of methanethiol via the transamination pathway of methionine in the gastrointestinal tract. The incubation of methanethiol with whole blood resulted in the formation of small amounts of dimethyl sulfide (0.5 to 1%). Experiments done with methanethiol, RBC membranes and RBC lysates showed that dimethyl sulfide was formed only during incubation of methanethiol with the membrane fraction. However during the incubation experiments done with methanethiol and whole blood, dimethyl disulfide was not formed in detectable amounts (<0.1% of the amount of methanethiol added to blood).

The authors suggested that their findings agreed with those of Canellakis & Tarver (1953) and Derr (1983) which showed a rapid oxidation of methanethiol into CO_2 and sulfate after ip administration. The liver most likely played a role but it was possible that blood played an important role in the *in vivo* oxidation of methanethiol. The oxidation in blood was most

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likely carried out by haemoglobin and the methylation of methanethiol to dimethyl sulfide was most likely the result of catalysis by RBC membrane associated thiol methyltransferase.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1. Oral Administration

4.1.1 Rats

Abernathy J, Lamb DW, Hoss HE, Brewer GJ, Mallicoat DR & Hayes RH (1980): A pilot study using technical methamidophos in rats. Mobay Report No. 68764. Study No. 80-971-01. Toxicology Report No. 94 (Revised). Lab: Stanley Research Centre, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 31 March 1980 - 2 May 1980. Report Date: 13 April 1980; revised 29 July 1980. Unpublished. (QA: Yes; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos technical Batch Number: 77-297-149 Purity: 75% Physical state: clear liquid at room temperature.
Test Species:	Fischer 344 Outbred Rats: males & females, 10 wk of age, of an unspecified bw obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts.
Dose:	0, 1, 2, 4, 8, 16, 32, 64 ppm in diet

Method

Groups of rats (5/sex) received methamidophos technical at 0, 1, 2, 4, 8, 16, 32, 64 ppm in diet for 34 d. Fresh batches of dietary admixture were made weekly. Corn oil was used as a vehicle to aid in the uniform distribution of the test substance in the food. A Hobart mixer was used for 10 min/batch. Between batches containers were washed with acetone and the acetone was allowed to evaporate. The food made for this study had 2% less fat than that commercially supplied to accommodate the contribution of the corn oil to the fat. The diet was stored in a freezer until needed. It was removed from the freezer on the day the food was to be used. A weighed amount of feed was placed into clean feeders and given to the rats. After 1 wk the uneaten food was weighed and disposed of by incineration. The food consumption of each rat was calculated.

Visual observations were made twice per d of each individual rat for mortality or clinical signs. Palpation of the rats' abdomen for masses or growths was also conducted. Bw was monitored weekly after an initial pretreatment weighing. Blood for ChE determinations was obtained from the orbital sinus in EDTA tubes. All rats were blood sampled during wk 3 and wk 5. Two concentration groups were bled on each of 4 consecutive days in those weeks. The rats were killed with chloroform on d 34 and the brain was taken from all rats except those which had received 32 & 64 ppm. Plasma, erythrocyte and brain cholinesterase activities were determined by the method of Talbott (Talbott, 1975). Haematological, additional clinical chemical and urinary measurements were not conducted. Gross necropsies were performed on all animals. The weights of organs were not recorded. All tissues and organs were grossly examined for pathological signs. Those tissues and organs showing pathological signs were saved. Statistical procedures were not performed.

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Results and discussion

Mortality and clinical observations: No deaths occurred. Clinical signs observed were corneal opacities in the right eye of 1 female (1 ppm) and 1 male (32 ppm). The time of appearance of these opacities was not reported. At necropsy it was reported that opacities were seen in the right eye of 2 male rats (1 at 2 ppm and 1 at 32 ppm) and 1 female rat (at 1 ppm). No other details were provided but the ocular effects as described are not among those usually attributed to organophosphates (Plestina & Piukovic-Plestina, 1978; Boyes *et al.*, 1994). The absence of a dose-response relationship suggests it is unlikely that the ocular opacities were treatment related.

Body weight and food consumption: There was a depressed bw gain in male rats at 64 ppm (mean of 30 g (225-195 g) as compared to 49 g (260-211 g) for control group males). At 32 ppm the mean bw gain in male rats was 49 g. On this basis the study authors concluded that 32 ppm was a maximum tolerated dose. There was no equivalent depression in bw gain in females and there was no decrease in food consumption at 64 ppm in either sex. The impaired food conversion in males at 64 ppm is likely to have been treatment-related.

The mean achieved doses of test substance were equivalent to approximately 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 mg/kg bw/d (Lehman, 1954).

The activity of plasma, RBC and brain ChE at 5 wk are tabulated below. The activities determined in the 3rd wk were those of RBC & plasma ChE only and are not shown. They were similar to the RBC & plasma ChE activities determined in the 5th wk, suggesting that maximum inhibition occurred within 3 wk.

The mean ChE activity and % ChE inhibition during the 5th wk

Dose Ppm	**Mean ChE activity and % inhibition during the 5th wk (Plasma & RBC U/mL, Brain U/g)**											
	RBC		**%**		**Plasma**		**%**		**Brain**		**%**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
0	1.10	1.20	-	-	0.58	1.80	-	-	10.2	10.6	-	-
1	1.02	1.10	7	8	0.51	1.83	12	-	9.9	10.6	3	-
2	1.21	1.18	-	2	0.59	1.71	-	5	9.5	9.3	7	12
4	0.90	0.79	18	34	0.54	1.38	7	23	8.0	8.1	22	24
8	0.51	0.63	54	48	0.42	0.93	28	48	6.5	6.2	36	42
16	0.24	0.30	78	75	0.31	0.61	47	66	4.4	4.2	57	60
32	0.08	0.10	93	92	0.26	0.40	55	78	-	-	-	-
64	0.02	0.01	98	99	0.15	0.21	74	88	-	-	-	-

Gr = Group, M = Male, F = Female

At 5 wk, dose-related biologically significant inhibition of plasma, RBC brain ChE (≥ 20% inhibition) occurred at a concentration of 4 ppm and above. The ChE activity seen at 1 and 2 ppm was within the limits of biological variation and assay precision.

Gross pathology: At necropsy 1 male rat (at 4 ppm) had a congested left thymus. Fat necrosis was observed in the mesentery of the broad ligament of 1 female at 4 ppm. One female (at 16 ppm) had a 10 cm ovarian cyst. A slight amount of clear fluid was found in the uterus of 1 female at 8 ppm, 1 at 32 ppm and 3 at 64 ppm. There were no gross lesions in the remaining 37 rats. Histological findings were not reported. The gross lesions seen were not considered to

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be treatment-related by the study authors. The historical data of this laboratory suggest that these observations in relation to the reproductive system are not uncommon in control rats. The other findings are sporadic in nature, are recorded in historical data and are unrelated to the known pharmacological action of organophosphates.

Conclusion

The NOEL in this study was 2 ppm (0.2 mg/kg bw/d) based on biologically significant (≥ 20%) inhibition of plasma, RBC and brain ChE activity at and above the next highest dose of 4 ppm.

Christenson WR, Clayton VA, Sangha GK, Stuart BP, Hoss HE, Jones RE, Mueller RE, Moore KD & Thyssen JH (1991): Technical grade methamidophos (MONITOR™): An eight week sub-chronic cholinesterase study in Fischer 344 rats. Study No. 89-972-CV. Report No: 5582. Lab: Health, Environment, Safety and Plant Management, Corporate Toxicology Department, Mobay Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Corporation, Kansas City, Missouri, USA. Study Duration: 26 March 1990 - 25 May 1990. Report Date: 19 March 1991. Unpublished. (QA: Yes; GLP: USEPA/FIFRA (1989) & OECD (1981); Test Guidelines: USEPA/FIFRA Pesticide Assessment Guidelines (1984); USEPA/TSCA Health Effects Testing Guidelines (1988) & OECD Guidelines for the Testing of Chemicals (1981)).

Test Substance:	Methamidophos technical Batch No. 0067009 Purity: 77.6% Physical state: Clear liquid.
Test Species:	Fischer 344 Rats, Strain CDF(F-344)/Crl/Br, males, females, aged 12 wk, of an unspecified bw, obtained from SASCO, Inc. Madison, Wisconsin.
Dose:	Nominal concentrations of 0, 0.5, 1.0, 2.0 and 4.0 ppm in diet. Actual concentrations: 0, 0.49, 0.97, 2.12 and 4.30 ppm in diet. Achieved doses were males: 0, 0.03, 0.07, 0.13 and 0.24 mg/kg bw/d; females: 0, 0.06, 0.06, 0.17 and 0.28 mg/kg bw/d.

Method

Rats were randomly assigned to 25 rats/sex/group. The rats were administered the test substance in the diet. Corn oil was used as the vehicle for the test substance at 1% w/w of the diet. The diet admixture was made weekly and stored at -23^{o}C until used. Fresh food was introduced weekly. This process continued for 8 wk (56 d). The concentration of test substance in the dietary admixture was tested during wk 1, 4 and 8. The homogeneity and stability of test substance in the mix was also monitored.

Observations for mortality and moribundity were made and recorded twice daily. A clinical history for each rat was maintained. Food consumption and bw measurements were made weekly. Plasma AChE, plasma BChE and RBC AChE activity was determined at d 0, 4, 28, 42 and 51. The determinations at d 14, 28 and 42 were conducted on the 1st 15 available male and female rats of each test group. Those at d 51 were conducted on all surviving rats. Brain AChE was measured at d 14 and 35 on 5 rats/sex/group and on all surviving rats at d 56. Blood was collected by orbital sinus bleeding and brains were removed at necropsy, divided

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in half and stored frozen for assay. BChE was specifically determined in plasma using a commercial kit employing butyrylthiocholine as the substrate. The other determinations were conducted using methodology previously described (Hackathorn *et al.*, 1983).

Continuous data were evaluated initially for equality of variance or homogeneity using Bartlett' test. Group means were further analysed by Analysis of Variance (ANOVA) followed by a Dunnett's test if a significant F was obtained in the ANOVA. With the exception of Bartlett's test ($p \leq 0.001$), probability values of $p \leq 0.05$ were accepted as significant.

There were no haematological counts, clinical chemistry determinations other than ChE, urinalysis, organ weighings, gross pathology or histological examinations. The expressed purpose of the study was to establish a NOEL for methamidophos-induced inhibition of plasma AChE, plasma BChE, RBC AChE and brain AChE in the Fischer 344 rat.

Results and discussion

Analytical Chemistry: A recovery sample was analysed with each group of samples during homogeneity, stability and test concentration analyses. The recoveries ranged from 84 to 95% for the rations containing methamidophos (mean recovery 90%, Coefficient of Variation 3%). The mean actual concentrations of methamidophos at the nominal concentrations of 0.5, 1.0, 2.0, and 4.0 ppm were 0.49 ± 0.06, 0.97 ± 0.03, 2.12 ± 0.08 and 4.30 ± 0.26 ppm respectively. After 28 d of freezer storage there was no significant decline in concentration of methamidophos at the 0.5 and 4.0 ppm nominal concentrations. For these two mixtures methamidophos was also considered homogeneously distributed in the food.

Mortality and Morbidity: There were no mortalities other than those scheduled. There were no treatment-related clinical signs.

Food consumption and body weight: There were no treatment-related effects on food consumption or bw gain.

Preliminary comment on cholinesterase: The study authors used the term "toxicologically significant" as the basis of their establishment of NOEL but did not define it. They did not specify what % inhibition they regarded as biologically significant (cf. accepted practice of regarding ≥ 20% inhibition as biologically significant in the absence of statistical significance). An examination of the data suggests that statistical significance and % ChE inhibition were considered together to arrive at a conclusion as to toxicological significance.

Cholinesterase: The mean plasma AChE values up to and including d 14 are tabulated below. The values for d 28, d 42 and d 51 are not shown.

Mean plasma AChE values prior to and at 14 d after the start of treatment

Time	**Mean plasma AChE values (U/mL) and % inhibition for each nominal concentration (ppm)**									
	Male					**Female**				
	0	**0.5**	**1.0**	**2.0**	**4.0**	**0**	**0.5**	**1.0**	**2.0**	**4.0**
d 0	0.52	0.50	0.52	0.51	0.50	1.98	1.88	1.93	1.87	1.83
d 14	0.57 -	0.58 -	0.53 7.0	0.50* 12.3	0.46* 19.3	2.3 -	1.92* 16.5	1.85* 19.5	1.50* 34.8	1.36* 40.8

* ANOVA and Dunnetts test (two sided), $p \leq 0.05$

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The degree of plasma AChE inhibition did not change substantially after d 14. A NOEL for plasma AChE inhibition was established at 0.5 ppm (0.03-0.06 mg/kg bw/d) for both sexes because that concentration was the highest at which no toxicologically significant inhibition occurred. The statistically significant inhibition in females at 0.5 ppm was not regarded by the study authors as toxicologically significant.

The mean plasma BChE values up to and including d 14 are tabulated below. The values for d 28, d 42 and d 51 are not shown. The degree of plasma BChE inhibition did not change substantially after d 14. A NOEL for plasma BChE inhibition was established at 0.5 ppm (0.03-0.06 mg/kg bw/d) for both sexes because that concentration was the highest at which no statistically or toxicologically significant inhibition occurred.

Mean plasma BChE values prior to and at 14 d after the start of treatment

Time	Mean plasma BChE values (U/mL) and % inhibition for each nominal concentration (ppm)									
	Male					Female				
	0	0.5	1.0	2.0	4.0	0	0.5	1.0	2.0	4.0
d 0	0.16	0.15	0.16	0.16	0.16	1.15	1.09	1.08	1.07	1.04
d 14	0.15 -	0.17 -	0.14 6.6	0.12* 20.0	0.11* 26.6	1.22 -	1.09 10.6	0.99* 18.8	0.85* 30.3	0.80* 34.4

* ANOVA and Dunnetts test (two sided), $p \leq 0.05$

The mean RBC AChE values are tabulated below. The degree of RBC AChE inhibition did not change substantially after d 14. The study authors established a NOEL for RBC AChE inhibition as 0.5 ppm (0.03-0.06 mg/kg bw/d) for both sexes. This decision was based on statistically significant inhibition except that at 0.5 ppm, the statistically significantly depressed value of 2.52 U/mL in females at d 42 was not regarded as toxicologically significant because the degree of RBC AChE inhibition was only 3.4%. That such a small decline in AChE activity was statistically significant was regarded by the study authors as a reflection of the precision of the AChE assay used. They did not apply the same argument to the statistically significant differences at 1.0 ppm which represented low depressions of RBC AChE activity (3.1-6.0%).

Mean RBC AChE values at each concentration

Time	Mean RBC AChE values (U/mL) and % inhibition for each nominal concentration (ppm)									
	Male					Female				
	0	0.5	1.0	2.0	4.0	0	0.5	1.0	2.0	4.0
d 0	2.51	2.46	2.55	2.55	2.61	2.43	2.46	2.54	2.51	2.59
d 14	2.72 -	2.72 -	2.79 -	2.59 4.8	2.27* 16.5	2.57 -	2.43 5.4	2.60 -	2.47 3.8	2.14* 16.7
d 28	2.79 -	2.67 4.3	2.66* 4.6	2.46* 11.8	2.08* 25.4	2.66 -	2.58 3.0	2.50 6.0	2.37* 8.1	1.94* 27.1
d 42	2.68 -	2.62 2.20	2.56* 4.4	2.43* 9.3	2.10* 21.6	2.61 -	2.52* 3.4	2.50* 4.2	2.38* 8.8	2.01* 7.7
d 51	2.62 -	2.60 0.7	2.51* 4.2	2.42* 7.6	2.19* 16.4	2.59 -	2.55 1.5	2.51* 3.1	2.36* 8.8	2.09* 19.3

* ANOVA and Dunnetts test (two sided), $p \leq 0.05$

The mean brain AChE values are tabulated below. The study authors established a NOEL for brain AChE inhibition as 0.5 ppm (0.03-0.06) mg/kg bw/d) for both sexes. This decision was

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based on statistically significant inhibition except that at 0.5 ppm, the statistically significantly depressed values of 13.9 U/g in males at 56 d and 14.3 U/g in females at 35 d were not regarded as toxicologically significant. There was a lack of a temporal relationship between the 2 values and the degree of RBC ChE inhibition of 3.3% and 4.6% respectively was regarded as inconsequential.

Mean brain AChE values at each concentration

Time	Mean brain AChE values (U/mL) and % inhibition for each nominal concentration (ppm)									
	Male					Female				
	0	0.5	1.0	2.0	4.0	0	0.5	1.0	2.0	4.0
d 14	14.8 -	14.1 4.7	13.8* 6.7	12.9* 12.8	11.6* 21.6	14.5 -	14.6 -	12.7* 12.4	13.1 9.6	11.3* 22.1
d 35	15.0 -	14.5 3.3	13.9* 7.3	13.4* 11.2	11.1* 26.0	15.0 -	14.3* 4.6	13.7* 8.6	12.6* 16.0	10.7* 28.6
d 56	14.4 -	13.9* 3.4	13.4* 6.9	12.4* 13.8	10.7* 25.7	14.2 -	13.7 3.5	13.3* 6.3	12.3* 13.4	9.7* 31.7

* ANOVA and Dunnetts test (two sided), $p \leq 0.05$

Conclusion

On the basis of statistically significant inhibition of ChE, the study authors determined NOEL's for plasma AChE, plasma BChE, RBC AChE and brain AChE as 0.5 ppm (0.03-0.06 mg/kg bw/d).

4.2 Dermal Application

4.2.1 Rats

Sheets LP, Gastner ME & Hamilton BF (1997): Repeated-dose 21-day dermal toxicity study with technical grade methamidophos (Monitor^{R}) in rats. Study No. 96-122-KQ & 97-922-KT. Report No. 107635. Lab: Toxicology, Agriculture Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Kansas City, Missouri, USA. Study Duration: 7 April 1997 - 29 April 1997. Report Date: 10 December 1997. Unpublished. (QA: Yes; GLP and Test Guidelines: 40 CFR Part 158, FIFRA Guideline 82-2, OECD Guideline 410 and Japan MAFF 59 NohSan No. 4200).

[Note: A supplemenal report to this study was also submitted as a separate report and is considered here (Moore, 1998). Study 97-922-KT was a 5 d repeated-dose dermal toxicity study conducted to assist in establishing doses for the main study (Study No. 96-122-KQ). The findings were incorporated in the main study report evaluated here]

Moore KD (1998): Supplemental report to repeated-dose 21-day dermal toxicity study with technical grade methamidophos (Monitor^{R}) in rats. Study No. 96-122-KQ. Report No. 107635-1. Lab: Toxicology, Agriculture Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Kansas City, Missouri, USA. Study Duration: 7 April 1997 - 29 April 1997. Report Date: 28 September 1998. Unpublished. (QA: Yes; GLP and Test Guidelines: 40 CFR Part 158, FIFRA Guideline 82-2, OECD Guideline 410 and Japan MAFF 59 NohSan No. 4200).

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Test Substance:	Methamidophos technical. Batch Number: 703-0001. Purity: 76.9%
Test Species:	Rat: Sprague-Dawley, males, females, 8 wk of age of an unspecified bw obtained from Sasco Inc., Kingston, New York.
Dose:	0, 1, 15, 50 mg/kg bw/d for 21 d at 6 h/d applied dermally.

Method

A 5 d repeated-dose dermal toxicity study (Study No. 97-922-KT) was conducted to assist in establishing doses for this study. The results of a 90 d dietary study with methamidophos (Sheets *et al.*, 1994b) were also examined to assist with dose selection. The doses selected for this study were 0 (vehicle control), 1, 15 or 50 mg/kg/bw/d. The 50 mg/kg dose was selected to produce evidence of cholinergic toxicity without causing a mortality rate that would interfere with data analyses. The 1 mg/kg bw/d dose was selected to produce no evidence of exposure or alternatively minimal inhibition of ChE activity and the 15 mg/kg bw/d dose level was selected to produce an intermediate level of cholinergic effects. The vehicle for the test substance was a phosphate buffer solution - pH 7.3. A dose volume of 1 mL/kg bw was used. Group sizes were 9-10 rats/sex/dose level.

The hair from the scapulae to the wing of the ileum and half-way down the flank on each side of each rat was removed. Rats were clipped 24 h before the 1st dose was administered and then 2/wk. Abrading of the skin was avoided. The test substance was administered as a single dose on each d of exposure. Doses were applied uniformly to a piece of gauze with plastic backing that was cut to a size that represented 10% of the rats total surface area, based on the allometric equation $A_b = km^{2/3}$, where A_b is the surface area, k the mass coefficient (8.79) and m the rat bw (g). Surface area assessments were adjusted each wk. Immediately after applying the dose to the gauze, the gauze was applied to the dose site and taped on. It was further secured with bandage. Treated rats were returned to their cages for the 6 h exposure period. After 6 h the bandages were removed and the dose site was wiped using paper towels dampened with water to remove as much of the test substance as possible without damaging the skin.

Females received 17 dose applications and males 18 over a period of 21 and 22 d respectively. All males and females were treated on 5 consecutive d over the first 2 wk (d 0-4 and d 7-11 respectively). In the 3rd wk females were treated on d 14-20 and males were treated on d 14-21. The continuous treatment in the 3rd wk was to ensure the determination of the maximum cumulative response using measures of ChE activity, gross pathology and histopathology.

Ophthalmological examinations: Pre-exposure and pre-terminal ophthalmic examinations were conducted on all rats in a semi-darkened room. The pre-terminal examination was conducted on d 18. The pupillary reflex was tested. After pupillary dilatation the eyelid, conjunctiva, cornea, uvea (ciliary body, iris and choroid), aqueous humor and lens were examined with a slit lamp and the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope with a condensing lens.

Clinical signs, body wt and food consumption: All rats were observed once daily for clinical signs. Body wt was measured just prior to initiation of the study and then weekly. Individual rat food consumption was determined weekly.

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Clinical pathology: Blood samples were collected from the orbital plexus 1 wk prior to the start of treatment for determination of plasma and RBC ChE activity (Ellman *et al.*, 1961). At 24 h after the last dose, blood samples were collected from the orbital plexus for terminal ChE activity determinations. Brain ChE activity was measured at termination using brain (left half, sagittal section) homogenate. Moribund rats were terminated (without blood collection) to minimise the likelihood of unobserved death and subsequent tissue autolysis.

Gross pathology and organ wt: On the d after the last dose was administered, all rats were terminated by CO_2 asphyxiation and subjected to gross necropsy. The tissues collected and preserved in 10% buffered formalin and organs weighed, were those as listed in Appendix III. In addition lungs were weighed.

Histopathology: Treated skin, liver, kidney, adrenal, brain, heart, lungs, parathyroids, pituitary, spleen, thyroid, gonads and any lesions observed during gross necropsy were processed using standard histologic techniques and stained with haematoxylin and eosin for examination. Processed tissues of all high-dose group rats were examined initially. Only if evidence of a response to treatment was observed were tissues from other treatment groups evaluated. Lungs, liver and kidneys of low- and mid-dose rats were routinely examined for evidence of infection.

Statistical analysis: Continuous data were subjected to Bartlett's test for homogeneity. If Bartlett's test showed homogeneity ($p > 0.001$) the data were subjected to an analysis of variance (ANOVA) followed by post-hoc analysis if the ANOVA F-test was significant at $p \leq 0.05$. Categorical data were visually inspected for trends that could indicate a treatment-related effect, then selected data were subjected to Chi-square examination followed by Fischer's Exact Test to show significant variations. All significant differences were reported at the 95% confidence level.

Results and discussion

Analysis of test substance in the vehicle: The analytically confirmed doses were 1.0, 15 and 47 mg/kg bw respectively. Stability of the test substance under storage conditions was verified with no appreciable decline in concentration with 21 d of storage.

Clinical signs: Treatment-related clinical signs were not observed in males or females at any dose level.

Ophthalmological examinations: All rats had clinically normal eyes at the initiation of exposure. No ocular abnormalities were observed in any rat at d 18.

Mortality: There were no treatment-related deaths.

Body wt and food consumption: In males, the mean gains in bw over the interval 0-21 d were 57, 52, 41 and 38 g for controls, 1, 15 and 50 mg/kg groups respectively. In females the respective gains in mean bw for each group over 0-21 d were 27, 27, 28 and 27 g. For either sex, there were no statistically significant differences in mean bw between the groups at 0, 7, 14 or 21 d. In males and females there were no statistically significant differences between groups with respect to food consumption at d 7, 14 or 21.

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Cholinesterase activity: Individual and mean pre-treatment plasma and RBC determinations for 50 males and 54 females were reported. Rats were assigned to groups after these determinations were available. It was not possible to trace the assignations as different identification numbers were reported for individual pre- and post-treatment rats. The mean pre-treatment plasma ChE activity for males and females was 0.56 and 0.96 IU/mL respectively and the respective mean pre-treatment RBC ChE activity for males and females was 1.28 and 1.25 IU/mL respectively. The study authors used control group mean values to calculate % inhibition.

The mean plasma, RBC and brain RBC activities and % inhibition determined at 24 h after the last dose are tabulated below. The degree of inhibition of plasma, RBC and brain ChE activity is dose-related in both sexes. Biologically significant inhibition (≥ 20%) of plasma ChE is not apparent in either sex at 1 mg/kg bw/d but was observed at 15 and 50 mg/kg bw/d.

Mean plasma, RBC and brain ChE activity and % inhibition[#]

ChE	**Mean plasma, RBC ChE activity (IU/mL) at 24 h after the last dose and brain ChE activity (IU/g) at termination and % inhibition for rats dosed dermally with doses shown (mg/kg bw/d) over 21 d**							
	Males				**Females**			
	0	**1**	**15**	**50**	**0**	**1**	**15**	**50**
Plasma	0.49 -	0.48 2	0.37* 24	0.20* 59	1.20 -	1.29 -	0.70** 42	0.40** 67
RBC	1.12 -	1.05 6	0.50* 55	0.28* 75	1.11 -	1.10 1	0.60* 46	0.27* 76
Brain	13.4 -	13.5 -	7.9* 41	4.5* 66	13.8 -	13.1 5	8.5* 38	5.3* 62

% inhibition compared to concurrent controls
* Statistically different from controls ($p < 0.05$) - Anova and Dunnetts (** - Kruskal-Wallis Anova and Mann-Whitney U-tests)

Gross pathology, histopathology and organ wt: No treatment-related gross or histological findings or differences in organ wt were observed following necropsy.

Conclusion

A NOEL of 1 mg/kg bw/d applied dermally was established on the basis that no biologically significant inhibition (≥ 20%) of plasma, RBC or brain ChE was observed at that dose.

4.2.2 Rabbits

Heimann KG, Nash G & Lorke D (1981b): SRA 5172. Subacute dermal toxicity on rabbits. Mobay Report No. 80323. Study No. T 0010270. Report No. 10330. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 1 May 1981 - 30 June 1981. Report Date: 30 October 1981. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance: Methamidophos technical. Batch No. 808902036. Purity: 64.5%
Physical state: emulsion (with Cremophor EL/distilled water)
No other details were provided.

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Test Species:	New Zealand White Rabbits: albino, male & female, bw 2.5-3 kg, age unspecified. Source: Hacking & Churchill, Huntingdon, England.
Dose:	0, 0.5 (0.1% in emulsion), 5.0 (1.0% in emulsion) mg/kg bw 6 h/d 15 d applied dermally.

Method

The back and flanks of each rabbit were shorn of hair 48 h before the start of treatment. Twenty four h before the start of the test, the skin areas to be treated were abraded with sand paper on 3/sex rabbits in each dose group. Redness and slight swelling appeared at the abraded sites. Hair which re-grew on the treated areas was reshorn twice weekly. Groups of rabbits (6/sex) received the test substance applied as an emulsion on 15 consecutive working days (5 times/wk) over a skin area of 42 cm^2. At the end of each daily 6 h exposure period, the treated areas were cleaned with soap and water.

The treated skin areas were examined before the start of the study and at the end of each 6 h exposure period for signs of inflammation. The severity of erythema present was graded and scored (Draize, 1959). The skin fold on the back, in the centre of the exposed area, was measured with a Cutimeter (Skinfold Caliper, Holtain Ltd., Crosswell, England). Before the start and after 15 d of treatment, blood and urine analysis was carried out on all rabbits. Between the 10th and 11th treatments, blood samples were taken from the ear vein for ChE determination. (The ChE results given by the study authors also indicate that the rabbits were bled before the 1st exposure and after the last exposure. They do not describe these bleedings in their methodology). The urine was collected from each rabbit over a period of 16 h overnight.

The following haematological and biochemical parameters were determined: RBC and WBC count, Hb, PCV, thrombocyte count, MCV, WBC-DC; AST, ALT, AP, BUN, blood glucose, CPK, and ChE. The following urinalysis was conducted: pH, protein, sugar, Hb, urobilinogen, and deposit; deposit constituents were identified as bacteria, epithelia, RBCs, WBCs, amorphous salts, triple phosphate, calcium oxalate and were graded 0, 1+, 2+, 3+ or 4+.

Twenty four to 48 h after the last treatment the rabbits were narcotised with sodium hexobarbitone, exsanguinated by the vena caudalis and grossly examined by disssection. The heart, lung, liver, spleen, kidneys, adrenals, testicles, ovaries and thyroid, were weighed. They and epididymes, uterus, treated and untreated dorsal skin, were fixed in Bouin's fluid or formol calcium (liver and one kidney). Brain samples were taken for ChE determination. The fixed organs or organ groups were embedded in Paraplast. The sections made were stained with hemalum-eosin, and additional sections of the kidneys stained by the Periodic Acid Schiff reaction. For the liver fat test, approximately 10 µm thick frozen sections were made from this organ, fixed in formol calcium and then stained with Oil Red O. The following organs from the rabbits in both dose groups and in the control group were histologically examined: heart, lung, liver, spleen, kidneys, adrenals, thyroid, testicles, epididymes, uterus, ovaries and skin (treated and untreated).

The daily appearance and behaviour of the rabbits was observed. The rabbits were weighed before the study and at the end of each study week. Food consumption was not measured. Statistical procedures were not performed.

Results and discussion

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Mortality and clinical observations: No deaths occurred. None of the rabbits showed abnormal behaviour or appearance during the treatment period. Erythema did not occur over the treatment period in rabbits with intact skin. In rabbits with abraded skin, erythema was noted. It had reversed by d 4. The severity of the erythema was equivalent in both treatment and control groups and so it was attributed to the abrasion process.

Body weight: The mean bw measurements are tabulated below. The treatment substance or the skin treatment had no effect on the bw of the rabbits.

Skin thickness: Abrasion induced a 0.4-1.2 mm increase in the skin thickness, but there were no effects on skin thickness that could be attributed to the test substance.

Haematology, Clinical Chemistry, and Urinary Measurements: Haematological, clinical chemical and urinalysis findings were reported for d 0 & d 15. For other than ChE, the differences over time or between individuals, sexes, skin treatments or control and dose groups were within ranges regarded by the study authors as typical for healthy rabbits held in their laboratory. A comparison with other data sources also indicated that the test results indicated typical haematological (Siglin & Rutledge, 1995), clinical chemical (Levine, 1995) and urinalysis (Hollinger, 1995) findings for the rabbit. The mean ChE determinations are tabulated below.

Biologically significant inhibition of plasma, RBC or brain ChE was not observed in rabbits which received 0.5 mg/kg bw/d. Brain ChE activity was not significantly altered at 5.0 mg/kg bw/d in rabbits of either sex with intact or abraded skin when compared to the control group. The degree of plasma or RBC ChE inhibition observed at 5.0 mg/kg bw/d is tabulated below:-

The mean plasma & RBC ChE activity and % inhibition* in rabbits at a dose of 5.0 mg/kg bw/d at d 0, d 10 & d 15

Gender/Skin treatment groups & ChE type	**ChE activity (U/mL) (% Inhibition*)**		
	Treatment group 5.0 mg/kg bw/d		
	d 0	**d 10**	**d 15**
Males/Intact Plasma	0.57	0.44 (20)#	0.37 (44)#
RBC	1.95	1.72 (30)#	1.57 (14)
Females/Intact Plasma	0.56	0.43 (25)#	0.43 (35)#
RBC	2.06	1.77 (11)	1.53 (30)#
Males/Abraded Plasma	0.64	0.54 (18)	0.52 (32)#
RBC	1.80	1.24 (40)#	1.53 (23)#
Females/Abraded Plasma	0.53	0.45 (22)#	0.44 (34)#
RBC	2.00	1.62 (13)	1.64 (19)

* % Inhibition was calculated using as a reference the control group ChE activity value at the same time
# Biologically significant inhibition

There were no differences that could be attributed to sex or skin treatment.

Absolute and relative organ weights: Absolute and relative organ wts were reported for those organs specified in the methodology. Apart from the observation that the mean liver wt and mean relative liver wt in males with abraded skin at the highest dose of 5.0 mg/kg bw were 85 g and 2.9 g/100 g bw respectively (compared to control values of 64 g and 2.4 g/100 g bw respectively), there was no indication of aberrant findings. The high mean wts in this group were the result of an unusually high liver wt for a single animal (112 g and 3.9 g/100 g bw).

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At necropsy this rabbit was found to have a "severely" increased liver glycogen level. It was the only rabbit with this finding. For the other rabbits in the same group, the mean absolute and relative liver wts were 72 g and 2.8 mg/100 g bw respectively. It is unlikely that this unusually large liver was a treatment-related response. The study authors noted that the occurrence was not unusual in the rabbit.

Gross necropsy and histopathological examination results: The findings of these examinations were limited to those associated with euthanasia and non-specific consequences ("spots, cysts, knots, lobules and scars") of inflammation in various organs in single rabbits attributable to parasitic invasion. Occasionally juvenile testicles and epididymes and focal atrophy in the testicles were noted. The study authors did not consider these observations to be treatment-related but rather as being within the range of variability for the rabbits of their age kept under conventional laboratory conditions.

Conclusion

Biologically significant (≥ 20%) inhibition of ChE activity was observed in the plasma and erythrocytes of rabbits treated dermally with methamidophos at 5 mg/kg bw/d for 15 d. The effect was equivalent irrespective of whether the skin was intact or abraded at the start of treatment. The NOEL was 0.5 mg/kg bw/d.

Comment

Abrasions were produced 24 h before the first dose of methamidophos was applied, but not subsequently. Hence, normal healing may have abolished any difference between dermal absorption of the test chemical across abraded skin compared to intact skin. This may account for the absence of any effect of abrasion on ChE acticity when measured at d 10 and 15.

4.3. Inhalational Administration

4.3.1 Rats

Pauluhn J & Machemer L (1987a): SRA 5172 TA. Study for acute inhalation toxicity to the rat to OECD Guideline No 403. Study Nos. T 8021834 & T 9021835. Report No. 15661. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 20 January 1986 - 3 March 1986. Report Date: 18 March 1987. Unpublished. (QA: Yes; GLP: T9021835 Yes (OECD), T 8021834 No; Test Guidelines: OECD Guideline No 403).

[Note: Both of the studies referred to in this report were range-finding studies for a planned inhalation exposure study of 3/4 wk duration. Study No T 8021834 was a 1x4 h dynamic spray inhalation exposure study. The results are reported in the acute toxicity tables of Section 3.1.1. of this review. Study T 9021835 is evaluated here. The 3/4 wk study (Pauluhn & Machemer, 1987b) is evaluated next].

Test Substance: Methamidophos technical.
Batch No. 808 526 309.
Purity: 75.7%.

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	Physical state: aerosol (test substance dissolved in polyethylene glycol E 400 (Lutrol)-ethanol 1:1 mixture).
Test Species:	Wistar Rats, Strain Bor:WISW (SPF-Cpb), males and females, aged 7-11 wk, 140-170 g bw (females) and 160-200 g bw (males), obtained from Winkelmann, Borchen, Kreis Paderborn.
Dose:	0 (vehicle) and analytical concentrations of 1.4, 5.4 or 33 mg/m^3 6 h/d inh for 5 d. Nominal concentrations of the test substance were 7, 30, 170 mg/m^3 using spray solutions of 0.035, 0.15 and 0.85 % w/v respectively.

Method

The test substance-vehicle solution was sprayed under dynamic conditions into a cylindrical inhalation chamber with a baffle chamber (Rhema Labor-technik, D 6238 Hofhaim, Am Stegskreuz 2, FRG). The operational methodology of the chambers has been published (Pauluhn, 1984b, 1987d). During exposure to the test aerosol, the rats were confined in perspex tubes. Head-nose exposure predominated. The vehicle-test substance solution was vaporised into the baffle section of the inhalation chamber at a rate of 200 µL/min by a jet of compressed air. The solution was supplied continuously to the jet. Each test substance concentration had the same vehicle concentration (10,000 µL Lutrol/m^3 as aerosol, 10,000 µL ethanol/m^3 as vapour). The nominal concentration of the test substance in the aerosol atmosphere was calculated from the quotient of the test compound (mg) sprayed into the baffle section and the total chamber air throughput. The analytical concentration in the test atmosphere was determined by subjecting chamber air samples to capillary gas chromatography. The lesser value of the analytical concentrations was attributed to precipitation of larger particles in the baffle chamber (Tillery *et al.*, 1976).

The system provided approximately 30 air changes per h in the test chamber. A steady state of operation was reached in approximately 6 min (MacFarland, 1976). The aerosol generation system was checked during exposure with an aerosol photometer monitoring system which permitted global recording of air input, air output, homogeneity of test atmosphere and stability of the test atmosphere. Air flows were continuously adjusted if necessary. The temperature and humidity of the inhalation chamber were also measured and maintained within the requirements of OECD Test Guideline 403.

An aerodynamic particle sizer with laser velocimeter was used for the particle size distribution analysis. Mass-related particle analyses were also conducted. The parameters MMAD (mass median aerodynamic diameter), NMAD (number median aerodynamic diameter) and the geometric standard deviation (distribution width, σ) were calculated (Dennis, 1976; Marple & Rubow, 1980; Pauluhn, 1982).

Observations were made of the appearance and behaviour of the rats when out of the tube. They were made several times on each day of exposure throughout the exposure period of 5 d and twice per d for 14 d thereafter. The bw of the rats was recorded on d 0, d 4, d11 and d 18.

The RBC and plasma ChE cholinesterase activity (Ellman *et al.*, 1961) and Htc, was determined for 5 males/group after the 1st and 5th exposures. Blood specimens were taken for these determinations from the retro-orbital venous plexus by means of a capillary 10-20 min, but no later than 1 h, after each of the two specified exposures. At the end of the 5 exposures, 5 males/group were anaesthetised with sodium hexobarbitone for pre-mortem removal of the brain. The study authors did not indicate that these were the same 5 males/group used for the

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RBC and plasma ChE determinations. Five females/group were humanely killed with sodium hexobarbitone (350 mg/kg bw ip) for post-mortem removal of lungs. These organs were removed 20-30 min, but no later than 1 h, after the end of the last exposure. The lung weights and brain ChE activities were determined. Gross necropsy was also conducted. The remaining 50% of the rats were humanely killed (sodium hexobarbitone) at the end of a 14 d observation period. Gross necropsy was conducted.

In other studies, 5 rats/sex/group were exposed inh for 4 h (head/nose) every 3 mo to air, water aerosol (nominal 500,000 $\mu L/m^3$) and polyethylene glycol E 400-ethanol (1:1) aerosol (nominal 20,000 $\mu L/m^3$). The bw and results of gross pathological examination of these rats were available for comparison with those of the vehicle control group.

Arithmetic group means, standard deviations, upper and lower confidence limits on the level of $(1 - \alpha) = 95\%$ and $(1 - \alpha) = 99\%$ were calculated. A modified Mann & Whitney 's rank (U) test (Mann & Whitney, 1947; Walter, 1951), was used at significance levels of $\alpha = 5\%$ and $\alpha = 1\%$. Values showing significant differences from the vehicle control group were identified with "+" for $p \leq 0.05$, "++" for $p \leq 0.01$. Also used was a one-way analysis of variance (ANOVA) program followed by Box's test for homogeneity of variances at $p = 0.0500$ level (1 tailed). If variation was found, the groups were post-hoc compared in pairs (1 and 2 tailed) with the Games and Howell modification of Tukey-Kramer's honestly significant difference test.

Results and discussion

Particle characterisation: All aerosol particles were of ≤ 5 μm diameter and therefore respirable. The MMAD for the aerosol generated was measured twice for each concentration. The results ranged from 1.3 to 1.48 μm.

Mortality and Clinical signs: No deaths occurred. No clinical signs were seen at the two lower concentrations. At 33 mg/m^3, all males and females developed clinical signs. The signs shown included weak tremor after the 1st and 2nd exposures. After the 2nd to 5th exposures, ungroomed coats, reduced mobility and weakness of the rear extremities were also observed. Except for the ungroomed coats these signs reversed before the next exposure. One female exhibited moderate tremor on the 5th exposure day before exposure. No marked sex-specific variations in the occurrence of clinical signs were noted. All of the clinical signs were regarded by the study authors to be characteristic of ChE inhibition.

Body weight: Mean bw was reported as associated with concentrations of 1, 5 and 25 mg/m^3, the origin of which was not explained. On d 4, male rats at the highest concnetration showed a significantly depressed mean bw ($p \leq 0.01$, U Test) as compared to the vehicle controls. By d 11, these male rats had gained bw and were no longer statistically different from the vehicle controls. No other treatment group of either sex showed a treatment-related decrease in bw.

ChE and Hct determinations: The results of the ChE determinations are tabulated below. Biologically significant inhibition (≥ 20%) of plasma, RBC and brain ChE occurred in male rats exposed to 33 mg/m^3. Hct was not affected by repeated blood sampling or concentration of test substance.

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Mean brain ChE activity at 33 mg/m^3 was significantly less (p = 0.0002, ANOVA/Box/Tukey-Kramer test) than that for the vehicle control group. Statistical comparisons of plasma, RBC and vehicle control group ChE activity were not provided.

Relative ChE activity in comparison to the vehicle control group*

Group (Analytical conc. - mg/m^3)	After 1st exposure		After 5th exposure		
	Mean activity (kU/L) & % Change in ChE activity		Mean activity RBC, plasma (kU/L), brain (U/g) & % Change in ChE activity		
	RBC #	Plasma#	RBC #	Plasma#	Brain
Control	2.24 na	0.44 na	2.16 na	0.38 na	1.49 na
1.4**	2.44 + 9	0.50 + 14	2.36 + 9	0.43 + 13	1.76 + 18
5.4**	2.21 - 1	0.42 - 5	2.19 + 1	0.33 - 13	1.53 + 3
33**	1.77 - 21	0.08 - 82	1.74 - 19	0.06 - 84	0.49 - 67

* All ChE activity estimations were conducted on male rats only

Sequential determinations conducted on 5 males/group

** The % changes in activity were given as being related to the three indicated analytical concentrations. However except for brain ChE, the mean activity measurements were reported as associated with concentrations 0, 1, 5 and 25 mg/m^3, the origin of which was not explained. The mean ChE activity measurements have been rounded to two decimal places

na = not available

There were no statistical differences between the concentrations 0, 1, 5 and 25 mg/m^3 in respect of the decline in RBC or plasma ChE mean activity between the 1st and 5th exposures.

Pathophysiology: The lungs removed from the female rats showed no gross alterations. There was no statistical difference in lung wt between the 4 concentration groups 0, 1.4, 5.4 and 33 mg/m^3 (ANOVA/Box). An excessive cholinergic stimulation may induce permeation pulmonary oedema due to vasodilation of the pulmonary capillaries (Nieman, 1985) leading to increased lung weights. However this effect was not noted in the female rats examined.

Necropsy: At necropsy, the vehicle control group showed a focus of hepatoid appearance in the right lung in 1 male rat. One female showed the same finding in a lung and another female showed a kidney with cystoid alteration and a ureter filled with urine. There were no gross lesions seen in the 1.4 mg/m^3 concentration group. Two males in the 5.4 mg/m^3 concentration group showed a dark red focus in a lung and a distended lung respectively. The 33 mg/m^3 concentration group contained 1 male rat with isolated red spots in a lung. The study authors did not regard these observations as treatment-related. It is possible that the consistent involvement of the lungs among the findings was due to the exposure technique.

Conclusion

Under the study conditions, the NOEL was 5.4 mg/m^3. This was based on inhibition of plasma, RBC and brain ChE activity and bw depression at the highest exposure concentration of 33 mg/m^3.

Pauluhn J & Machemer L (1987b): Study for sub-acute inhalation toxicity to rats in accordance with OECD Guideline No 412. Study No. T 0022105. Report No. 16069. Lab:

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Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 1 April 1986 - 30 April 1986. Report Date: 30 September 1987. Unpublished. (QA: Yes; GLP: not specified; Test Guidelines: OECD Guideline No 412 & FIFRA Guideline 82-4).

[Associated with this evaluation are the two preliminary range-finding studies T 8021834 (1x4 h exposure) outlined in the acute toxicity tables of Section 3.1.1 & T 9021835 (5x6 h/d for 5 d) reviewed above (Pauluhn & Machemer, 1987a)]

Test Substance:	Methamidophos technical. Batch No. 808 526 309. Purity: 73%. Physical state: aerosol (test substance dissolved in polyethylene glycol E 400 (Lutrol)-ethanol 1:1 mixture).
Test Species:	Wistar Rats, Strain Bor:WISW (SPF-Cpb), males, females, aged 9-12 wk, ≈ 180 g bw (females) and ≈ 210 g bw (males), obtained from Winkelmann, Borchen, Paderborn district, FRG.
Dose:	Air, vehicle and analytical concentrations of 2.6, 12 or 48 mg/m^3 6 h/d 5 d/wk inh for 3 wk. Nominal concentrations of the test substance were 20, 80, 320 mg/m^3 using three spray solutions of 0.1, 0.4 and 1.6 % w/v. Nominal dose levels were 0.5, 2.4 and 9.5 mg/kg bw/d assuming a respiratory minute volume of 0.75 L/kg bw.

Method

The study schedule was as follows. On d -6/-5, the rats were subjected to veterinary examination and allocated randomly to 5x10/sex groups. Preliminary ChE activity estimations were also conducted on those days. On d 0 the rats were weighed. The 1st exposure occurred on d 1. The last exposure occurred on d 25. Interim ChE activity determinations were conducted on males only (5/group) on d 8. Urinalysis was conducted on d 17-18. Necropsy examinations were conducted at a rate of 20/d and occurred over d 21-25. The rats were weighed at the end of each wk. Clinical chemistry, including the final ChE determinations, and haematology tests were performed at the time of necropsy.

The rats to be necropsied were exposed to the test substance up to 1 d prior to killing by exsanguination (cardiac puncture) under anaesthesia with diethyl ether. On the day of necropsy, the rats were exposed for at least 3 h in order to obtain realistic values for the ChE determinations. The study authors indicated that for some rats, a 4 wk exposure period was thus possible but that these rats would be classified as having been exposed for 3 wk.

The vehicle and test substance-vehicle solution was sprayed under dynamic conditions into a cylindrical inhalation chamber with a baffle chamber (Rhema Labor-technik, D 6238 Hofhaim, Am Stegskreuz 2, FRG). The operational methodology of the chambers has been published (Pauluhn, 1984b, 1987d). During exposure to the test aerosol, the rats were confined in perspex tubes. Head-nose exposure predominated. The vehicle-test substance solution was vaporised into the baffle section of the inhalation chamber at a rate of 200 µL/min by means of a jet of compressed air. The solution was supplied continuously to the jet. Each test substance concentration had the same vehicle concentration (10,000 µL Lutrol/m^3 as aerosol, 10,000 µL ethanol/m^3 as vapour). The nominal concentration of the test substance in the aerosol atmosphere was calculated from the quotient of the test compound (mg) sprayed into the baffle section and the total chamber air throughput. The analytical

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concentration in the test atmosphere was determined by subjecting chamber air samples to capillary gas chromatography. The lesser value of the analytical concentrations was attributed to precipitation of larger particles in the baffle chamber (Tillery *et al.*, 1976). The 5 groups of rats were exposed in the test atmosphere to air, vehicle, and three concentrations of test substance, as required by OECD Test Guideline 412.

The system provided approximately 30 air changes per h in the test chamber. A steady state of operation was reached in approximately 6 min (MacFarland, 1976). The aerosol generation system was checked during exposure with an aerosol photometer monitoring system which permitted global recording of air input, air output, homogeneity of test atmosphere and stability of the test atmosphere. Air flows were continuously adjusted if necessary. The temperature and humidity of the inhalation chamber were also measured and maintained within the requirements of OECD Test Guideline 412.

An aerodynamic particle sizer with laser velocimeter was used for the particle size distribution analysis. Mass-related particle analyses were also conducted. The parameters MMAD (mass median aerodynamic diameter), NMAD (number median aerodynamic diameter) and the geometric standard deviation (distribution width, σ) were calculated (Dennis, 1976; Marple & Rubow, 1980; Pauluhn, 1982).

Observations were made of the appearance and behaviour of the rats before and after exposure. Evaluation in the exposure tube was performed only if clear signs occurred. At the end of exposure, the rats were evaluated for the appearance of the eyes and respiratory tract, the general condition of the rhinarium and pinna of the ear, the condition of the hair coat, grooming activity, breathing, circulation, somatomotor activity and behaviour pattern (including tremors, convulsions, hypersalivation, dyspnea, diarrhoea, lethargy, sedation and coma), central nervous and autonomic signs.

The blood samples for the determination of plasma and RBC cholinesterases were obtained from the retro-orbital venous plexus. For the d 8 interim determinations the blood was taken 10-15 min after the end of exposure. The blood samples for clinical chemistry and haematology were obtained by cardiac puncture. Blood samples required for glucose determination were obtained at the penultimate wk from the caudal veins of fasted non-anaesthetised rats. Urine was collected from individual rats in metabolism cages (without watering bottles) overnight during the penultimate week of the study.

The clinical chemistry parameters examined were:-

Blood: ChE-RBC, ChE-Plasma, WBC, RBC, Hb, Hct, MCV, MCH, MCHC, Thrombocyte count (THRO), Thromboplastin time (HQUICK), WBC-DC, AST, ALT, AP, Glutamate dehydrogenase (GLDH), LDH, Sorbitol dehydrogenase (SDH or IDH), Triglycerides (TRIGL), Creatinine (CREA), BUN, Bilirubin (BILI, BILI-t = total bilirubin), Total protein (PROT), Blood glucose (GLUC), Cholesterol (CHOL), Na, K, Ca, Cl, Mg.

Organs: ChE-Brain (One hemisphere of the brain (median section) was used for the brain ChE determinations), Liver: N-Demethylase (aminopyrine-N-demethylase, N-DEM), O-Demethylase (p-nitroanisole-N-demethylase, O-DEM), Cytochrome P-450 (P450), TRIGL.

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The urinalysis parameters examined were: pH, PROT, GLUC, Blood (BLO), Urobilinogen (URO), Ketone bodies (KETO), BILI. Examination of sediment for bacteria, epithelial cells, RBC WBC, amorphous salts, triple phosphate crystals and calcium oxalate crystals.

The absolute organ wt and relative organ wt (organ wt mg/100 g bw) were determined for adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus and thyroid. The bw value used was determined prior to necropsy. The organ wt were those of the exsanguinated and unfixed organ. The following organ tissues for all rats were fixed for histological examination: Adrenals, bone (femur including marrow), brain (cerebrum and cerebellum), coagulating gland, efferent ducts, epididymides, oesophagus, eyes, Harderian gland (lacrimal gland), head (nasopharynx, oropharynx, sinus nasales and paranasales), heart, intestines (stomach, duodenum, jejunum, colon, rectum), kidneys with pelvis, larynx, liver, lungs (with main stem bronchi), lung associated lymph nodes, muscle (quadriceps femoralis), ovaries, parathyroids, prostrate, salivery glands, sciatic nerve, seminal vesicles with vas deferens, skin (rhinarium/nose region), spleen, sternal marrow, sternum, testes, thymus, thyroid, tongue, trachea, uterus. All other organs exhibiting gross changes were also fixed.

In other studies, 5 rats/sex/group were exposed inh for 4 h (head/nose) every 3 mo to air, water aerosol (nominal 500,000 $\mu L/m^3$) and polyethylene glycol E 400-ethanol (1:1) aerosol (nominal 20,000 $\mu L/m^3$). The bw and results of gross pathological examination of the rats used were available for comparison with those of the vehicle control group.

Arithmetic group means, standard deviations, upper and lower confidence limits on the level of $(1 - \alpha) = 95\%$ and $(1 - \alpha) = 99\%$ were calculated. A modified Mann & Whitney 's rank (U) test (Mann & Whitney, 1947; Walter, 1951), was used at significance levels of $\alpha = 5\%$ and $\alpha = 1\%$. Also used was a one-way analysis of variance (ANOVA) program followed by Box's test for homogeneity of variances at $p = 0.0500$ level (1 tailed). If variation was found, the groups were *post-hoc* compared in pairs (1 and 2 tailed) with the Games and Howell modification of Tukey-Kramer's honestly significant difference test. The semiquantitatively determined urinalysis data were not statistically evaluated. Verbal descriptions only of other urine abnormalities were made. The histological findings were statistically evaluated using the "Pairwise Fisher's Test" and all results were presented as incidences, in the form of a stochastic evaluation.

The study authors stated that a statistically significant difference ($p < 0.05$) in a treatment group was regarded as toxicologically relevant only if the difference was statistically significant in comparison with both control groups.

Results and discussion

Statistics: For bw and ChE determinations the statistical comparison groups were specified as vehicle control and air control group respectively. For others the comparison group was not specified. An inspection of the data suggests that the air control group was the comparison group except when pair-wise *post hoc* tests were conducted.

Particle characterisation: All aerosol particles were ≤ 5 μm diameter and therefore respirable. The MMAD for the vehicle aerosol was 1.25 μm. The MMAD's for the test atmosphere aerosols were 1.42-1.52 μm; in all groups, the particle characteristics were nearly the same. The particles would have reached the alveoli and other potential target structures of the respiratory tract.

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Mortality and morbidity: There was no mortality. At the concentration of 48 mg/m^3, all rats showed cholinergic signs after each exposure. They exhibited a slight reduction in activity, moderate to severe tremors, and an ungroomed hair coat. The muscle tremors occurred about 3-4 h after the start of exposure. The study authors specified a duration of signs of 1 d to necropsy. The duration of particular signs was not specified except that after 10 exposures, ungroomed hair coat was continually present. The severity of the signs did not increase with the number of exposures. There were no qualitative or quantitative differences between the sexes.

Body weight: A statistically significant ($p \leq 0.01$) decrease in mean bw occurred in male rats at the highest concentration of 48 mg/m^3. It was first observed in wk 1 ($p \leq 0.05$) and persisted at wk 2. The mean bw for each group at 3/4 wk is tabulated below:-

Mean body weight of rats in each group at wk 3/4

Sex	Mean bw (g) for each group at 3/4 wk (after 15 x 6 h exposures)				
	Air Control	**Vehicle Control**	**2.6 mg/m^3**	**12 mg/m^3**	**48 mg/m^3**
Male	246	232	232	224	217*
Female	179	181	184	177	173

* Statistically significant difference (U Test, $p \leq 0.01$) [cf. vehicle control]

Haematology: The study authors remarked on the differences in MCV (decrease) and MCHC (increase) that were observed in both sexes at 12 and 48 mg/m^3. They are shown in the table below. At 2.6 mg/m^3, the male MCV was decreased and the female MCHC was increased. The study authors dismissed the possibility of a treatment-related effect on MCV and MCHC because there were no treatment-related differences between the groups for Hb, RBC and Hct, the three parameters used to calculate MCV and MCHC. It is possible that dehydration influnced the MCV and MCHC values. On this basis no toxicological significance can be ascribed to the statistical differences observed. There was no treatment-related effect on any other haematological parameter.

Mean MCV and MCHC values in each group#

Parameter	Sex	Mean MCV and MCHC values for each group				
		Air Control	**Vehicle Control**	**2.6 mg/m^3**	**12 mg/m^3**	**48 mg/m^3**
MCV FL	Male Female	65 63	63 62	62** 61##	62** 61*	61** 60**
MCHC g/L	Male Female	313 314	316 318	319 321*	322* 324**	330** 331**

The control group used as a comparison was not specified
No statistical significance was ascribed to this value
* U Test, $p \leq 0.01$, ** U Test, $p \leq 0.05$

Clinical chemistry: The study authors remarked on the BUN and CREA observations shown in the table below. Statistically significant depressions in BUN were observed in both sexes at each concentration. CREA values were reduced in females at 12 mg/m^3 and in both sexes at 48 mg/m^3. The study authors attributed the reductions in BUN and CREA to the daily 8 h periods of food and water deprivation. The CREA precursor creatine phosphate depletes steadily during intense muscular activity and it is possible that at 48 mg/m^3 a secondary decrease in CREA production following cholinergic muscle tremors has occurred. Otherwise

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no toxicological significance can be ascribed to the statistical differences observed. There was no treatment-related effect on any other clinical chemical parameter except for ChE.

Mean creatinine and blood urea nitrogen values in each group#

	Mean CREA and BUN values for each group					
	Sex	**Air Control**	**Vehicle Control**	**2.6 mg/m^3**	**12 mg/m^3**	**48 mg/m^3**
CREA µmol/L	Male	66	70	76	64	50**
	Female	75	70	67	60**	53**
BUN mmol/L	Male	7.08	6.33*	5.78**	5.84**	5.41**
	Female	6.88	6.46	5.76*	5.83*	5.55**

The control group used as a comparison was not specified
* U Test, $p \leq 0.01$, ** U Test, $p \leq 0.05$

Cholinesterase: The results of the ChE determinations are tabulated below. Dose-related biologically significant inhibition of plasma ChE occurred in females at concentrations of 2.6 mg/m^3 and above and in males at 12 and 48 mg/m^3. RBC ChE activity was inhibited to a statistically and/or biologically significant extent in females at 2.6 mg/m^3 and above and in males at 48 mg/m^3. At 48 mg/m^3 brain ChE inhibition was severe. It was inhibited to a lesser extent at the next lowest concentration of 12 mg/m^3.

Preliminary and final ChE determinations in each group

Group (Anal. Conc. mg/m^3)	**Week of Study**	**Mean Cholinesterase Concentration & % Inhibition**					
		Male			**Female**		
		RBC ChE kU/L %	**Plasma$^+$ ChE kU/L %**	**Brain$^+$ ChE U/g %**	**RBC ChE kU/L %**	**Plasma$^+$ ChE kU/L %**	**Brain$^+$ ChE U/g %**
Air Control	0	2.18 7	0.45 4	-	2.18 8	1.21 -	-
	3	2.63 5	0.43 9	1.14 7	2.97 -	1.21 -	1.85 -
Vehicle Control	0	2.35 -	0.46 -	-	2.38 -	1.31 -	-
	3	2.76 -	0.47 -	1.22 -	2.95 -	1.21 -	1.96 -
2.6	0	2.42 0	0.44 0	-	2.27 0	1.2 0	-
	3	2.68 0	0.40 6	1.26 -	2.6* 13	1.0 21	1.59 13
12	0	2.40 0	0.50 0	-	2.45 0	1.21 0	-
	3	2.49 5	0.30* 26	0.96 14	2.35* 21	0.41* 66	1.07* 39
48	0	2.48 0	0.48 0	-	2.45 0	1.2 8	-
	3	2.10* 19	0.09* 72	0.38* 62	2.2* 26	0.14* 88	0.5* 68

* $p < 0.01$, Statistical test not specified. [cf. air control group]

Liver tissue: N-DEM values are tabulated below together with liver wt. At 12 and 48 mg/m^3 there were increases in N-DEM in females and at 48 mg/m^3 there was a decrease in N-DEM in males.

Mean N-Demethylase values in each group#

Parameter	Mean N-Demethylase values for each group					
	Sex	**Air Control**	**Vehicle Control**	**2.6 mg/m^3**	**12 mg/m^3**	**48 mg/m^3**
N-Dem mU/g	Male	116.2	118.0	106.9	113.8	95.0*
	Female	45.3	52.3	52.0	57.3*	61.4*

The control group used as a comparison was not specified
* U Test, $p \leq 0.01$

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The opposite sex trends in N-demethylase values were regarded by the study authors as having no diagnostic relevance. The data is difficult to interpret. N-demethylation of methamidophos is not possible because its molecule does not have a $N\text{-}CH_3$ moiety. Biotransformation of methamidophos involves three processes O-demethylation, S-demethylation and deamination (Salama *et al.*, 1992). There were no differences between the groups in P450 and O-DEM values and no toxicological significance can be ascribed to the liver enzyme findings.

Urinalysis: The findings were unremarkable except in one respect. Statistically significant acidification of the urine was observed at 48 mg/m^3 (vehicle control pH 7: 48 mg/m^3 group pH 6). This suggests metabolic acidosis. The study authors suggested that it could have been secondary to a severe cholinergic effect causing lactic acidosis as a result of muscle activity and stress. It is also possible that respiratory impedance had elevated blood pCO_2 and contributed to acidosis.

Organ weights: The absolute and relative wt of thymus, lung, liver, spleen and adrenal for males and females in the air control, vehicle control and 48 mg/m^3 groups are tabulated below. In males at 48 mg/m^3 there occurred a statistically significant decrease in absolute and relative thymus, liver and spleen wt and a statistically significant increase in relative lung and adrenal gland wt. In females there occurred a statistically significant decrease in absolute liver wt and a statistically significant increase in relative lung and adrenal wt.

Absolute and relative organ wt in males at 48 mg/m^3

Group	Mean absolute (mg) and relative wt (Organ wt mg/100 g bw)				
	Thymus	**Lung**	**Liver**	**Spleen**	**Adrenal**
Air	315 128	967 394	9245 3763	426 174	38 15
Vehicle	295 126	970 419	8466** 3648	426 184	37 16
48 mg/m^3	191* 28*	925 428**	7238* 3341*	336* 155**	43 20*

* U Test, $p \leq 0.01$, ** U Test, $p \leq 0.05$ [cf air control group]

Absolute and relative organ wt in females at 48 mg/m^3

Group	Mean absolute and relative wt (Organ wt mg/100 g bw)				
	Thymus	**Lung**	**Liver**	**Spleen**	**Adrenal**
Air	195 109	805 449	6000 3347	306 171	54 30
Vehicle	215 119	861 477*	6173 3423	342** 190	56 31
48 mg/m^3	174 100	828 478**	5409** 3118	289 167	56 32**

* U Test, $p \leq 0.01$, ** U Test, $p \leq 0.05$ [cf air control group]

There was a lack of gross or histological abnormalities or enzymatic findings likely to have an impact on organ size or wt. Stress, vehicle, loss of bw in males, sex or treatment may have contributed to the differences observed. An unequivocal attribution to any or all of these possible causes is not possible. It is not likely however that these changes are treatment-related. No toxicological significance has been ascribed to them.

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Necropsy results: Gross necropsy showed no indications of treatment-related organ damage. No histological evidence of test substance induced organ damage or specific damage to the respiratory tract was found.

Conclusion

Under the conditions of this study, a 3 wk inhalation exposure of rats to methamidophos technical resulted in cholinergic signs expected from the known pharmacological profile of this substance, at the highest concentration of 48 mg/m^3. In females at all 3 test concentrations, plasma ChE activity was inhibited to a biologically significant extent (≥20%) and RBC ChE inhibition was statistically significant. Consequently a NOEL could not be established.

5. SUBCHRONIC STUDIES
5.1. Oral Administration
5.1.1 Mice

Foster D, Lamb DW, Hoss HE, Brewer GJ, Mallicoat DR & Hayes RH (1980): A pilot study using technical methamidophos in mice. Mobay Report No. 68763. Study No. 79CCM01(P). Toxicology Report No. 95. Lab: Stanley Research Centre, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 3 December 1979 - 14 January 1980. Report Date: 13 May 1980; revised 22 August 1980. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos technical. Batch Number: 77-297-194. Purity: 75%. Physical State: Clear liquid.
Test Species:	Mice: CD_1 outbred strain albino, aged 6 wk of an unspecified bw obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts.
Dose:	0, 2, 10, 50 and 100 ppm in diet.

Method

Fresh batches of dietary admixture were made weekly. Corn oil was used as a vehicle to aid in the uniform distribution of the test substance in the food. A Hobart mixer was used for 10 min/batch. The food made for this study had 2% less fat than that commercially supplied to accommodate the contribution of the corn oil to the fat. The diet was stored in a freezer until needed. It was removed from the freezer on the day the food was to be used. A weighed amount of feed was placed into clean feeders and given to the mice. After 1 wk the uneaten food was weighed and disposed of. The food consumption of each mouse was calculated. The mice received the test diet for 43 d. The control group contained 20 mice/sex. The groups at 2 and 10 ppm contained 24 male and 23 female, and 25 male and 23 female mice respectively. The groups at 50 and 100 ppm each contained 25 male and 24 female mice.

Visual observations of behaviour, clinical signs or mortality were made twice daily. Body palpation, bw measurements were conducted weekly. Blood for ChE determinations was obtained from the orbital sinus. Five mice/sex were selected for blood sampling during wk 1, wk 2 and wk 5 on the basis that a mouse was bled only once. Control mice and mice at 2 ppm

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were bled at wk 6 to determine if peak ChE depression had occurred in the mice at 2 ppm at wk 5. At termination of the study, the brain was taken from 5 mice/sex/group. Plasma, RBC and brain ChE activity were determined (Talbott, 1975). Necropsies were conducted only on mice that died during the study.

Statistical evaluation of the results was not conducted.

Results and discussion

Clinical signs and mortality: The signs observed were wet abdomens on 2 male mice and a tarry-like substance around the anal region of 1 female at 10 ppm. Temporary lesions were seen in a few of the mice used for bleeding. Nine mice died - 2 in the control group, 3 at 10 ppm, 1 at 50 ppm and 3 at 100 ppm. Gross necropsy of the dead mice did not reveal lesions lesions attributable to the test substance.

Mean plasma and RBC ChE activity for each treatment group

Time	ChE activity (µmol/mL/min or µmol/g/min) for each treatment group (ppm) and % ChE inhibition									
	Males					Females				
	0	2	10	50	100	0	2	10	50	100
Plasma ChE										
wk 1	2.91 -	2.81 3	1.23 58	0.28 90	0.28 90	3.53 -	2.68 24	1.43 59	0.28 92	0.36 90
wk 5	3.18 -	2.14 33	1.08 66	0.34 89	0.22 93	4.43 -	3.60 19	1.64 63	0.62 86	0.26 94
wk 6	2.6 -	1.92 26	-	-	-	4.3 -	3.63 16	-	-	-
RBC ChE										
wk 1	0.81 -	0.93 -	0.57 30	0.14 83	0.21 74	0.87 -	0.62 29	0.50 43	0.19 78	0.27 69
wk 5	0.89 -	0.70 21	0.34 62	0.12 87	0.13 85	0.80 -	0.60 25	0.34 58	0.17 79	0.13 87
wk 6	0.97 -	0.84 13	-	-	-	0.93 -	0.64 31	-	-	-
Brain ChE										
d 43	14.6 -	11.7 20	6.2 58	1.9 87	1.4 90	15.0 -	10.7 29	5.5 63	2.0 87	1.6 89

Food consumption and bw: The study authors presented the weekly food consumption rates but did not attempt to interpret them. There were wide variations from wk to wk in the wt of food left in the feeders at weekly weighings. A considerable amount of food was wasted due to food being scratched from the feeders. Recovery for weighing of the scratched out food was not feasible because of faecal and urine contamination. The feed waste was more evident for females during wk 2 at 10 and 50 ppm and during wk 4 for the control and 50 and 100 ppm groups. The mean achieved test chemical intakes were equivalent to approximately 0.3, 1.5, 7.5 and 15 mg/kg bw/d (Lehman, 1954).

The initial and final bw of the control males was 28.2 and 33 g respectively. The respective bw for control females were 21 and 25 g respectively. For the 0, 2, 10, 50 and 100 ppm groups, the ratio of the final to initial mean bw in male mice was 1.2, 1.1, 1.2, 1.1 and 1.0 respectively. For female mice the ratios were 1.2, 1.1, 1.2, 1.2 and 1.1 respectively. Males at

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50 ppm made no bw gain (27 to 27 g mean bw) in the 1st wk but consistently gained bw to end at 31 g. Males at 100 ppm lost 4 g (27 to 23 g mean bw) during the 1st wk. They then gained bw consistently to end at 28 g. In the 1st wk their food consumption was not different from other males. Female mice at 50 and 100 ppm lost 1 g (22 to 21 g mean bw) and 2 g (21 to 19 g mean bw) respectively in the 1st wk but recovered to grow consistently to end at 26 and 24 g respectively. In that wk their food consumption was not different from other females. It is likely that the effect on bw in males and females at 50 and 100 ppm in the 1st wk, was treatment related.

Cholinesterase: The plasma and ChE activity determinations for each concentration are tabulated above. The results for wk 2 are not shown because they were similar to those of wk 1. Biologically significant plasma, RBC and brain ChE inhibition occurred at 2 ppm and above. The degree of inhibition was dose-dependent reaching a plateau at 50 ppm. At 2 ppm the degree of inhibition of plasma and RBC ChE peaked at 5 wk in males and 1 wk in females.

Conclusion

Biologically significant plasma, RBC and brain ChE inhibition occurred at all concentrations tested. On this basis a NOEL for methamidophos in mice could not be determined. The LOEL was 2 ppm (approximately equivalent to 0.3 mg/kg bw/d).

5.1.2 Rats

Loser E & Lorke D (1970a): Bay 71 628. Subchronic toxicological studies on rats (Three-month feeding experiment). Mobay Report No. 28043. Study No. not specified. Report No. 2165. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: not specified. Report Date: 29 June 1970. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

This evaluation includes histopathology examinations which were the subject of a separate report as follows.

Groning P & Lorke D (1976a): Bay 71 628 (Tamaron). Subchronic toxicity study on rats (Three-month feeding experiment). Histopathology. Mobay Report No. 49871. Study No. not specified. Report No. 6313. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 31 August 1976. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos technical. Purity: 50% (in a Silkasil S premix)
Test Species:	Wistar Rats, SPF derived, males, females, 28-32 d of age, bw 45-55 g obtained from Winkelmann, Kirchborchen, Kreis Paderborn.
Dose:	0, 2, 6, 20, 60 ppm in diet.

Method

Rats (30/sex/control group and 15/sex/treatment group) received the test substance in the diet for 3 mo. Clinical pathology examinations were conducted on 5 rats/sex/group after 4 wk and

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3 mo of feeding. Hb, Hct, RBC, WBC, MCH, MCV, reticulocyte count, WBC-DC, thrombocyte count and prothrombin time (PT) were determined. The activities of AP, ALT, AST, ornithine-carbamyl transferase (OCT) were measured. The bilirubin and total protein content of serum was also determined. Serum urea, serum creatinine, blood sugar and blood cholesterol levels were determined. The urine was examined for sugar, protein, and blood using Combi-Uristex test strips (Merck, Darmstadt) and for bile pigment. The meaning of the term "sugar" was not specified. The sediment was microscopically examined. Plasma and RBC ChE activity was measured (Eben & Pilz, 1967) at 8 d, 4 wk, 8 wk, and 13 wk in 5 rats/sex/group. Blood was taken from the retro-orbital venous plexus.

The appearance and behaviour of the rats throughout the study were observed. Those which died were necropsied. All survivors were anaesthetised with ether and killed by exsanguination. Gross necropsies were conducted during which the thyroids, thymus, heart, lung, liver, spleen, kidneys, adrenals and gonads were weighed.

Tissues from brain, heart, lung, liver, kidneys, spleen, stomach, intestinal tract, urinary bladder, testes, ovaries, uterus, thyroid, thymus, adrenals and pituitary were excised *post-mortem* from 5/sex rats at each dietary concentration of test substance. Sections of the fixed tissues were cut at approximately 5µ and stained with hemalum and eosin. Frozen sections of liver cut at approximately 15µ were stained for fat with Oil Red O. Sections of heart, lung, liver, spleen, kidneys, adrenals, pituitary, thyroid, testes, ovaries and uterus from each group were histopathologically examined. The remaining tissues were also examined but only for the control and 60 ppm groups.

The non-parametric rank test (Wilcoxon, 1947) was used for the statistical evaluation of the results.

Results and discussion

Test substance intake: The intake of test substance for the 2, 6, 20 and 60 ppm groups was for males, 0.04, 0.12, 0.41 and 1.14 mg/rat/d respectively, and for females 0.04, 0.10, 0.34 and 1.06 mg/rat/d respectively.

Initial and final doses at each concentration

Time	Dose (mg/kg bw/d) at each concentration (ppm)									
	Males					Females				
	0	**2**	**6**	**20**	**60**	**0**	**2**	**6**	**20**	**60**
1 wk	0	0.6	2.5	8.7	24.2	0	0.7	2.2	7.5	23.5
13 wk	0	0.08	0.3	1.1	3.9	0	0.1	0.4	1.5	5.3

Clinical signs and mortality: The rats at 2, 6 and 20 ppm respectively, did not differ from the control group in appearance and behaviour. The rats at 60 ppm were less lively and slightly weakened. Cholinergic signs were not observed. Three rats died. One female in the 6 ppm group and 1/sex in the 60 ppm group were accidently killed while being blood sampled.

Food consumption and bw: Food consumption in male rats for the control and 60 ppm group was 1.9 and 1.7 kg/rat respectively. This was the largest difference between the groups in food consumption. Food consumption by females for the same two groups was 1.5 and 1.6 kg/rat respectively. The mean initial bw for each of the male groups was 47 g and for each of the female groups 45 g. For the groups 0, 2, 6, 20 and 60 ppm, expressing the final bw as a

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multiple of the initial bw showed for males 7.5, 7.5, 7.5, 7.6 and 6.2 respectively and for females 4.9, 4.7, 4.8, 4.8 and 4.5 respectively. Each group showed a steady rate of growth over the study period. From d 14 males and females at 60 ppm had a consistently lower rate of growth than other groups. There was no week in which they lost bw. The lower final mean bw of the males and females of the 60 ppm group and the lower food consumption of the males at 60 ppm, were not specified as statistically significant differences from the control group. It is likely however that these findings were toxicologically significant.

Haematology, clinical chemistry and urinalysis: There were no treatment related differences at 4 wk or 3 mo between the treated groups and the control groups in the parameters examined.

Cholinesterase: The plasma and RBC ChE activity determinations are tabulated below. The determinations at 4 and 8 wk are not shown since the ChE activity determinations were similar from wk 4. Biologically significant plasma or RBC ChE inhibition (≥20%) was observed at and above 6 ppm in males. It was observed in females at 20 and 60 ppm.

Mean plasma and RBC ChE activity for each group at 1 and 13 wk*

Time	ChE activity for each treatment group (ppm) ("µmol acetylcholine") and % ChE inhibition									
	Males					Females				
	0	2.0	6	20	60	0	2.0	6	20	60
Plasma ChE										
1 wk	2.64 -	2.77 -	2.18 17.4	1.61 39.0	1.29 51.0	2.85 -	3.03 -	2.39 16.1	1.54 46.0	1.01 64.5
13 wk	2.11 -	1.95 7.6	1.64 22.3	1.27 39.8	0.98 53.5	4.74 -	5.30 -	4.03 14.9	2.42 49.0	1.36 71.3
RBC ChE										
1 wk	3.11 -	3.06 1.6	2.61 16.1	1.76 43.4	1.08 65.3	3.16 -	2.93 7.3	2.78 5.1	1.61 49.0	0.97 30.6
13 wk	4.23 -	3.65 13.7	3.22 23.9	1.58 62.6	1.03 75.6	4.03 -	4.04 -	3.45 14.4	2.30 42.9	1.19 70.5

* A pretreatment determination was not conducted

Organ weights: At 60 ppm, male rats showed a statistically significant decrease in the wt of thyroid, thymus, heart, lung, liver, spleen, kidney, adrenal and gonads compared to the control group. This decrease was not reflected in relative organ wt and the smaller male organs at 60 ppm were the result of a lower bw compared to the control group. There were no statistically significant differences between the 2, 6 and 20 ppm treatment groups and the controls in relative organ wt for either sex. In female rats at 60 ppm there was a statistically significant increase in the mean relative wt of lung (426 to 468 mg/100 g, $p < 0.01$) and heart (276 to 294 mg/100 g, $p < 0.05$) and a decrease in thymus wt (134 to 114 mg/100 g, $p < 0.05$) compared to the female control group. It is difficult to specify the cause of these changes. Similar differences were not observed in males at that concentration. There were no findings at necropsy in these organs likely to impact on their size or wt. The relative wt changes observed may have have been secondary to cholinergic stimulation but the absence of similar changes in males at 60 ppm makes this conclusion equivocal.

Gross necropsy: There were no treatment-related gross necropsy findings.

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Histopathology: The cells of the "zona arcuata" in the adrenal cortex of rats from the treated groups were often seen to be greatly dilated and containing many small vacuoles. The zone was hypertrophic. The extent of vacuolation in the zone is tabulated below. Vacuolation was not seen in the control group. The vacuolation was treatment-related and its extent was dose-dependent. The relationship between concentration and vacuolation parallels that between concentration and ChE inhibition.

Extent of vacuolation of the "zona arcuata" of the adrenal glands

Grade (*)	**Vacuolation in the "zona arcuata" of the adrenal gland - number of rats with each grade (in sequence) at each concentration (ppm) (n = 5/sex)**									
	Males					**Females**				
	0	**2**	**6**	**20**	**60**	**0**	**2**	**6**	**20**	**60**
+, 1, 2	0, 0, 0	1, 0, 0	3, 1, 0	0, 1, 4	0, 0, 5	0, 0, 0	1, 0, 0	3, 0, 0	0, 2, 3	0, 0, 5

* Grades of vacuolation: + = very minimal, 1 = minimal, 2 = moderate

The alterations seen in the "zona arcuata" of the adrenal cortex were considered by the study authors to be an adaptation to stress induced by the test substance. Hypertrophy of varying degree in the zona fasciculata of the adrenal cortex is an adaptive response. In this instance it is most likely secondary to cholinergic stimulation of the adrenal medulla. The subsequent stimulation, via the pituitary, of corticosteroid release from the zona fasciculata of the adrenal cortex is likely to induce hypertrophy of corticosteroid producing cells.

Minimal and random occurrence of inflammatory cell infiltrations in various organs, small fat droplets in the liver, physiological thymus involution, ovarian cysts and atrophy of the seminal vesicle were also noted. These findings were not considered treatment-related.

Conclusion

Under the conditions of this study a NOEL for methamidophos in diet was established at 2 ppm (0.03 mg/rat/d or males 0.08, females 0.1 mg/kg bw/d respectively), based on biologically significant plasma and RBC ChE inhibition at the next highest dose.

Guo L, Chen Y, Lu R, Liu Y, Xia L, Chen J, Zhang X & Mao J (1986d): Acute, cumulative and sub-chronic toxicity, delayed neurotoxicity and experimental treatment of intoxication. Part 3: Sub-chronic toxicity. Zhonghua Laodong-Weisheng Zhiyebing Zazhi, 4(5):257-260.

Test Substance:	Methamidophos "direct isomerisation crystal". Purity: 90.4%. Source: Synthetised at Zhejiang Research Institute.
Test Species:	Rat: Wistar, male and female, approximately 209 g bw of an unspecified age and obtained from an unspecified source.
Dose:	0, 1.0, 2.5, 5.0, 10.0, 20.0 ppm in diet

Prelude

Only limited information was provided by the study authors. The techniques for whole blood ChE activity measurement were not described and the measurements of activity, except for pre-dosing activity, were not provided. ChE activity was presented as percentages of pre-dosing activity which are presumed to be group mean activity percentages. The timing of various experimental activities of various experimental activities was given in imprecise terms.

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Method

Ninety one rats were randomly allocated to 6 groups (5 treatment and 1 control) containing 7-10/sex/group. The five treatment groups were fed an unspecified diet admixed with methamidophos for 12 wk. The dose groups at 5.0 ppm and above were observed for a further 2-4 wk after withdrawal of methamidophos. General appearance and behaviour were observed at an unspecified frequency. Body wts and food utilisation were measured weekly. Whole blood ChE activity was determined before exposure and once/2 wk during exposure and in the post-dosing observation period. Serum ALT and BUN were determined before exposure, at mid-term exposure and at the end of exposure. The blood collection procedures and site/s of collection were not described. Gross necropsies were conducted. The time and method of termination of the rats was not specified. Liver and kidney wts were determined. Histological examinations of lung, liver, kidney, heart and testes or ovaries from 4 rats/sex/group were conducted. Statistical analysis ("F-distribution") was applied to bw and food consumption measurements, pre-dosing levels of ChE activity measurements in males and females, ALT and BUN determinations and relative organ wts.

Results and discussion

Dose equivalents: The approximate dose equivalents were 0.1, 0.25, 0.5, 1.0 and 2.0 mg/kg bw/d (Lehman, 1954).

Appearance and behaviour: At 20 ppm an unspecified number of rats (sex unknown) exhibited dishevelment of the coat, huddling posture and activity avoidance after 1 mo of exposure.

Food consumption and bw: Measurements of food consumption and bw were not provided. There were no statistical differences between treatment groups and controls.

Whole blood ChE activity: In 44 male and 47 female rats, the respective mean pre-dosing ChE activity was 1.57 and 1.87 μmol ACh hydrolysed/50 μL at 37^{o}C. The difference between the two values was statistically significant ($t = 7.33 > 2.66$, $P < 0.01$).

At 1 ppm at 12 wk, ChE activity decreased to 89% of the pre-dosing level in males but otherwise remained above 90% in both sexes. The study authors stated that the t-distribution test showed no notable difference. At 2.5 ppm in the 8-12 wk post-dosing interval, ChE activity in males varied between 80 and 90% of the pre-dosing level. Activity in females remained above 90% of the pre-dosing level throughout the exposure period.

At 5 ppm at 6 wk in males ChE activity was 72.8% of the pre-dosing level and varied between 70 and 80% thereafter. The activity in females varied between 80 and 90% of the pre-dosing level at 4, 8 and 12 wk. In both sexes ChE activity recovered in the 2nd wk after withdrawal of methamidophos.

At 10 and 20 ppm at 2 wk of exposure, ChE activity in male rats decreased to 82.2 and 69.6% of the pre-dosing level respectively. In females at 2 wk, activity decreased to 68.5 and 54.1% respectively. After 12 wk, these levels had decreased in males to 60.6 and 34.2% respectively and in females to 63.5 and 38.9% respectively. In males, ChE activity had not been completely restored to pre-dosing levels 4 wk after withdrawal of methamidophos. In females ChE activity was completely restored 2 wk after withdrawal.

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ALT and BUN values: No statistically or toxicologically significant differences were observed in any group at various exposure times.

Pathological findings: There were no treatment-related differences between groups in gross or histological findings or in relative liver or kidney wts.

Conclusion

Biologically significant inhibition (≥ 20% ChE inhibition) of whole blood ChE was observed at the second lowest concentration of 2.5 ppm (≈ equivalent to 0.25 mg/kg bw/d) in male rats. Under the conditions of this study a NOEL of 1 ppm (≈ 0.1 mg/kg bw /d) was therefore established.

Comment

The absence of the determined ChE values does not allow verification of percentages presented. The conclusion must be qualified accordingly.

5.1.3 Dogs

Loser E & Lorke D (1970b): Bay 71 628. Subchronic toxicological studies on dogs (Three-month feeding experiment). Mobay Report No. 27986. Study No. not specified. Report No. 2164. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: not specified. Report Date: 26 June 1970. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

This evaluation includes histopathology examinations which were the subject of a separate report as follows.

Groning P & Lorke D (1976b): Bay 71 628 (Tamaron). Subchronic toxicity studies on dogs. (Three-month feeding experiment). Histopathology. Mobay Report No. 51187. Study No. not specified. Report No. 6213. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Chemagro Agricultural Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 9 July 1976. Unpublished. (QA: No; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos technical. No further details provided
Test Species:	Dog, beagle breed, males and females, 5-6 mo old, unspecified bw obtained from breeder Velaz.
Dose:	0, 1.5, 5 and 15 ppm in diet

Method

Dogs were allocated to 3 treatment groups: 2 dogs/sex/group, and 1 control group: 3 dogs/sex. The test substance was mixed with the food twice weekly for 3 mo. In the first 4 wk they were supplied 250 g/d and from the 5th week they received 300 g/d. Each ration was mixed to a paste with twice the quantity of water immediately before feeding.

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The dogs were observed for any changes of appearance or behaviour. At the end of the study their eyes were examined with an ophthalmoscope. Dogs were weighed weekly and if the full ration was not consumed the left-over food was weighed. Clinical pathology examinations were conducted at initiation of treatment and at 1 and 3 mo. Blood was obtained from the saphenous vein. The haematological parameters determined were Hb, Hct, MCH, MCV, ESR, RBC, WBC, WBC-DC, reticulocyte count, thrombocyte count and prothrombin time (PT). Enzymes determined were AP, ALT, AST, sorbitol dehydrogenase (SDH), LDH and ornithine carbamyl transferase (OCT). Serum bilirubin, urea, creatinine, sugar, cholesterol and total protein content were also determined. Liver function using bromophthalein retention was examined. Urine was analysed for sugar, protein, blood, bile pigment and sediment. Inulin and p-aminohippuric acid clearance tests were conducted on all dogs at the end of the study. Plasma and RBC ChE were determined using the hydroxamate method (Eben & Pilz, 1967) at 1, 4, 8 and 13 wk.

At 3 mo, the dogs were anaesthetised with sodium hexabarbitone and killed by opening the thorax. Gross necropsies were conducted during which the thyroid, heart, lung, pancreas, liver, spleen, kidneys, adrenal gland and gonads were weighed.

Tissues from brain, pituitary, thymus, heart, lung, diaphragm, liver, gall bladder, kidneys, adrenals, spleen, stomach, duodenum, ileum, jejunum, caecum, colon, urinary bladder, tonsils, salivary glands, cervical lymph nodes, testes, prostate gland, ovaries, uterus, thyroid and parathyroid were excised *post-mortem* from every dog and fixed in 10% buffered formalin. Sections of the fixed tissues were cut at approximately 5µ and stained with hemalum and eosin. Sections of heart, lung, liver, spleen, kidneys, adrenals, pituitary, thyroid, parathyroid, testes, prostate gland and ovaries were histopathologically examined. The remaining tissues were also examined but only for the control and 15 ppm groups.

Statistical methods were not used in this study.

Results and discussion

Preliminary note: The preliminary haematological results were given for test concentrations 0, 1, 3 and 10 ppm. Haematological results at 1 mo and 3 mo were given for concentrations 0, 3, 10 and 30 ppm. Body wt measurements were given for concentrations specified as: 0, 3(1.5), 10(5) and 30(15) ppm.

Test substance intake: For the 1.5, 5 and 15 ppm treatment groups, the average intake of test substance was: males 0.43, 1.42 and 4.37 mg/dog/d respectively, and females: 0.43, 1.41 and 4.27 mg/dog/d respectively. Since the increase in mean bw in the dogs over the study was $\leq$ 10%, the median mean bw of 10 kg (males) and 9.5 kg (females) was used to establish doses for males: 0.04, 0.14 and 0.44 mg/kg bw/d and females: 0.04, 0.15 and 0.45 mg/kg bw/d.

Clinical signs and mortality: There was no difference in appearance, behaviour or appetite of treated dogs when compared to the control groups. No mortalities occurred. The ophthalmoscopic examination showed no corneal or lens opacity. There were no pathological changes to the ocular fundi.

Food consumption and bw: Steady growth occurred in all groups. There were no treatment-related effects on food consumption or bw.

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Haematology, clinical chemistry and urinalysis: Preliminary haematological, enzyme activity, liver and kidney clearance and urinary parameters were within ranges regarded as physiologically typical for young dogs. No treatment-related differences between groups were observed at 1 mo or at 3 mo except for ChE.

Cholinesterase: The plasma and RBC ChE determinations are tabulated below. The study authors indicated that the activity values for plasma and RBC ChE at 1.5 ppm were within the limits of biological variation and assay precision. However the inhibition of RBC ChE in females at 1.5 ppm at 3 mo (19.7%), approaches biological significance. Biologically significant plasma or RBC ChE inhibition (≥20%) was observed in both sexes at and above 5 ppm.

Mean plasma and RBC ChE activity for each group of dogs*

Time	**Mean plasma and RBC ChE activity for each treatment group (ppm) ("µmol acetylcholine") and % ChE inhibition**							
	Males				**Females**			
	0	**1.5**	**5**	**15**	**0**	**1.5**	**5**	**15**
Plasma ChE								
d 0	8.9 -	8.0 -	7.8 -	10.6 -	10.5 -	10.7 -	9.3 -	8.8 -
1 wk	9.4 -	8.6 8.5	7.0 25.5	-# -	10.5 -	10.7 -	8.7 17.1	8.7 17.1
1 mo	6.9 -	7.1 -	6.5 5.8	5.7 17.4	9.2 -	8.4 8.7	5.7 38.0	4.2 54.3
2 mo	8.3 -	7.0 15.7	6.6 20.5	6.4 22.9	9.4 -	9.0 4.2	6.0 36.2	3.7 60.6
3 mo	8.8 -	7.9 10.2	7.2 18.2	6.2 29.5	10.0 -	9.2 8.0	6.5 35.0	6.8 32.0
RBC ChE								
d 0	6.5 -	8.0 -	5.4 16.9	6.5 -	7.0 -	6.8 2.8	6.8 2.8	6.3 10.0
1 wk	6.8 -	7.5 -	5.7 16.2	-# -	7.0 -	6.4 8.6	6.5 7.1	4.4 37.1
1 mo	5.9 -	6.7 -	4.7 20.3	3.0 49.1	7.1 -	6.7 5.6	5.1 28.2	3.4 52.1
2 mo	6.3 -	6.0 4.8	3.7 41.3	2.5 60.3	6.9 -	5.9 14.5	4.0 42.0	1.3 81.1
3 mo	5.5 -	6.7 -	5.0 9.1	2.4 56.4	7.6 -	6.1 19.7	4.5 40.8	2.0 73.7

* Statistical evaluation was not reported for this data
# The reason for the missing datum was not specified

Organ weights: The absolute organ wt and relative organ wt showed no treatment-related differences between groups.

Necropsy findings: The necropsy examinations showed that all dogs were well nourished. None of the organs showed any pathological findings which could be attributed to the test substance. Any other pathological findings were not specified.

Histopathology: There were no treatment-related pathological changes in the tissues examined.

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Conclusion

The study authors concluded that at 1.5 ppm (0.04 mg/kg bw/d) there was no inactivation of plasma or RBC ChE. They did not specify a NOEL. In this evaluation, a NOEL of 1.5 ppm is assigned, based on biologically significant (≥ 20%) plasma and RBC ChE inhibition at higher doses.

5.2 Inhalational administration

5.2.1 Rats

Pauluhn J & Machemer L (1988): SRA 5172. (Methamidophos): Study of the subchronic inhalation toxicity to rats in accordance with OECD Guideline No. 413. Bayer Report No. 16578. Study No. T 9022366. Report No. 98370. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 30 March 1988. Unpublished. (QA: Yes; GLP: OECD Principles of Good Laboratory Practice (Bundesanzeiger No 42a (1983)) & FIFRA Good Laboratory Standards (40 CFR Part 160 and 792); Test Guidelines: OECD Guideline No 413).

The following addendum report (16578A) was undertaken to evaluate the significance of effects on liver weights. Agencies stated that the data in the original report No. 16578 (Pauluhn & Machemer, 1988) did not support the conclusion that relative liver weights were within the "range of variation" of controls. The addendum demonstrated via a more refined statitistical evaluation, that the conclusions drawn were correct

Pauluhn J & Machemer L (1990): Addendum to SRA 5172. (Methamidophos): Study of the subchronic inhalation toxicity in rats in according to OECD-Guideline No. 413. Bayer Report No. 16578A. Study No. T 9022366 (Addendum). Report No. 98370-1. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 30 March 1988. Unpublished. (QA: Yes; GLP: Yes: OECD Principles of Good Laboratory Practice (Bundesanzeiger No 42a (1983)) & FIFRA Good Laboratory Standards (40 CFR Part 160 and 792); Test Guidelines: OECD Guideline No 413).

Test Substance:	Methamidophos technical. Batch No. 77-297-149. Purity: 73.4%. Physical state: aerosol (test substance dissolved in polyethylene glycol E 400 (Lutrol)-ethanol 1:1 mixture). Source: Mobay Chemical Corporation, Kansas City, Missouri, USA.
Test Species:	Wistar Rats, Strain Bor:WISW (SPF-Cpb), males, females, aged 7-11 wk, ≈ 170 g bw (females) and ≈ 210 g bw (males), obtained from Winkelmann, Borchen, Paderborn district, FRG.
Dose:	Mean analytical concentrations of 1.0, 5.4 or 23 mg/m^3 6 h/d 5 d/wk inh for 3 mo. Nominal concentrations of the test substance were 6.2, 38, 204 mg/m^3 using three spray solutions of 0.03, 0.19 and 1.02 % w/v. Nominal dose levels were not specified.

Method

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The study schedule was as follows. On d 0, the rats were allocated randomly to 7 groups of 10/sex. These groups included an air and vehicle control group and 2 satellite groups. The satellite groups received vehicle aerosol and the high concentration of test material respectively. The first exposure occurred on d 1 and the last in wk 13/14.

The test groups were necropsied on several successive days starting in wk 13. The satellite groups were observed for 42 d after the end of exposure (recovery period) and were necropsied in wk 19. The test group rats to be necropsied were exposed to the test substance up to 1 d prior to killing. On the day of necropsy, they were exposed for at least 3 h in order to obtain realistic values for the ChE determinations. The study authors indicated that for some rats, a 14 wk exposure period was thus possible but that these rats would be classified as having been exposed for 13 wk.

The vehicle and test substance-vehicle solution was sprayed under dynamic conditions into a cylindrical inhalation chamber with a baffle chamber (Rhema Labor-technik, D 6238 Hofhaim, Am Stegskreuz 2, FRG). The operational methodology of the chambers has been published (Pauluhn, 1984b, 1987d). During exposure to the test aerosol, the rats were confined in perspex tubes. Head-nose exposure predominated. The vehicle-test substance solution was continuously vaporised into the baffle section of the inhalation chamber at a rate of 200 µL/min by a jet of compressed air. Each test substance concentration had the same vehicle concentration (10,000 µL Lutrol/m^3 as aerosol, 10,000 µL ethanol/m^3 as vapour). The nominal concentration of the test substance in the aerosol atmosphere was calculated from the quotient of the test compound (mg) sprayed into the baffle section and the total chamber air throughput. The analytical concentration in the test atmosphere was determined by subjecting chamber air samples to capillary gas chromatography. The lesser value of the analytical concentrations was attributed to precipitation of larger particles in the baffle chamber (Tillery *et al.*, 1976). The 5 groups of rats were exposed in the test atmosphere to air, vehicle, and three concentrations of test substance, as required by OECD Test Guideline 413.

The system provided approximately 30 air changes per h in the test chamber. A steady state of operation was reached in approximately 6 min (MacFarland, 1976). Air input, air output, homogeneity of test atmosphere and stability of the test atmosphere were monitored. Air flows were continuously adjusted if necessary. The temperature and humidity of the inhalation chamber were also measured and maintained within the requirements of OECD Test Guideline 413.

An aerodynamic particle sizer with laser velocimeter was used for the particle size distribution analysis. Mass-related particle analyses were also conducted. The parameters MMAD (mass median aerodynamic diameter), NMAD (number median aerodynamic diameter) and the geometric standard deviation (distribution width, σ) were calculated (Dennis, 1976; Marple & Rubow, 1980; Pauluhn, 1982).

Observations were made of the appearance and behaviour of the rats before and after exposure. Evaluation in the exposure tube was performed only if clear signs occurred. At the end of exposure, the rats were evaluated for the appearance of the eyes and respiratory tract, the general condition of the rhinarium and pinna of the ear, and the hair coat, grooming activity, breathing, circulation, somatomotor activity and behaviour pattern (including tremors, convulsions, hypersalivation, dyspnea, diarrhoea, lethargy, sedation and coma), central nervous and autonomic signs.

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Body wt was determined at d 0 and weekly thereafter prior to the start of exposure. Declining bw provoked the weekly measuring of food consumption/cage (5 rats/cage) from wk 6.

Urinalysis of test groups occurred in wk 12/13. Clinical chemistry and haematology determinations were conducted in test groups in wk 1, 4, 8 and 11 and at necropsy (wk 13/14). In satellite groups selected clinical chemistry and the complete haematological parameters were determined at the end of the exposure in wk 12 and during the recovery period in wk 16 and 19.

The blood samples for the determination of plasma and RBC ChE, interim clinical chemistry and haematological parameters were obtained from 5 rats/sex/group from the retro-orbital venous plexus. For all examinations involving ChE activity, the blood was obtained from rats in < 5 min and brain < 1 h after exposure. One hemisphere of the brain was used for brain ChE determination. The termination blood samples for clinical chemistry and haematology were obtained by cardiac puncture. Blood samples required for glucose determination were obtained from fasted non-anaesthetised rats. Urine was collected from individual rats in metabolism cages (without watering bottles) overnight.

The clinical chemistry and haematological parameters examined were:-

Blood: ChE-RBC, ChE-Plasma, WBC, RBC, Hb, Hct, MCV, MCH, MCHC, Thrombocyte count (THRO), Thromboplastin time (HQUICK), WBC-DC, AST, ALT, AP, Glutamate dehydrogenase (GLDH), LDH, Sorbitol dehydrogenase (SDH or IDH), Triglycerides (TRIGL), Creatinine (CREA), BUN, Bilirubin (BILI, BILI-t = total bilirubin), Total protein (PROT), Blood glucose (GLUC), Cholesterol (CHOL), Na, K, Ca, Cl, Mg.

Organs: ChE-Brain, Liver: N-Demethylase (aminopyrine-N-demethylase, N-DEM), O-Demethylase (p-nitroanisole-N-demethylase, O-DEM), Cytochrome P-450 (P450), TRIGL.

The urinalysis parameters examined were: pH, PROT, GLUC, Blood (BLO), Urobilinogen (URO), Ketone bodies (KETO), BILI. Examination of sediment for bacteria, epithelial cells, RBC WBC, amorphous salts, triple phosphate crystals and calcium oxalate crystals.

Lung function tests were performed on 2 males and 2 females/group at the end of the exposure period or at the end of the recovery period. Parameters measured were respiratory rate, respiratory minute volume, pleural pressure, dynamic compliance, specific compliance, lung resistance, change in resistance following ACh provocation, forced expiratory measurements, tidal volume, inspiratory capacity, expiratory capacity, vital capacity (forced), total lung capacity, residual volume, functional residual capacity and CO diffusion capacity.

The ACh provocation aerosol was generated using a DeVilbiss aerosol generator (10 L air/min). The number median aerodynamic diameter of the provocation aerosol was 0.9 µm. The provocation substance was ACh hydrochloride 25% in deionised water. The average ACh concentration in the aerosol was approximately 590 mg/m^3 air.

Eye examinations were performed on 5 test group rats/sex/group prior to 1st exposure and at the end of exposure (91-100 d). The eyes were examined for changes in the retina, vitreous body, lens, cornea and the external eye surface.

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The absolute and relative organ wt were determined for adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus and thyroid. The bw value used was determined prior to necropsy. The organ wts were those of the exsanguinated and unfixed organ. The following organs and tissues for all rats were fixed for histological examination:-

Adrenals, bone (femur including marrow), brain (cerebrum and cerebellum), coagulating gland, efferent ducts, epididymides, oesophagus, eyes, Harderian gland (lacrimal gland), head (nasopharynx, oropharynx, sinus nasales and paranasales), heart, intestines (stomach, duodenum, jejunum, colon, rectum), kidneys with pelvis, larynx, liver, lungs (with main stem bronchi), lung associated lymph nodes, muscle (quadriceps femoralis), ovaries, pancreas, parathyroids, prostrate, salivary glands, sciatic nerve, seminal vesicles with vas deferens, skin (rhinarium/nose region), spleen, sternal marrow, sternum, testes, thymus, thyroid, tongue, trachea, uterus. All other organs exhibiting gross changes were also fixed.

Arithmetic group means, standard deviations, upper and lower confidence limits on the level of $(1 - \alpha) = 95\%$ and $(1 - \alpha) = 99\%$ were calculated. A modified Mann & Whitney 's rank (U) test (Mann & Whitney, 1947; Walter, 1951) was used at significance levels of $\alpha = 5\%$ and $\alpha = 1\%$. Also used was a one-way analysis of variance (ANOVA) program followed by Box's test for homogeneity of variances at $p = 0.050$ level (1 tailed). If variation was found, the groups were *post-hoc* compared in pairs (1 and 2 tailed) with the Games and Howell modification of Tukey-Kramer's honestly significant difference test. The semiquantitatively determined urinalysis data were not statistically evaluated. Verbal descriptions only of other urine abnormalities were made. The histological findings were statistically evaluated using the "Pairwise Fisher's Test" and all results were presented as incidences, in the form of a stochastic evaluation.

Results and discussion

Particle characterisation: All aerosol particles were ≤ 5 µm diameter and therefore respirable. The MMAD for the vehicle aerosol was 1.30 µm. The MMAD's for the test atmosphere aerosols were 1.52-1.53 µm; in all groups, the particle characteristics were nearly the same. The particles would have reached the alveoli and other potential target structures of the respiratory tract.

Concentrations: The results of test substance monitoring analyses are tabulated below.

Nominal and mean analytical concentrations of test substance

Concentration			
mg/m^3 air			**µL/m^3 air**
"Target"	**Nominal**	**Analytical**	**Vehicle**
0	-	Air	-
0	-	Vehicle	20000
0.8	6.2	1.05	20000
6.0	38.0	5.35	20000
30.0	204	23.05	20000

Mortality and morbidity: There were no treatment-related mortalities. During the entire exposure period, slight to moderate muscle tremors were observed at 23 mg/m^3 on the day of exposure but not prior to exposure the following day. The severity of the tremors was constant. On exposure free days they were not observed.

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Body weight: Body wt measurements in controls and test groups are tabulated below. The air control group was used as the reference group in statistical evaluations. The statistically significant ($p \leq 0.01$) deficit in mean bw occurring in males at 23 mg/m^3 was first observed at 2 wk and continued to wk 13. Vehicle control males showed a statistically significant deficit in mean bw at wk 4 and wk 6-12 ($p \leq 0.05$) and at wk 13 ($p \leq 0.01$). Females at 23 mg/m^3 showed a statistically significant deficit in mean bw from wk 4 to 11 ($p \leq 0.05$ except at wk 5 when $p \leq 0.01$). Rats at 1 and 5.4 mg/m^3 showed no significant difference from air controls. Body wt measurements were also provided for the satellite vehicle control and 23 mg/m^3 groups. Satellite males at 23 mg/m^3 showed a statistically significant deficit in bw at wk 5 ($p \leq 0.01$) and wk 7 ($p \leq 0.05$). Satellite females at 23 mg/m^3 and satellite vehicle control rats were not affected.

Mean body weight of rats in each test group at selected intervals

Sex/Interval	Mean bw (g) for each group (mg/m^3) at selected times									
	Male					Female				
	AC	VC	1.0	5.4	23.0	AC	VC	1.0	5.4	23.0
d0 (initial)	171	171	170	170	174	174	172	174	170	170
wk 5	229	214	222	227	191*	177	175	169	175	167*
wk 10	252	234#	249	247	209*	185	183	178	181	176#
wk 13 (final)	256	232*	258	249	203*	182	179	178	179	173

AC = air control; VC = vehicle control

* Statistically significant decrease in bw ($p \leq 0.01$) [cf. air control]

Statistically significant decrease in bw ($p \leq 0.05$) [cf. air control]

The study authors regarded the inhibited bw gain observed as "implausible" and introduced food consumption measurements at wk 6. Amid a high variablity in food consumption measurements a decrease in food consumption from wk 11-13 was discernible. They regarded the decrease as an artifact induced by collecting blood and urine samples. They suggested that the inhibited bw gain observed at 23 mg/m^3 was due to decreased food consumption. It is likely that the depression in bw gain at that concentration was treatment-related.

Haematology: Incidental statistically significant differences ($p \leq 0.01$ cf air control) occurred in Hct, MCV, MCH and MCHC at 5.4 mg/m^3 at wk 1 and at 23 mg/m^3 at wk 1, 4 and 19 wk (satellite group). The differences were mostly within the range of variation in air and vehicle control groups and there was no relationship with concentration or time. Local inflammatory cellular infiltrates at the retro-orbital sinus (wk 1, 4, 8 and 11) may have lead to haematological differences compared with blood collected by cardiac puncture at necropsy (wk 13). The intergroup differences were therefore attributable to individual biological variation, variations induced by sampling techniques and variations in assay precision rather than the test substance. No toxicological significance was ascribed to the differences.

Clinical chemistry: Incidental statistically significant differences ($p \leq 0.01$ cf air control)) were seen in males at 23 mg/m^3 at 13 wk. They were increased AST (91 cf 57 U/L), increased LDH (354 cf 164 U/L), decreased PROT (52 cf 59 g/L), decreased CREA (52 cf 76 µmol/L) and GLUC (3.6 cf 4.2 µmol/L). Statistically significant differences ($p \leq 0.01$ cf air control)) were also seen in females at 23 mg/m^3 at 13 wk. They were decreased PROT (52 cf 59 U/L), decreased BUN (6.6 cf 7.5 mmol/L), decreased CREA (50 cf 58 µmol/L), decreased CHOL (1.5 cf 1.8 mmol/L) and GLUC (3.4 cf 3.8 µmol/L). Statistically significant differences ($p \leq 0.01$ cf air control) in CREA were also observed in males and females at 13 wk at the two lower concentrations. In the satellite 23 mg/m^3 group there were no significant differences at

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19 wk in these parameters. The study authors attributed the differences in BUN and CREA in the test groups to physiological aberrations induced by experimental activity despite their being unchanged in air or vehicle control groups. They also proposed that increases in AST and LDH were not caused by liver damage since AP, ALT, GLDH and IDH were unchanged. This view would be supported by the absence of treatment-related histological abnormalities in the liver. It should be noted however that large amounts of AST and LDH are present in skeletal muscle from which these enzymes can become liberated without concomitant elevation in AP or ALT. Given the large magnitude of the increases above control levels, a treatment-related increase in AST and LDH cannot be excluded. Slight decreases in PROT, CHOL and GLUC were attributed to secondary stress factors and are considered to be of equivocal significance.

Cholinesterase: The results of the ChE determinations are tabulated below. Statistically significant differences (cf air controls) in inhibition of RBC, plasma or brain ChE were observed at 5.4 and 23 mg/m^3. The 23 mg/m^3 test group plasma ChE activity inhibition had partially reversed by 13 wk. The 23 mg/m^3 satellite group RBC and plasma ChE activity inhibition had fully reversed by 19 wk.

ChE determinations in each group at selected intervals

Group (Anal. Conc. mg/m^3)	**Week of Study†**	**Mean ChE Concentration & % Activity**					
		Male			**Female**		
		RBC ChE kU/L %	**Plasma ChE kU/L %**	**Brain ChE U/g %**	**RBC ChE kU/L %**	**Plasma ChE kU/L %**	**Brain ChE U/g %**
Air Control	1 13	2.83 94 2.07 88	0.49 88 0.46 79	- 1.46 105	2.26 88 2.81 98	1.11 94 1.24 93	- 1.34 103
Vehicle Control	1 13	3.02 100 2.35 100	0.56 100 0.58*100	- 1.39 100	2.58 100 2.86 100	1.18 100 1.33 100	- 1.30 100
1.0	1 13	2.95 98 2.29 97	0.46 82 0.43 74	- 1.28 92	2.24 87 2.67 93	0.91 77 1.08 81	- 1.16 89
5.4	1 11 13	2.36* 78 2.16# 75 2.03 86	0.24* 43 0.21* 41 0.36* 62	- - 0.99* 71	2.18 85 2.09 80 2.35* 82	0.65* 55 0.56* 37 0.75* 56	- - 0.97* 75
23.0	1 11 13	2.07* 69 2.01* 70 1.81 77	0.05* 9 0.08* 16 0.27* 47	- - 0.73* 53	1.53* 59 1.74* 67 2.04* 71	0.11* 9 0.13* 9 0.45* 34	- - 0.72* 55
Satellite Vehicle	12 16	2.63 100 2.46 100	0.52 100 0.45 100	- -	1.95 100 2.46 100	1.45 100 1.85 100	- -
Satellite 23.0	12 16	1.76* 67 2.46 100	0.08* 15 0.53 118	- -	1.27* 65 2.47 100	0.18* 12 1.79 97	- -

† Pre-dosing determinations were not conducted
* Statistically significant decrease in ChE activity ($p \leq 0.01$) [cf. air control]
# Statistically significant decrease in ChE activity ($p \leq 0.05$) [cf. air control]

Liver tissue: Statistically significant ($p \leq 0.05$, air controls) decreases in N-DEM concentration were observed in males at 23 mg/m^3 and in females at 5.4 and 23 mg/m^3 at necropsy. No toxicological significance was ascribed to these decreases.

Urinalysis: The findings were unremarkable except in one respect. Statistically significant (cf acidification of the urine was observed in females of the vehicle control and 23 mg/m^3 groups (air control females pH 7.1; vehicle controls 6.6 and the 23 mg/m^3 group pH 6.2) at wk 12. This suggests metabolic acidosis. The study authors suggested that it could have been

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secondary to a cholinergic effect causing lactic acidosis as a result of muscle activity and stress. It is also possible that respiratory impedance has elevated blood pCO_2 and contributed to acidosis. The urinalysis showed no evidence of damage to the urogenital tract.

Lung function tests: Because of small sample sizes, the study authors applied statistical analyses only to the parameters shown in the table below. They did not provide the results but indicated that the measurements for dynamic compliance showed an increase at 5.4 and 23.0 mg/m^3 compared to air controls (indicating that at these concentrations lung elasticity decreased). This effect was attributed to an increase in tone of intrathoracic musculature caused by vagal nerve stimulation. There were no substantial differences between the groups in other parameters. There was no chronic obstructive or restrictive treatment-induced lung damage occurred.

Selected respiratory tract function measurements at the completion of the study

Respiratory tract function parameter#	Combined male and female measurements for each treatment group (mg/m^3) at 12 wk and at 19 wk for satellite groups						
	AC	VC	1.0	5.4	23.0	S VC	S 23.0
Dynamic compliance	0.31	0.27	0.32	0.37	0.38	0.34	0.33
Resistance	0.33	0.25	0.28	0.41	0.33	0.29	0.34
Δ Resistance*	0.16	0.07	0.15	0.25	0.27	0.08	0.11

AC = air control; VC = vehicle control; S VC = satellite VC; S 23.0 = satellite 23.0
# Units for dynamic compliance: mL/cm H_2O; for lung resistance: cm H_2O/mL/s
* Δ Resistance = resistance after acetylcholine provocation minus resistance before provocation

There was no treatment-related variation in the basal respiratory tract resistance. The increased resistance at 5.4 mg/m^3 was attributed to mechanically induced increase in resistance after introduction of the orotracheal catheter. Increased reactivity of the bronchial musculature to acetylcholine aerosol was observed at 5.4 and 23.0 mg/m^3. This finding and the results for ChE activity are mutually consistent and show that when the respiratory tract is the primary absorption organ methamidophos aerosol does not cumulate in the respiratory tract.

Eye examinations: The results provided no indications of treatment-related changes in the refractive media or in the fundus.

Organ weights: Statistically significant differences (cf air control) observed in males at 23 mg/m^3 are tabulated below. In females a decrease ($p \leq 0.01$) in absolute and relative spleen wt and an increase ($p \leq 0.01$) in relative adrenal wt at 23 mg/m^3 was observed. Incidental differences ($p \leq 0.05$) in males and females were observed at 1.0 and 5.4 mg/m^3.

Absolute and relative organ wt in males

Group (mg/m^3)	Mean absolute (mg) and relative wt (organ wt mg/100 g bw)						
	Brain	Heart	Lung	Liver	Kidney	Spleen	Adrenal
AC -absolute	1360	772	997	8663	1563	415	40
-relative	533	302	390	3387	612	162	16
VC -absolute	1320	717	986	7528*	1408*	401	35
-relative	575#	309	427*	3239	608	173	15
23 -absolute	1322	652*	898*	6328*	1265*	313*	34#
-relative	654*	322	441*	3107#	623	153	17

AC = Air Control; VC = Vehicle Control
* $p \leq 0.01$, # $p \leq 0.05$ (cf air control)

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There was a lack of gross or histological abnormalities or enzymatic findings likely to have an impact on organ size or wt. All observed effects on organ wt are probably secondary to the depressed terminal bw at 23 mg/m^3. Absolute and relative organ wt in males and females of satellite groups at 23 mg/m^3 showed that differences had fully reversed at 19 wk.

Necropsy results: Gross necropsy showed no indications of treatment-related organ damage.

Histopathology: No histological evidence of test substance-induced tissue damage or specific damage to the respiratory tract was found.

Conclusion

Under the conditions of this study, 13 wk inhalation exposure of rats to methamidophos technical resulted in decreased bw gain and cholinergic muscle tremors at the highest concentration of 23 mg/m^3. Statistically significant inhibition of RBC, plasma and brain ChE was observed at 5.4 and 23 mg/m^3 but not at 1.0 mg/m^3. On this basis a NOEL of 1.0 mg/m^3 (equivalent to a nominal inhalation dose of 0.3 mg/kg bw/exposure d) was established.

6. CHRONIC STUDIES

6.1 Oral Administration

6.1.1 Mice

Hayes RH, Stuart BP & Lamb DW (1984a): Oncogenicity study of methamidophos technical (Monitor^R) on mice. Mobay Report No. 87479. Study No. 80-332-01. Toxicology Report No. 507. Lab: Environmental Health Research, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsors: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA & Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 14 January 1980 - 21 January 1982. Report Date: 6 August 1984. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: 40 CFR Part 160).

Test Substance:	Methamidophos technical. Batch Number: 77-297-149. Purity: 70%. Source: Mobay Chemical Corporation, Stilwell, Kansas, USA.
Test Species:	Mouse: CD_1 outbred strain, albino, males and females, 6 wk old, of an unspecified bw, obtained from Charles River Breeding Laboratories, Massachusetts, USA.
Dose:	0, 1, 5, 25 ppm in diet.

Method

Mice for the oncogenicity study groups were randomly allocated at 50 mice/sex/dietary level. A further 160 mice were randomly allocated to a reserve group (10 mice/sex/dietary level) and a replacement group (10 mice/sex/dietary level). The diets were fed to the reserve group for 53 wk and to the oncogenicity groups for 105 wk. The replacement group also received the diets in order to replace oncogenicity groups at their respective levels. Where replacement occurred, the bw, food consumption and observation data of replacement mice were substituted for the those of the original mice. In this study, 2 males and 3 females at 1 ppm, 2 females at 5 ppm and 3 females at 25 ppm, died within the 1 st mo and were replaced. After 1 mo the remaining replacement mice were killed.

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Prior to the initiation of the study, a complete material accountability study was conducted on a composite of five batches of methamidophos prepared for toxicological investigation. The percentage of methamidophos technical in the test substance was determined by high-pressure liquid chromatography at 6 mo intervals during the study. The homogeneity of methamidophos technical in the diet was determined by gas liquid chromatography (GLC) on 9 random samples taken from the top, middle or bottom thirds of the mixing bowl of the 1 and 54 ppm batches. The stability of methamidophos in the diet was determined by fortifying a sample of mixture with ^{14}C-methamidophos to obtain a concentration of 1 ppm. The test mixture was divided equally, with 1 portion placed in the freezer and the other kept at room temperature. A 50 g aliquot of each portion was analysed for concentration of methamidophos by GLC on d 1, 3, 7 and 16 after preparation. A sample was taken weekly from each batch of diet mixed. A duplicate sample was taken monthly from the current weekly batch to analytically determine the concentration of methamidophos.

Observations for toxicological effects, moribundity and mortality were made twice daily. Weekly observations for abnormalities and masses by palpation were made. Weekly measurements of food consumption and bw were recorded.

Mice in the satellite groups were used for haematological determinations at 6 mo and at termination. Haematological determinations conducted were RBC, WBC, Hct, Hb, WBC-DC, platelet count, MCH, MCHC, MCV and blood smear. Clinical chemistry or urinalysis were not performed.

All rats found dead or killed were subjected to gross necropsy. Killing was by carbon dioxide asphyxiation. A gross examination was made of the external surface, all orifices, cranial cavity, external surface of the brain, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissue and organs. Tissues were embedded in paraffin, cut 6 µm thick, mounted on slides and stained with haematoxylin-eosin. A histopathological examination was conducted on all available tissues from all rats at all study levels. The organs weighed and tissues examined were adrenals, brain, gonads, heart, kidneys, liver, lungs and spleen. The tissues subjected to histopathological examination were those listed in Appendix III except for Zymbal's gland.

Body wt, feed consumption, haematology and absolute and relative organ wt were subjected to an Analysis of Variance followed by Least Significant Difference or Duncan's New Multiple Range Test. All statistical differences were reported at the 95% confidence level ($p \leq 0.05$).

Results and discussion

Material accountability, stability, homogeneity and concentration: The study was initiated with a test substance containing methamidophos technical at a purity of 72.4%. Successive semi-annual analyses showed the purity of methamidophos technical in the test substance to be 70.0, 71.5. 70.5 and 70.5%. Changes in purity were compensated for in diet preparation. The degradation of methamidophos in the food was determined to be <15% in 16 d. This was accepted as sufficient stability for the 7 d mix used here. Methamidophos was distributed homogeneously in the food. The limits of variation in the concentrations of methamidophos in the diet are tabulated below.

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The mean, standard deviation and range of the concentration of methamidophos technical at each dietary level

Dietary level (ppm)	Mean ± SD (ppm)	Range (ppm)
1	0.97 ± 0.10	0.65 to 1.10
5	4.78 ± 0.50	3.89 to 5.90
25	24.24 ± 2.67	20.20 to 30.70

Intake of test substance: The calculated achieved doses are tabulated below.

The achieved doses of methamidophos

Sex	Dietary level (ppm)	Total food consumption (kg)	Total dosage of methamidophos		
			mg	mg/kg bw	mg/kg bw/d
Male	0	4	0	0	0
	1	4	4	106	0.1
	5	3	16	357	0.7
	25	3	80	2017	3.5
Female	0	3	0	0	0
	1	4	4	133	0.2
	5	2	9	301	0.8
	25	3	75	2229	4.0

Clinical signs and mortality: Changes in appearance or behaviour were described as rough coat, urine stain, enlarged abdomen and spontaneous seizures when handled. These changes were considered to be age-related. The incidence of palpable masses was low and the body distribution pattern was unremarkable. The occurrence of these masses was not attributable to the test substance.

The mortality rates are tabulated below. The mortality rate in females at 5 ppm is high compared to control females. This appears to be coincidental. There are no trends in mortality rates that suggested a treatment-related effect.

Mortality rates at each dietary level over time

Time (wk)	Number/% mortalities at each dietary level (ppm)							
	Males				Females			
	0	1	5	25	0	1	5	25
52	5/10	4/8	2/4	2/4	5/10	1/2	3/6	4/8
78	9/18	9/18	11/22	6/12	12/24	9/18	16/32	11/22
106	22/44	25/50	23/46	27/54	22/44	24/48	35/70	28/56

Food consumption: The weekly mean food consumption data for the 1st 13 wk was presented in graphic form only. The graphic data showed considerable fluctuation of values which precluded definite conclusions. The data for selected intervals is tabulated below. At 25 ppm mean weekly food consumption was statistically significantly lower than controls at 13, 39, 78 and 106 wk in males and at 39, 78 and 106 wk in females. The depression of food consumption at 25 ppm increased with time in both sexes. The depression was attributed to the test substance.

Mean weekly food consumption at selected times

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Dietary level (ppm)	Mean weekly food consumption (g) at the specified times (wk)									
	Males					Females				
	2#	13	39	78	106	2#	13	39	78	106
0	41	39	38	44	42	42	41	42	44	42
1	39	40	38	42	37*	38	42	39*	41	37
5	39	39	37	42	37*	38	42	39*	44	37*
25	38	38*	36*	40*	37*	40	39	38*	39*	35*

* Statistically significantly lower than controls ($p \leq 0.05$)

Statistical evaluation was not performed on the initial food consumption data

Body wt: The weekly mean bw for the 1st 13 wk was presented in graphic form only. The graphic data showed a progressive increase in bw with the slowest growing group being the 25 ppm males. The mean bw of control and 25 ppm mice at selected times is tabulated below. At 25 ppm the mean weekly bw was statistically significantly lower than controls at 78 and 106 wk in males and females. This slower rate of growth is coincident with decreased food consumption. No treatment-related differences between the groups at 1 and 5 ppm were noted.

Mean bw at selected dietary levels and times

Dietary level (ppm)	Mean weekly bw (g) at the specified times (wk)									
	Males					Females				
	0#	13	39	78	106	0#	13	39	78	106
0	29	37	40	41	39	23	32	35	36	36
25	29	36	39	38*	36*	24	32	35	33*	32*

* Statistically significantly lower than controls ($p \leq 0.05$)

Statistical evaluation was not performed on the initial bw measurements

Haematology: There were no treatment-related differences between treated groups and controls.

Gross necropsy, histopathology and organ wt: There were no treatment-related differences between the treated groups and controls in gross or cellular pathological findings or mean absolute or relative organ wt. The study authors indicated that the neoplastic histopathological observations concerned spontaneous or naturally-occurring neoplasms in male and female mice at all dietary levels as confirmed by historical data for this strain of mouse (Percy & Jonas, 1971). There were no unusual or rare neoplasms observed. A treatment-related effect was not seen. There was no evidence of induced oncogenicity in this study.

Conclusion

(1) Methamidophos was not oncogenic in mice at 25 ppm or less in the diet (3.5 or 4.0 mg/kg bw/d in males and females respectively).

(2) On the basis of decreased food consumption and rate of growth a NOEL of 5 ppm in the diet (0.7 or 0.8 mg/kg bw/d in males and females respectively) was established.

6.1.2 Rats

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Hayes RH, Stuart BP & Lamb DW (1984b): Chronic feeding/oncogenicity study of technical methamidophos (Monitor^R) to rats. Mobay Report No. 88687. Study No. 81-271-01. Toxicology Report No. 554. Lab: Environmental Health Research, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsors: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA & Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 9 February 1981 - 23 February 1983. Report Date: 13 November 1984. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: 40 CFR Part 160).

Test Substance:	Methamidophos technical. Batch Number: 77-297-149 Purity: 70%. Source: Mobay Chemical Corporation, Stilwell, Kansas, USA.
Test Species:	Rats: Fischer 344, males and females, 6 wk old, obtained from Charles River Breeding Laboratories.
Dose:	0, 2, 6, 18 and 54 ppm in diet.

Method

The dietary admixture was prepared weekly with corn oil (1% w/w) as the vehicle for the test substance. Rats for the chronic study groups were randomly allocated at 50 rats/sex/dietary level. A further 200 rats were randomly allocated to a reserve group (10 rats/sex/dietary level) and a replacement group (10 rats/sex/dietary level). The diets were fed to the reserve and chronic study groups for 12 and 24 mo respectively The replacement group rats also received the diets in order to replace chronic study or reserve group rats at their respective dietary levels. Where replacement occurred, the bw, food consumption and observation data of each replacement rat was substituted for that of the original rat. In this study as a result of death or moribundity, 3 female rats (chronic study: 1 at 0 and 1 at 1 ppm; reserve: 1 at 1 ppm) were replaced during the 1st mo. After 1 mo the replacement rats were killed. Surviving reserve and chronic study rats were killed at 12 and 24 mo respectively.

Prior to the initiation of the study, a complete material accountability study was conducted on a composite of five batches of methamidophos prepared for toxicological investigation. At termination of the study components of the composite sample with quantities of 1% or greater were identified. The percentage of methamidophos technical in the diet was determined by high-pressure liquid chromatography at 6 mo intervals during the study. The homogeneity of methamidophos technical in the diet was determined by gas liquid chromatography (GLC) on 9 random samples taken from the top, middle or bottom thirds of the mixing bowl of the 1 and 54 ppm batches. The stability of methamidophos in the diet was determined by fortifying a sample of mixture with ^{14}C-methamidophos to obtain a concentration of 1 ppm. The test mixture was divided equally, with 1 portion placed in the freezer and the other kept at room temperature. A 50 g aliquot of each portion was analysed for concentration of methamidophos by GLC on d 1, 3, 7 and 16 after preparation. A sample was taken weekly from each batch of diet mixed. A duplicate sample was taken monthly from the current weekly batch to analytically determine the concentration of methamidophos.

Observations for toxicological effects, moribundity and mortality were made twice daily. Weekly observations for abnormalities and masses were made by palpation. Weekly measurements of food consumption and bw were made and recorded.

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Haematology and general blood chemistry parameters were determined at initiation, 6 and 12 mo in reserve rats and at 18 and 24 mo in chronic study rats. ChE activity (Ellman *et al.*, 1961) was determined as follows: Plasma, RBC and brain ChE activity at 0 and 2 ppm in all replacement rats when terminated at 1 mo; plasma and RBC ChE activity at all dietary levels in all reserve rats at initiation, 6 and 12 mo; plasma and RBC ChE activity at all dietary levels in 10 chronic study rats/sex at 12, 15, 18 and 24 mo. Brain ChE was determined at 12 mo in all terminated reserve rats and at 24 mo in 10 terminated chronic study rats/sex. The 12 and 15 mo determinations specified above for chronic study rats were prompted by a slight decrease in plasma ChE activity at 12 mo in 2 ppm female reserve rats. The parameters determined are tabulated below. Urinalysis was not performed.

Haematology	**Clinical Chemistry**
RBC	albumin, AP, ALT
WBC	AST
Hct	bilirubin (total)
Hb	calcium, sodium, potassium, chloride,
WBC-DC	cholesterol (total), creatinine (blood)
platelet count	ChE activity (plasma, RBC and brain) (Ellman *et al.*, 1961)
MCH	glucose (blood)
MCHC	LDH
MCV	protein (total)
blood smear	urea nitrogen (blood)

All rats found dead or killed due to moribundity, for interim determinations or at study termination were subjected to gross necropsy. Killing was by carbon dioxide asphyxiation. A gross examination was made of the external surface, all orifices, cranial cavity, external surface of the brain, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissue and organs. Tissues were embedded in paraffin, cut 6 μm thick, mounted on slides and stained with haematoxylin-eosin. A microscopic examination was conducted on all available tissues from all rats at all study levels except those killed for interim determinations.

The organs weighed were: adrenals, brain, gonads, heart, kidneys, liver, lungs, spleen.

The tissues examined were: blood smear, bone marrow, spleen, thymus, lymph nodes (cervical), heart, aorta (dorsal), salivary gland (submaxillary),oesophagus, stomach, duodenum, liver, pancreas, ileum, jejunum, cecum, colon, rectum, pituitary, adrenals, thyroid, parathyroids, gross lesions, skin, muscle (skeletal), brain, spinal cord, eyes (and Harderian glands), optic nerve, epididymes, mammary gland, ovaries, seminal vesicle, prostate gland, testes, uterus, cervix, larynx, trachea, lungs, bone, ribs (costochondral junction), sternum, kidneys, urinary bladder.

Body wt, feed consumption, haematology, clinical chemistry and absolute and relative organ wt were subjected to an Analysis of Variance followed by Least Significant Difference or Duncan's New Multiple Range Test. All statistical differences were reported at the 95% confidance level ($p \leq 0.05$).

Results and discussion

Material accountability, stability, homogeneity and concentration: The study was initiated with methamidophos technical at a purity of 72.4%. Successive semi-annual analyses

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showed the purity of methamidophos technical to be 70.0, 71.5. 70.5 and 70.5%. Changes in purity were compensated for in diet preparation. The degradation of methamidophos in the food was determined to be 15% in 16 d. The limits of variation in the concentrations of methamidophos in the diet are tabulated below.

The mean, standard deviation and range of the concentration of methamidophos technical at each dietary level

Dietary level (ppm)	Mean ± SD (ppm)	Range (ppm)
2	1.99 ± 0.26	1.51 to 2.50
6	5.92 ± 0.49	5.02 to 7.00
18	17.37 ± 1.74	14.20 to 22.40
54	51.96 ± 4.28	40.20 to 59.00

Intake of test substance: The achieved doses are tabulated below.

The achieved doses of methamidophos technical

Sex	Concentration in food (ppm)	Total food consumption (kg)	Total dosage of methamidophos		
			Mg	mg/kg bw	mg/kg bw/d
Male	0	14.43	0	0	0
	2	10.90	21.8	58.5	0.09
	6	12.38	74.3	213.9	0.29
	18	13.05	234.9	629.0	0.85
	54	13.73	741.5	2112.2	2.85
Female	0	9.08	0	0	0
	2	9.52	19.0	85.9	0.11
	6	10.04	60.2	260.1	0.35
	18	10.77	193.9	783.4	1.06
	54	10.17	549.0	2589.7	3.49

Clinical signs and mortality: Signs seen in all groups throughout the course of the study were lacrimation and enlarged abdomen. At 18 and 54 ppm, "most" of the rats also showed loose stools, urine stain, rough coat and skin lesions (predominantly tail rash). The study authors did not attribute these signs to any cause. There were no differences between the groups in the occurrence of palpable masses.

There had been no mortalities in males by 12 mo. One control female and 1 female at 18 ppm had died by 12 mo. Most mortalities occurred in the last 6 mo of the study. There were no differences between the dietary levels or the sexes at any of the specified times.

Food consumption and bw: A treatment-related effect on food consumption was not observed. From wk 4 to wk 105 the mean bw of males at 54 ppm was consistently lower each week ($p \leq 0.05$) than that of the control group males (wk 4, 213 g cf control 221 g; wk 105, 335 g cf control 382 g). The same statistical significance was observed in lower male bw at 18 ppm from wk 5 to wk 84 except for wk 61 and 77. From wk 11 to wk 105 the mean bw of females at 54 ppm was consistently lower each week ($p \leq 0.05$) than that of the control group females (wk 11, 164 g cf control 168 g; wk 105, 234 g cf control 265 g). In males at 6 ppm there was a statistically significantly lower bw than controls over the 1st 11 wk. Otherwise incidental statistical differences, mainly bw increases in both sexes, were

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noted. The lower rates of growth in males at 18 ppm and 54 ppm and in females at 54 ppm were most likely treatment related.

Haematology and blood chemistry: Occasional individual parameters at various dietary levels were statistically significiantly different from those of the control rats. The differences were slight, inconsistent in occurrence, and not sex-related or dose-related. They were within the limits of biological variation and assay precision and were not toxicologically significant. There was no evidence of loss of liver or kidney function.

Brain ChE: The brain ChE activity determinations are tabulated below. Biologically significant brain ChE inhibition (≥ 20% inhibition) was detected in females at 12 mo at 2 ppm. It was also detected in both sexes at 12 and 24 mo at 6 ppm and above. The degree of inhibition was dose-related.

Brain ChE activity determinations at each dietary level

Time	Brain ChE activity (µmol/g/min) for each treatment group (ppm) and % ChE inhibition									
	Male					Female				
	0	2	6	18	54	0	2	6	18	54
1 mo	14.7 -	12.3 16.3	-	-	-	14.6 -	12.0 17.8	- -	- -	- -
12 mo	13.7 -	12.4 9.5	8.0 41.6	4.5 67.1	3.2 76.6	14.1 -	10.7 24.1	7.8 44.7	4.2 60.7	2.9 79.4
24 mo	12.1 -	10.6 12.4	7.4 38.8	4.3 64.5	2.5 79.3	11.8 -	11.0 6.8	8.2 30.5	4.3 63.5	2.9 75.4

Plasma ChE: The plasma ChE activity determinations are tabulated below. The results at 12 and 15 mo were not substantially different from the results at 12 mo for the reserve rats and are not shown.

Plasma ChE activity determinations at each dietary level

Time	ChE activity (µmol/mL/min) for each treatment group (ppm) and % ChE inhibition									
	Males					Females				
	0	2	6	18	54	0	2	6	18	54
Init.	0.55 -	0.56 -	0.48 -	0.45 -	0.48 -	0.79 -	0.78 -	0.77 -	0.81 -	0.79 -
1 mo	0.47 -	0.38 19.1	- -	- -	- -	1.52 -	1.21 20.4	- -	- -	- -
6 mo	0.55 -	0.48 12.7	0.44 20.0	0.22 60.0	0.11 80.0	2.38 -	1.88 21.0	1.40 58.8	0.64 73.1	0.20 91.5
12 mo	0.74 -	0.61 17.6	0.46 37.8	0.28 62.2	0.12 83.8	2.77 -	2.03 26.7	1.37 50.5	0.61 55.5	0.20 92.7
18 mo	1.22 -	1.08 11.5	0.71 41.8	0.44 63.9	0.15 87.7	2.89 -	2.69 6.9	1.78 61.6	0.83 71.3	0.14 95.1
24 mo	1.55 -	1.11 28.4	0.82 47.1	0.46 70.3	0.14 71.6	2.42 -	2.16 10.7	1.78 73.5	0.70 71.1	0.21 91.3

There was significant plasma ChE inhibition (≥ 20%) in males at 24 mo, in females at 1, 6 and 12 mo at 2 ppm and in both sexes at every time interval from and including 6 mo at 6 ppm and above. The degree of inhibition was dose-related.

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RBC ChE: The RBC ChE activity determinations are tabulated below. The results for the 12 and 15 mo determinations in the chronic study rats are not shown. They were not substantially different from the results at 12 mo for the reserve rats. The results of statistical analysis of this data was not given. Biologically significant RBC ChE inhibition (≥ 20% inhibition) was detected in males at 18 mo at 2 ppm. It was also detected in both sexes at every time interval from and including 6 mo at 6 ppm and above. The degree of inhibition was dose-related.

RBC ChE activity determinations at each dietary level

Time	RBC ChE activity (µmol/mL/min) for each treatment group (ppm) and % ChE inhibition									
	Male					Female				
	0	2	6	18	54	0	2	6	18	54
Init.	1.94 -	1.90 -	1.88 -	1.85 -	1.87 -	1.93 -	1.90 -	1.94 -	2.00 -	1.84 -
1 mo	1.71 -	1.57 8.2	- -	- -	- -	1.63 -	1.49 8.5	- -	- -	- -
6 mo	1.59 -	1.49 6.3	1.03 25.2	0.43 72.9	0.34 78.6	1.47 -	1.28 12.9	0.84 42.8	0.47 68.0	0.38 74.1
12 mo	1.77 -	1.48 16.4	1.03 41.8	0.61 65.5	0.38 78.5	1.48 -	1.30 12.2	0.90 39.2	0.51 65.5	0.44 70.3
18 mo	1.86 -	1.48 20.4	1.09 41.2	0.57 69.3	0.35 81.2	1.58 -	1.42 10.1	0.92 41.7	0.53 66.4	0.32 79.7
24 mo	1.51 -	1.32 12.6	1.03 31.8	0.53 64.9	0.37 75.5	1.55 -	1.26 18.7	0.99 36.1	0.50 67.7	0.30 80.6

Organ wt:

The absolute organ wts (g) and relative organ wts (% bw) which were were statistically different from those of controls ($p < 0.05$) are tabulated below. No statistical difference was observed in adrenal, lung or spleen wts. With the exception of absolute brain wt and relative kidney wt in females, absolute and relative organ wts were not statistically different from controls at 2 and 6 ppm. The significant increase in relative brain weights of 54 ppm male and female rats was attributed decreased bw. The decrease in absolute wt of liver and kidney in males at 18 and 54 ppm can also be attributed to decreased body wt.

Absolute and relative organ wt in males at 18 and 54 ppm concentration

Group	Mean absolute (g) and relative wt (% bw)				
	Brain	Heart	Liver	Kidney	Testes
Controls	2.074 0.596	1.292 0.373	16.915 4.794	3.775 1.080	4.918 1.348
18 ppm	2.091 0.633	1.273 0.386	15.374* 4.412*	3.432* 1.010	3.446* 0.962*
54 ppm	2.118* 0.690*	1.174* 0.380	14.369* 4.588	3.216* 1.039	3.057* 0.965*

* statistically significantly different ($p < 0.05$) (cf controls)

At 18 and 54 ppm, 5/49 and 11/50 males respectively showed atrophied testicles compared to 2/50 control males. The decrease in absolute and testicular wt in males at 18 and 54 ppm is likely to be a consequence of atrophy. All other statistically significant differences in

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relative and absolute organ weights were within the normal range of untreated mature rats according to historical data available to the study authors (Hazleton Inc, 1984).

Absolute and relative organ wt in females at 18 and 54 ppm concentration

Group	Mean absolute (g) and relative wt (% bw)				
	Brain#	Heart	Liver	Kidney##	Ovaries
Controls	1.857 0.76	0.963 0.398	10.60 4.263	2.418 0.984	0.240 0.097
18 ppm	1.884* 0.752	0.969 0.386	11.391 4.474*	2.601* 1.028	0.193 0.076
54 ppm	1.909* 0.911*	0.946 0.453*	10.038 4.501	2.345 1.102*	0.116 0.052

* statistically significantly different ($p < 0.05$) (cf controls)

Absolute brain wt was also statistically significantly greater ($p < 0.05$) than controls at 2 and 6 ppm (1.886 and 1.899 g respectively).

Relative kidney wt was also statistically significantly greater ($p < 0.05$) than controls at 2 and 6 ppm (1.071 and 1.066 % bw respectively).

Gross and histopathological pathology: Gross examination showed pathological changes common to controls and treated groups. The significant depression in testicular wt was associated with testicular change characterised by single to multiple, yellow to tan, soft nodules often associated with cysts containing clear fluid. Unaffected testes (unilateral or bilateral) were often small, shrunken and grey. Histologically the testes were often atrophied both in testes affected by interstitial cell tumour and those not affected by that tumour. The incidence of testicular interstitial cell tumour, the most frequently occurring tumour in males, was 42/50, 45/50, 46/50, 41/49 and 43/50 at 0, 2, 6, 18 and 54 ppm respectively. The numbers of rats with interstitial cell tumour and testicular atrophy was not specified but the number of rats without interstitial cell tumour identified as having testicular atrophy was 2/50, 3/50, 6/50, 5/49 and 11/50 at 0, 2, 6, 18, 54 ppm respectively. The incidence of testicular atrophy at 54 ppm may have been treatment-related. Seminal vesicles were small, shrunken with minimal secretary content. Prostrate glands often had multifocal pinpoint white to tan zones which were sites of chronic active inflammation.

The types of non-neoplastic lesions seen were comparable to those reported for ageing F344 rats (Goodman, 1979; NIH/USA, 1981). Apart from testicular atrophy at 54 ppm, evidence of a treatment-related effect was not observed.

Number of benign and malignant tumours

Parameter*	Neoplasms at each concentration (ppm)									
	Male					Female				
	0	2	6	18	54	0	2	6	18	54
1	98	100	100	100	100	84	90	90	92	88
2	60	68	56	52	76	50	44	54	52	54
3	158	157	200	198	141	134	229	193	154	127
4	59	56	64	50	57	43	29	39	47	43

* 1 = % rats with tumours; 2 = % rats with benign tumours only; 3 = total number tumours found; 4 = % of the total number of tumours which were benign

The numbers of benign and malignant neoplasms found are tabulated above. The neoplasms seen were common to the F344 rat (NIH/USA, 1981; Hazleton Inc, 1984). There was no treatment-related increase in total tumours found and no single type of cancer was found

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more frequently in treated rats than among controls. For example, the incidence of the most common tumour found in females, endometrial stromal polyp, was 13/50, 14/50, 18/50, 11/50 and 16/49 at 0, 2, 6, 18 and 54 ppm respectively.

Conclusion

1. On the basis of biologically significant (≥ 20%) plasma, RBC and brain ChE inhibiton, at 2 ppm (or 0.09 or 0.11 mg/kg bw/d for males or females respectively), a NOEL could not be established for this study.

2. Methamidophos was not oncogenic for male or female rats consuming up to 54 ppm (2.85 mg/kg/bw/d for males and 3.49 mg/kg bw d for females) in food over a period of 24 mo.

6.1.3 Dogs

Hayes RH, Hoss HE, Mallicoat DR, Cox LM & Lamb DW (1984c): One-year feeding study of methamidophos (MonitorR) in dogs. Mobay Report No. 87474. Study No. 81-174-01. Toxicology Report No. 497. Lab: Environmental Health Research, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsors: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA & Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 4 January 1982 - 13 January 1983. Report Date: 26 June 1984. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: 40 CFR (Part 160).

Test Substance:	Methamidophos technical. Batch number: 77-297-149. Purity: 71.5%. Source: Mobay Chemical Corporation, Stilwell, Kansas, USA.
Test Species:	Dog, beagle breed, males and females, 7 mo old, unspecified bw obtained from Laboratory Research Enterprises, Kalamazoo, Michigan.
Dose:	0, 2, 8, 32 ppm in diet.

Method

Dogs (6/sex) received methamidophos technical at 0, 2, 8, 32 ppm in diet for 12 mo. The dietary admixture was prepared every 3/4 d with corn oil (1% w/w) as the vehicle for the test substance. A weighed amount of food was offered to each dog daily. Weekly determinations of food consumption were made.

Prior to the initiation of the study, a complete material accountability study was conducted on a composite of five batches of methamidophos prepared for toxicological investigation. At termination of the study components of the composite sample with quantities of 1% or greater were analysed. The percentage of methamidophos technical in the test substance was determined by high-pressure liquid chromatography at 6 mo intervals during the study. The homogeneity of methamidophos technical in the diet was determined by gas liquid chromatography (GLC) on 9 random samples taken from the top, middle or bottom thirds of the mixing bowl of the 2 and 32 ppm batches. The stability of methamidophos in the diet was determined by using ^{14}C-labelled methamidophos mixed in the dog ration. The batch was sampled on the day of preparation and placed in a freezer. On d 7 the batch was sampled again and the remaining food divided in half. One half of the food was stored in the freezer and sampled on d 14 and 21. The other half was stored at room temperature and sampled on d

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10, 14, 17, 21 and 24. GLC was used to assay the actual amount of methamidophos in the ration and liquid scintillation counting assay for total radiocarbon residue from the methamidophos and decomposition products. A duplicate sample was taken monthly from the current weekly batch to analytically determine the concentration of methamidophos.

Observations for toxicological effects were made twice daily. The dogs were weighed weekly and examined at the same time for abnormalities and palpable masses. Three plasma and RBC ChE determinations were made at weekly intervals prior to the initiation of the study. After initiation, plasma and RBC ChE determinations were made 2/mo for 3 mo then every second mo, and at termination. Brain ChE was determined at termination.

Haematology, blood chemistry and urinalysis were performed on all dogs prior to initiation, 1/mo for 3 mo then every second mo, and prior to termination of the study. The dogs were fasted for 16-18 h prior to collection of blood. Blood was collected from the jugular with Vacutainer tubes. Urine was collected from the floor of the cage with a syringe. The determinations made are tabulated below.

Haematology	RBC, WBC, Hct, Hb, WBC-DC, reticulocyte and platelet count, MCH, MCHC, MCV, blood smear
Blood Chemistry	albumin, AP, ALT, AST, bilirubin (total), calcium, sodium, potassium, chloride, cholesterol (total), creatinine (blood), ChE activity (Ellman *et al.*, 1961), glucose (blood), LDH, protein (total), urea nitrogen (blood)
Urinalysis	pH, ketones, glucose, bilirubin, occult blood, protein, urobilinogen, RBC, WBC, casts, crystals, other cells, spermatozoa, bacteria

Prior to the initiation and termination of the study, all dogs were examined with an ophthalmoscope and slit lamp biomicroscope for evidence of changes to eyes. Photographs of the retina were taken.

At termination all dogs were necropsied. A gross examination was made and tissues were preserved in 10% buffered neutral formalin, embedded in paraffin and stained with haematoxylin-eosin. Eyes were fixed in Bouin's solution for 24 h, washed and preserved in 70% alcohol. The left hemisphere of the brain of each dog was taken for ChE activity determinations and the right hemisphere for histopathological studies. A microscopic examination was conducted on all available tissues from all dogs at all dietary levels except those killed for interim determinations. The organs weighed and tissues examined were:-

The organs weighed were: Adrenals, brain, gonads, heart, kidneys, liver, lungs, spleen, thyroid (with parathyroid).

The tissues examined were: blood smear, bone marrow, spleen, thymus, lymph nodes (mesenteric and non-mesenteric), heart, aorta (dorsal), salivary gland (submaxillary), oesophagus, stomach, duodenum, liver, gall bladder, pancreas, ileum, jejunum, cecum, colon, rectum, pituitary, adrenals, thyroid, parathyroids, gross lesions, skin, muscle (skeletal), brain (forebrain, midbrain and hindbrain), spinal cord, eyes, optic nerve, sciatic nerve, epididymes, mammary gland, ovaries, seminal vesicle, prostate gland, testes, uterus, cervix, larynx, trachea with mainstem bronchi, lungs, bone, ribs (costochondral junction with attached muscle), sternum, kidneys, urinary bladder.

Body wt, food consumption, haematology, blood chemistry, urinalysis, relative and absolute organ wt were subjected to an Analysis of Variance followed by Least Significant Difference

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test. All significant differences were reported at $p \leq 0.05$. The frequencies of lesions on gross and micropathology were also analysed in two-way cross tabulation tables.

Results and discussion

Material accountability, stability, homogeneity and concentration: The study was initiated with methamidophos technical at a purity of 71.5%. Changes in purity were compensated for in diet preparation. The % limits of variation in the concentrations of methamidophos at 2, 8 and 32 ppm were 90-113, 89-116 and 82-112 respectively.

Intake of test substance: The achieved doses are tabulated below. The study authors did not indicate which bw parameter or interval of time was used to calculate the dose given as mg/kg bw or mg/kg bw/d respectively.

The achieved doses of methamidophos technical

Sex	**Concentration in food (ppm)**	**Total food consumption (kg)**	**Total dosage of methamidophos**		
			mg	**mg/kg bw**	**mg/kg bw/d**
Male	0	144.1	0	0	0
	2	145.7	293	21.7	0.06
	8	136.9	1095	89.2	0.24
	32	139.4	4462	335.3	0.90
Female	0	114.9	0	0	0
	2	114.2	228	20.6	0.06
	8	117.6	941	81.9	0.22
	32	110.8	3545	327.8	0.88

Clinical signs and mortality: The low-level occurrence of lacrimation, diarrhoea, loose stool and vomiting in both sexes and at all dietary levels was noted. Other incidental signs were also recorded. These findings were not treatment-related. There was no mortality. Observations on palpable masses were not recorded.

Food consumption and bw: There was no treatment-related effect on food consumption or bw in either sex.

Haematology: Statistical analysis of haematology data revealed occasional significant differences which were not regarded as biologically significant. A statistically significant increase in the platelet count of 32 ppm females was not considered treatment-related because the platelet count was elevated prior to initiation of the study.

Cholinesterase: The plasma, RBC and brain ChE determinations are tabulated below. The determinations in the period 6 wk-11 mo for plasma and RBC ChE are not shown. There were no substantial differences in the determinations after 2 wk. The statistical significance of any value was not given. The degree of inhibition was dose-related. The study authors did not regard the inhibition of ChE activity at 2 ppm as toxicologically significant.

ChE activity determinations

	ChE activity (µmol/mL/min or µmol/g/min) for each treatment group (ppm) and % ChE inhibition	
Time	**Male**	**Female**

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	0	2	8	32	0	2	8	32
Plasma ChE								
Init.*	1.59 -	1.64 -	1.62 -	1.59 -	1.49 -	1.51 -	1.64 -	1.55 -
2 wk	1.74 -	1.63 6.3	1.15 33.9	0.60 65.5	1.54 -	1.38 10.4	1.21 21.4	0.62 59.7
1 mo	1.62 -	1.53 5.5	1.20 25.9	0.90 44.4	1.41 -	1.37 2.8	1.23 12.8	0.97 31.2
12 mo	2.00 -	1.60 20.0	1.29 35.5	0.87 56.5	1.66 -	1.46 12.0	1.54 7.2	1.02 38.5
RBC ChE								
Init*	1.20 -	1.42 -	1.24 -	1.49 -	1.27 -	1.36 -	1.49 -	1.40 -
2 wk	1.25 -	1.39 -	0.83 33.6	0.37 70.4	1.26 -	1.39 -	0.98 22.2	0.33 73.8
1 mo	1.39 -	1.28 7.9	0.61 56.1	0.38 72.7	1.44 -	1.21 15.9	0.77 46.5	0.37 74.3
12 mo	1.44 -	1.29 10.4	0.55 61.8	na[#] -	1.57 -	1.39 11.5	0.69 56.0	0.26 83.4
Brain ChE								
12 mo	6.17 -	5.05 18.1	2.79 54.8	1.80 70.8	6.01 -	5.33 11.3	3.28 45.4	2.02 66.4

* The mean of 3 pre-study determinations
# Datum not decipherable

Blood chemistry: Except at 8 ppm at 7 mo, mean ALT values for male dogs were significantly less than those of the control males at all dietary levels and intervals after initiation. These differences were due to increasing levels of ALT in control males (32 U/mL at initiation to 70 U/mL at 11 mo) compared to treated dogs whose ALT values remained relatively constant. Male dogs at all dietary levels had significantly lower AST values than controls at four intervals (1, 3, 9 and 11 mo). At 7 mo the AST values for the 8 and 32 ppm males were significantly higher than controls. Statistically significant differences occurred occasionally in other parameters. The clinical chemistry findings were not attributable to the test substance.

Urinalysis: Statistically significant differences for urinalysis values occurred occasionally. There were no trends or patterns evident in their occurrence. The findings were not attributable to the test substance.

Ophthalmology: There was no ocular discharge, opacity or haemorrhage. There were no differences in findings between pre-treatment and terminal examination and it was concluded that the test substance did not cause detectable changes in the eye.

Organ Weights: Relative and absolute thyroid wt were significantly decreased at 2 ppm in males and relative pituitary and relative lung wt for 8 ppm males were significantly increased. Significant differences in female relative or absolute organ wt between dietary levels did not occur. These differences were not attributable to the test substance.

Necropsy: The distribution of abnormalities between groups did not indicate a treatment-related effect.

Histopathology:

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Microscopic examination of all available tissues showed randomly occurring incidental inflammatory and degenerative changes. The occurrences were indistinguishable from those seen in untreated dogs of comparable age. The severity of the changes was minimal to mild within and between groups. There were no findings that were attributable to the test substance.

Conclusion

On the basis of biologically significant (≥ 20%) plasma, RBC and brain ChE inhibiton at 8 ppm (0.22 mg/kg bw/d), a NOEL of 2 ppm (0.06 mg/kg bw/d) was established.

7. REPRODUCTION STUDIES

7.1 Rats

Hixson EJ, Hoss HE, Mallicoat DR & Lamb DW (1984b): Effect of methamidophos (Monitor^R) on reproduction in rats. Mobay Report No. 88686. Study No. 82-671-01. Toxicology Report No. 553. Lab: Environmental Health Research, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsors: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA & Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 19 April 1982 - 12 March 1984. Report Date: 8 November 1984. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: 40 CFR (Part 160).

Test substance:	Methamidophos technical. Batch Number: 77-297-149. Purity: 70.5% Source: Mobay Chemical Corporation, Kansas City, Missouri, USA.
Test Species:	Rats: CD, male and female, 5 wk old, of an unspecified bw obtained from Charles River Laboratories, Kingston, New York.
Dose:	0, 3, 10, 33 ppm in diet.

Method

Rats were randomly assigned to numbered cages and held for 14 d prior to the beginning of the study. They were fed Purina Rodent Chow 5001-4 and water *ad libitum* and maintained under standard environmental conditions. During periods before mating rats were housed 2 of the same sex and dietary group per stainless steel cage. Clean steel cages were provided every 3rd wk. After mating, bred females were individually housed in plastic cages.

When preparing the dietary admixture, the test substance was dissolved in acetone and the acetone solution was mixed with corn oil and dripped over the food which was mixed with a Hobart mixer. Acetone was used to rinse the glassware. It evaporated from surfaces and food during the food mixing process. Each batch of food and corn oil used was free of contaminants. The test substance and prepared food were stored in a freezer at -10^{o}F prior to use. Samples of each batch of food were stored frozen for analysis. Samples were analysed monthly for verification of test substance concentration by HPLC. Rats were randomly allocated to groups of 26 rats/sex/dietary level to constitute the F_0 generation. The control group received diet prepared with corn oil and acetone.

After at least 100 days of dosing, the F_0 rats were mated to produce the F_1 generation. The F_1 pups were weaned at 21 d post-partum, and 26 pups/sex/dietary level were randomly selected

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as parents of the F_2 generation. These rats were maintained on treated feed for 120 d and then mated to produce the F_{2a} generation. The F_{2a} pups were weaned at 21 d postpartum and killed. The dams were allowed to rest for approximately 1 mo. The F_1 rats were mated again to produce the F_{2b} generation. The F_{2b} pups were weaned at 21 d postpartum and all rats were killed.

Breeding was accomplished by co-housing 2 females with 1 male. All females at each dietary level were placed for mating in an attempt to attain a minimum of 20 bred females per group. Because mating was done on a 2 to 1 (females to male) basis, only half of the males in each group (the first 13 animal numbers) were used for mating. The remaining 13 males per group were continued on treated feed for possible use as replacement breeders. No mating pair was co-housed for breeding for more than three weeks. Males were replaced if they failed to breed either female in three weeks. Females being bred were checked each morning. If a vaginal plug or sperm-positive smear was observed, that day was considered gestation d 0 and the female was placed in a plastic cage. If a plug was not found or the smear was not sperm positive, the female was maintained for an additional three weeks after the breeding period in case she had been bred.

Indices used to assess dam reproductive performance were number mated, number spermatazoa-positive, number delivering, number delivering as % mated, number delivering as % spermatozoa-positive

Toxicologic and pharmacologic signs were recorded daily. Body weights and feed consumption were measured weekly prior to mating. Bred females were weighed on GD 0, 6, 13, 20 and 21, and weekly feed consumption was measured on GD 7, 14 and 21. Beginning on GD 21, bred females were observed closely at least once daily for signs of impending delivery.

The day delivery was complete was designated LD 0. Occasionally dams whose litters were thought to be complete delivered additional pups between days 0 and 1. Food consumption was measured on LD 4, 7, 14 and 21. Total litter wt and dam bw were recorded on LD 0, 1, 4, 7, 14 and 21. The number of live and dead pups, sex and total weight of live pups was recorded. Other litter indices used were viability index for a particular d [(number live pups on that d/number pups born alive) x 100] and lactation index [(number live pups d 21/number live pups d 4) x 100]. Physical and behavioural abnormalities observed in dams and pups were recorded.

Rats were killed by carbon dioxide asphyxiation. Abnormal tissues were sampled, preserved in 10% neutral buffered formalin and processed using routine histopathologic procedures for microscopic examination.

After the F_1 pups were weaned, the F_0 parents were killed for complete gross necropsy. The testes and secondary sex organs from the F_0 males were preserved and processed for microscopic examination. The F_1 pups not selected to continue on study were killed for complete gross necropsy. Complete tissues were collected and preserved from 10 randomly selected pups/sex/dietary level.

When the F_{2a} pups were weaned, 10 randomly selected pups/sex/dietary level were killed for gross necropsy and complete tissue collection. The remaining pups were sacrificed for gross necropsy. When the F_{2b} pups were weaned, all pups were sacrificed for gross necropsy. The F_1 parents were sacrificed for gross necropsy.

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The gonads were weighed and together with secondary sex organs were preserved and processed for microscopic examination. The other organs and tissues examined but not weighed were as listed in Appendix IV with the exception of Zymbal's gland. Clinical pathology determinations were not performed.

Body weight and feed consumption data were summarized using means and standard deviations. Group mean body weight and feed consumption data were tested for differences from control using Duncan's Multiple Range Test. Duration of gestation, litter counts and reproductive performance indices were tested for differences using the Kruskal-Wallis test. When the Kruskal-Wallis test identified a significant difference ($\alpha = 0.05$) overall, pair-wise comparisons were performed using Dunn's Procedure. Mean litter and mean pup weights were tested for significant differences ($\alpha = 0.05$) using Duncan's Multiple Range Test.

Results and discussion

Intake of test substance: The determined ranges of concentration of methamidophos at each treatment level are tabulated below.

The mean, standard deviation and range of the concentration of methamidophos at each treatment level

Treatment level (ppm)	Mean ± SD (ppm)	Range (ppm)
3	3.0 ± 0.4	2.4 to 3.7
10	9.1 ± 1.3	7.4 to 12.0
33	29.9 ± 4.0	26.3 to 37.2

Achieved dose: The intial to terminal achieved dose range for each dietary level was calculated as being equivalent to 0, 0.3 to 0.15, 1.0 to 0.5 and 3.3 to 1.65 mg/kg bw/d (Lehman, 1954).

Clinical signs and mortality: No mortalities were reported. The most common clinical sign observed was alopecia. The alopecia was of variable duration, usually not observed until wk 8 and generally persiting until term. There was no clear relationship between its occurrence and generation, sex or dietary level. Lacrimation, laceration and sundry minor maladies were also observed.

Food consumption: There were no treatment-related differences between the groups at any generation in food consumption except that at 33 ppm the mean combined food consumption of the F_1 (F_{2b}) lactating dams and their litters was statistically significantly less ($p \leq 0.05$) at d 7, 14 and 21 than that of controls (96, 291 and 351 g respectively cf controls 121, 370 and 479 g).

F_0 pre-mating bw: At 3, 10 and 33 ppm, mean bw in females was statistically significantly higher ($p \leq 0.05$) each wk from wk 8 to 15 than that of controls. At 33 ppm, mean bw in males was statistically significantly less ($p \leq 0.05$) each wk from wk 6 to 15 than that of controls. At wk 6 in males mean bw was 358 g (cf controls 373 g). At wk 15 it was 470 g (cf controls 504 g). The depressed bw gain may have been treatment-related.

F_0 pregnant dams bw: No statistically significant differences in mean bw were observed between groups throughout gestation. At 33 ppm, mean bw gain over gestation was

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statistically significantly less ($p \leq 0.05$) than that of controls (98 g cf control 123 g). The depressed bw gain may have been treatment-related.

F_0 lactating dams bw: No statistically significant differences in mean bw were observed between groups throughout lactation. At 33 ppm, mean bw gain over lactation was 14 g (cf controls 24 g). These means were not statistically different but the depressed bw gain may have been treatment-related.

F_1 litter wt and pup bw: At 33 ppm, litter wt and pup bw were statistically significantly less ($p \leq 0.05$) at LD 7, 14 and 21 post-delivery than that of controls. At LD 7, 14 and 21 litter wt was 108.5, 199.6 and 297.9 g respectively (cf controls 151.7, 282.2 and 423.2 g). Pup bw was 11.2, 21.2 and 32.4 g respectively (cf controls 13.6, 25.3 and 38.2 g). The gain in mean pup bw over the lactation was 26 g (cf controls 32 g). The depressed bw gain may have been treatment-related.

F_1 litter size and viability: There were no statistically significant differences in litter size or reproductive indices between the groups.

F_0 reproductive performance: This is tabulated below. Decreased reproductive performance occurred in all treated groups compared to control rats. There were no dose-related responses in reproductive performance and it was not likely that the decreased litter production was a result of treatment.

Reproductive performance of F_0 dams

Reproductive Parameter	Dietary level (ppm)			
	0	**3**	**10**	**33**
Number mated	26	26	26	26
Number spermatazoa-positive	25	23	25	24
Number delivering	25	15	19	15
Number delivering as % mated	96.2	57.7	73.1	57.7
Number delivering as % spermatozoa-positive	100	65.2	76.0	62.5

F_1 premating bw: At 10 ppm in females mean bw was statistically significantly higher ($p \leq 0.05$) each wk from wk 5 to 16 than that of controls. At 33 ppm mean bw in males was statistically significantly less ($p \leq 0.05$) each wk from wk 1 to 16 than that of controls. In females the same bw pattern occurred from wk 1 to 15. At wk 1 in males mean bw was 134 g (cf controls 159 g). At wk 16 it was 476 g (cf controls 530 g). At wk 1 in females mean bw was 107 g (cf controls 129 g). At wk 15 it was 255 g (cf controls 271 g). The depressed bw gain in both sexes may have been treatment-related.

F_1 (F_{2a}) pregnant dams bw: At 10 ppm mean bw was statistically significantly higher ($p \leq 0.05$) throughout gestation than that of controls. At 33 ppm, mean bw was statistically significantly less ($p \leq 0.05$) throughout gestation than that of controls. At GD 6 mean bw was 267 g (cf controls 292 g). At GD 21 it was 343 g (cf controls 379 g). At 33 ppm, mean bw gain over gestation was 84 g (cf controls 101 g). These means were not statistically different but the depressed bw gain may have been treatment-related.

F_1 (F_{2a}) lactating dams bw: At 10 ppm mean bw was statistically significantly higher ($p \leq 0.05$) throughout lactation than that of controls. At 33 ppm, mean bw was statistically significantly less ($p \leq 0.05$) throughout lactation than that of controls. At 33 ppm, mean bw

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gain over lactation was 8 g (cf controls 22 g). These means were not statistically different but the depressed bw gain may have been treatment-related.

F_{2a} litter wt and pup bw: At 33 ppm, mean litter wt and pup mean bw were statistically significantly less ($p \leq 0.05$) at LD 1, 4, 7, 14 and 21 than that of controls. The mean bw gain in pups over the lactation was 26 g (cf controls 33 g). This effect may have been treatment-related.

F_{2a} litter size and viability: At 33 ppm, litter size and viability were statistically significantly less at LD 14 than that of controls (6.7, 76.4% cf controls 9.1, 98.1%). Otherwise there were no treatment-related effects on litter size, viability index or lactation index. This effect is not likely to have been treatment-related

F_1 (F_{2a}) reproductive performance: This is tabulated below. There were no toxicologically significant differences between the groups in the production of F_{2a} litters.

Reproductive performance of F_1 (F_{2a}) dams

Reproductive Parameter	Dietary level (ppm)			
	0	**3**	**10**	**33**
Number mated	26	26	25	26
Number spermatazoa-positive	22	24	23	26
Number delivering	16	19	13	14
Number delivering as % mated	61.5	73.1	52.0	53.8
Number delivering as % spermatozoa-positive	72.7	79.2	56.5	53.8

F_1 (F_{2b}) pregnant dams bw: At 10 ppm mean bw at GD 0, 6 and 13 was statistically significantly higher ($p \leq 0.05$) than that of controls. No statistically significant differences in bw gain were observed between groups throughout gestation. At 33 ppm, mean bw gain over gestation was 93 g (cf controls 115 g). These means were not statistically different but the depressed bw gain may have been treatment-related.

F_1 (F_{2b}) lactating dams bw: At 10 ppm mean bw at LD 1, 4 and 7 was statistically significantly higher ($p \leq 0.05$) than that of controls. At 33 ppm mean bw at LD 14 was statistically significantly less ($p \leq 0.05$) than that of controls. At 33 ppm, mean bw loss over gestation was 26 g (cf controls 6 g). These means were not statistically different but the greater loss in mean bw was associated with statistically significant depression in food consumption. These effects may have been treatment-related.

F_{2b} litter wt and pup bw: At 33 ppm, mean litter wt was statistically significantly less ($p \leq 0.05$) at LD 0 (birth wt), 1, 7, 14 and 21 than that of controls; pup mean bw was statistically significantly less ($p \leq 0.05$) at LD 7 than that of controls. The mean bw gain in pups over the lactation was 26 g (cf controls 32 g). These effects may have been treatment-related.

F_{2b} litter size and viability: At 33 ppm, litter size was statistically significantly less ($p \leq 0.05$) at LD 0 (at birth) and at LD 14. Mean litter size on LD 0 was 7.2 cf controls 11.2 and on d 14, 7.1 cf controls 10.9. For LD 0, 1, 4, 7, 14 and 21 the litter count was 7.2, 8.8, 8.3, 7.9, 7.1 and 7.0 respectively (cf controls 11.2, 11.3, 11.1, 11.1, 10.9 and 10.9). The viability at LD 4, 7, 14 and 21 was 84.6, 81.4, 74.3 and 73.1% respectively (cf controls 99.4, 98.9, 97.7 and 97.7%). The lactation index was 75.4% cf controls 98.3%. There were 8.9 mean live births (95.7%) cf controls 11.3 (98.3%). The mean number born dead was 0.5 (4.3%) cf controls 0.1 (1.7%).

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F_1 (F_{2b}) reproductive performance: This is tabulated below. During production of F_{2b} litters, the % spermatozoa-positive dams delivering litters at 33 ppm was reduced.

Reproductive performance of F_1 (F_{2b}) dams

Reproductive Parameter	Dietary level (ppm)			
	0	**3**	**10**	**33**
Number mated	26	26	26	26
Number spermatazoa-positive	20	22	10	21
Number delivering	16	13	6	8
Number delivering as % mated	61.5	50.0	23.1	30.8
Number delivering as % spermatozoa-positive	80.0	59.1	60.0	38.1

Gonad wt: Mean absolute and relative gonad wt at each dietary level were provided for F_0 adult males, F_1 pup males and females, F_1 adult males and females and F_{2a} male and female pups. Incidental statistically significantly lower ($p \leq 0.05$) absolute or relative testes wt in F_0 males (cf controls) were observed. The differences were not dose-related. The mean relative wt of testes was statistically significantly higher ($p \leq 0.05$) than controls in F_1 males at 33 ppm. This was attributed to reduced bw in this group. In F_1 adult females the absolute wt of ovaries in the 33 ppm group was statistically significantly lower ($p \leq 0.05$) than that of controls but the relative wt was not. No differences between groups were found in wt of gonads in pups.

Gross necropsy all generations: Occasional gross changes seen in pups were not indicative of a treatment-related effect and were considered incidental. Gross lesions found in adults were typical of adult rats of this age and strain and were not considered treatment-related.

Histopathological examination: A variety of microscopic lesions were observed in control and test groups of each generation. The occurrence of these lesions was considered spontaneous and unrelated to treatment.

Conclusion

At 33 ppm, the parenteral generations showed depression in premating bw and depression in gestational and lactational bw gain. The production of F_{2a} and F_{2b} litters was characterised by inhibited lactational bw gain in pups. The production of F_{2b} litters was further characterised by decreased proportion of spermatozoa-positive dams that delivered live pups, reduced litter size at birth, and reduced viability and lactation indices. The NOEL for parental toxicity and reproductive toxicity was 10 ppm (0.5 mg/kg bw/d), based on reduced bodyweight gain in the parents and pups, and reduced viability in the offspring at higher doses (Hixson *et al*, 1984b).

Eigenberg DA, Freshwater KJ & Lake SG (1998): A two-generation dietary reproduction study in rats using technical methamidophos. Study No. 95-672-GJ. Report No.108040. Lab: Agricultural Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Kansas City, Missouri, USA. Study Duration: 26 February 1996 - 1 May 1997. Report Date: 5 January 1998. Unpublished. (QA: Yes; GLP: USEPA FIFRA Guideline 83-4, USEPA TSCA 40 CFR 798.4700, Japan MAFF 59 NohSan 4200; Test Guidelines: OECD Guideline 416).

[The content of the following 2 separately reported supplements, the purpose of which was to introduce additional statistical analyses (ANOVA dose group trends analyses) of pup wts

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(Astroff & Eigenberg, 1998) and to provide additional analytical chemistry information (Moore, 1999), have been considered in this evaluation]

Astroff BA & Eigenberg DA (1998): A two-generation dietary reproduction study in rats using technical methamidophos. Supplemental submission. Study No. 95-672-GJ2. Report No. 108040-1. Lab: Toxicology, Agricultural Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 9 October 1998. Unpublished. (QA: Yes; GLP: USEPA FIFRA Guideline 83-4, USEPA TSCA 40 CFR 798.4700, Japan MAFF 59 NohSan 4200; Test Guidelines: OECD Guideline 416).

Moore KD (1999): A two-generation dietary reproduction study in rats using technical methamidophos (Monitor^{R}). Supplemental submission. Study No. 95-672-GJ. Report No. 108040-2. Lab: Toxicology, Agricultural Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Bayer Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 23 February 1999. Unpublished. (QA: Yes; GLP: USEPA FIFRA Guideline 83-4, USEPA TSCA 40 CFR 798.4700, Japan MAFF 59 NohSan 4200; Test Guidelines: OECD Guideline 416).

Test Substance:	Methamidophos technical. Batch Number: 0067009. Purity: 73%. Source: Bayer Corporation, Stilwell, Kansas, USA.
Test Species:	Rat: Sprague-Dawley males and females, 7 wk old of an unspecified bw obtained from SASCO Inc, Saint Louis, Missouri, USA.
Dose:	0, 1, 10, 30 ppm in diet

Method

The diet containing methamidophos was prepared by dissolving the required amount of methamidophos in acetone, mixing it in corn oil and then food. Diet batches were prepared weekly and stored in a freezer. The food, the corn oil vehicle and drinking water were analysed for contaminants. The purity of the test substance was determined prior to, during and after the study period by NMR and MS. The concentration, homogeneity and stability of methamidophos in food was systematically assessed by GC analysis over the duration of the study.

The doses for this study were selected based on the results of a subchronic ChE study in rats (Christenson *et al.*, 1991), a 2 yr chronic/oncogenicity study in rats (Hayes *et al.*, 1984b), a subchronic neurotoxicity study in rats (Sheets *et al.*, 1994b), and a previous 2-generation reproductive study in rats (Hixson *et al.*, 1984b). At initiation 240 rats were divided into 4 dose groups of 30 rats/sex/group. For the production of the 2nd generation, a total of 120 male and 120 female F_{1b} pups were randomly selected using 1 or more pups/sex/litter. The selected pups were divided into 30 rats/sex/group and genealogy was checked to prevent the mating of litter mates.

All F_0 adults were fed the prepared diets from the 1st d of premating until termination. All F_1 adults (F_{1b} pups) were fed the treated feed from weaning until termination. After receiving methamidophos for 10 wk the F_0 adults were bred to produce the F_{1a} and F_{1b} litters.

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The F_{1b} pups selected to be parents for the F_{2a} and F_{2b} generations were placed on treated food following weaning. One wk after the weaning of the last litter, these rats received treated food for an additional 10 wk prior to mating.

Breeding was accomplished by co-housing 1 female with the same male for up to 21 consecutive d. Approximately 1/4 of the rats from each dose group began cohabitation on each of the first 4 d of the mating phase. Co-housing for the 2nd breeding (F_{1b} and F_{2b}) began approximately 2 wk following the termination of the last litter in the F_{1a} and F_{2a} litters respectively. The day of positive identification of sperm or observation of an internal vaginal plug was designated GD 0.

Adult males were terminated after completion of the mating phase for the second breeding. Adult females were terminated after each dam's pups were weaned or died, when GD 24 was reached, or 24 d after the last d of co-housing in the second breeding of each generation.

During the 10 wk period prior to the F_{1a} and F_{2a} matings, individual adult bw and food consumption were measured weekly. Food consumption was measured 1/wk during gestation, 2/wk during wk 1 of lactation and 1/wk during wk 2 and 3 of lactation. Body wt was measured on GD 0, 6, 13 and 20 and on LD 0, 4, 7, 14 and 21. Body wt and food consumption was measured 1/wk for each male over the study except during co-housing periods. Individual pup bws were determined at LD 0, 4 (pre and post-cull), 7, 14 and 21.

[*Note*: In the results section of this study, F_0/F_{1a} female and male premating mean weekly food consumption and mean weekly bws were given for 10 and 20 wk periods respectively. F_0/F_{1b} male premating bws were given for a further 4 wk period. F_1/F_{2a} female and male premating food consumption and bws are given for 10 and 21 wk periods respectively. F_1/F_{2b} male premating bw gains were given for 4 wk. There were no corresponding food consumption data for the 4 wk periods].

The number of live and still born pups was recorded for each litter. Litter counts and clinical observations were performed daily from LD 0-21. Individual pup wts were determined as soon as possible after delivery (LD 0) and on LD 4, 7, 14 and 21. Each litter was randomly culled on LD 4 to yield 8 pups/litter (4/sex if possible). Culled pups were killed and subjected to a gross necropsy examination. For F_{1b} pups kept beyond LD 21, to be F_1 parents, daily observations were made for moribundity, mortality and clinical signs.

Plasma, RBC and brain ChE were determined for 10 F_0 and F_1 adults/sex/dose group. Plasma and RBC ChE were measured during wk 8 of the F_0 and F_1 pre-mating periods and just prior to termination. Blood was collected via the orbital sinus. Plasma, RBC and brain ChE were determined for 1 male and 1 female pup from 10 litters/dose group at the time of culling (LD 4) and at weaning (LD 21).

At termination, adult rats and weanlings were killed by CO_2 asphyxiation. Pups culled on LD 4 and pups born to dams which died or were terminated while delivering were killed by intracranial injection of Fatal-Plus™ (Vortech Pharmaceuticals, Dearborn, Michigan, USA). All adults and pups were necropsied. Pups found dead on LD 0 were subjected to lung water-flotation testing to establish stillbirth. In females the uterus was examined for the presence of implantation sites without counting the sites. Terminal bw and gonad wts were measured for all F_0 and F_1 adults. Relative organ wts were calculated for all organs weighed. The tissues collected from the F_0 and F_1 adults and fixed in buffered 10% formalin were coagulating

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gland, cervix, epididymides (caput, corpus and cauda), pituitary gland, prostate gland, seminal vesicles, uterus, vagina and gross ledions. The ovaries and testes were collected from F_0 and F_1 adults and fixed in Bouin's fluid. All tissues were examined histologically except skulls containing maloccluded teeth without any other accompanying morphological lesions. Tissues were processed routinely and stained with haematoxylin and eosin.

The cumulative % of inseminated females at each day of mating and the median number of d to insemination were determined. The length and number of oestrus cycles were examined for the F_0/F_{1a} and F_1/F_{2a} matings. The following indices were calculated for all matings: female mating index, female fertility index, gestation index, live birth index, viability index and lactation index. These indices were as defined in Appendix IV of this review.

Statistical significance was determined at $p \leq 0.05$ for all tests except Bartlett's test where $p \leq 0.001$ was used. All tests were two-tailed, except for adult pathology evaluations. Body wt and food consumption data were anlayzed by analysis of variance (ANOVA) and if significant differences were shown by ANOVA, Dunnett's test was used to identify significant differences from the control group. The number of oestrus cycles and oestrus cycle length were analysed by the Kruskal-Wallis test. If significant differences were shown, the Mann-Whitney U-test was used to identify statistical significance between groups. Insemination length, gestation length, litter size, viability index, live birth index and % of male pups were anlaysed by the Kruskal-Wallis test. If significant differences were shown by Kruskal-Wallis, Dunn's test was used to identify significant differences from the control group. Clinical signs, number of dams with cannibalised pups, mating index, fertility index and gestation index were analysed by the Chi-square test. If significant differences were shown by Chi-square, Fisher's exact test was used to identify significant differences from the control group. A Bonferroni adjustment of the p value (adjusted p = p/number of comparisons) was used with Fisher's exact test for clinical signs, mating index, fertility index and gestation index. Cholinesterase data, term bws and organ wts were evaluated by Bartlett's test for homogeneity. If the data were homogeneous, an ANOVA was performed followed by Dunnet's t-test on parameters showing a significant effect by ANOVA. If the data were non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical differences between groups.

Pup wt trend analysis was conducted, by breeding/d, with ANOVA (Astroff & Eigenberg, 1998). The primary trend analysis was conducted with a linear contrast across all 4 dose groups (CLMH trend test - the 4 groups were designated CLMH for control, low (1.0 ppm), mid (10 ppm) and high (30 ppm)). If $p > 0.05$, the dose-response trend was deemed not statistically significant and no further analyses were conducted. If $p \leq 0.05$, the dose-response trend was deemed statistically significant and secondary trend analyses were conducted. A contrast was constructed from the ANOVA to test the linear trend across the CLM dose groups. If the CLM trend test was significant, a contrast was constructed from the ANOVA to compare the CL groups. If the CLM trend test was not significant, no further analyses were conducted. (Note: The linear contrasts for the secondary CLM group and CL group trend analyses were individual group comparisons of the M and L dose groups with the control respectively). In addition to the above dose-response trend analyses, analyses were conducted with an ANCOVA model (covariate = litter size). If the overall ANCOVA indicated a significant group effect ($p \leq 0.05$) then individual group comparisons with the control group were conducted with Dunnett's test.

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Twice daily observations of adults and daily observations of F_1 pups kept beyond LD 21, were conducted for moribundity, mortality and clinical signs.

Results and discussion

Test substance: The chemical identity of the methamidophos batch used was confirmed by NMR and MS. Its purity was established at 73% and its stability in the freezer during the conduct of the study was verified. It was also confirmed as stable at room temperature over a 4 wk period. The mean recovery of methamidophos from food was 90.1% with a CV of 3%. Methamidophos was homogeneously distributed in food and was stable in food at freezer temperature for 28 d and at room temperature for at least 14 d. The methamidophos mean concentration for the 1 ppm test level was 1.00 ppm (100%, CV = 12%). The 10 ppm level mean was 9.66 ppm (96.6%, CV = 12%) and the 30 ppm level mean was 26.1 ppm (87.1%, CV = 10%).

Test substance intake: The intake of methamidophos in mg/kg bw/d is tabulated below.

Intake of methamidophos

Concentration in food (ppm)	**Intake of methamidophos in mg/kg bw/d**			
	Females			
	Premating	**Gestation**	**Lactation**	**Males**
1	0.1	0.1	0.2	0.1
10	0.9	0.7	1.5	0.9
30	2.4	1.9	3.9	2.5

Clinical signs: The clinical signs observed in adults at each dose level were reported. There were no treatment-related clinical signs in adults.

Mortality: The number and cause of mortalites in F_0 and F_1 adults at each dose level were reported. There were no treatment-related mortalities.

Adult food consumption, adult and viable pup bw:

***1. F_0 adults/F_{1a} generation pups*:**

F_0/F_{1a} premating mean food consumption: There were no treatment-related effects on food consumption in females at any dietary concentration nor in males at 1.0 and 10 ppm. At 30 ppm, male food consumption was significantly less ($p \leq 0.01$) than that of the controls at wk 1 (99 g/kg/d cf controls 107) and significantly higher ($p \leq 0.05$ or $p \leq 0.01$) than that of the controls from wk 3-20 (wk 3: 83 g/kg/d cf controls 78; wk 9: 64 g/kg/d cf controls 58; wk 17: 57 g/kg/d cf controls 52; wk 20: 56 g/kg/d cf controls 53). The increase in male food consumption at 30 ppm from wk 3 was considered treatment-related.

F_0/F_{1a} premating mean bw: There were no treatment related effects in females at any dietary concentration nor in males at 1.0 or 10 ppm. At 30 ppm, male bws were significantly less ($p \leq 0.05$ or $p \leq 0.01$) at wk 1-6, wk 9 and wk 17-20 (wk 1: 248 g cf controls 260; wk 9: 367 g cf controls 392; wk 17: 412 g cf controls 438; wk 20: 419 g cf controls 451). The reduction in mean male bw at 30 ppm was considered treatment-related.

In females, the respective mean bw gains in controls and at 1.0, 10, 30 ppm over 10 wk were 85, 83, 75 and 83 g. In males over 20 wk they were 236, 230, 231 and 201 g respectively

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(mean bw gains were not analysed statistically). In males at 30 ppm a biologically significant reduction in food conversion efficiency occurred.

F_0/F_{1a} mean food consumption and bw during gestation: There were no significant differences between treatment groups and controls.

F_0/F_{1a} mean food consumption during lactation: At 30 ppm, maternal consumption during LD 14-21 was significantly lower ($p \leq 0.01$) than that of controls (64.0 g/rat/d cf controls 72 respectively). Otherwise there were no differences between the groups. The deficit in food consumption at 30 ppm was considered treatment-related.

F_0/F_{1a} mean maternal bw during lactation: At 1 ppm there were no significant differences between the groups. At 10 ppm, bws were significantly less ($p \leq 0.05$) than that of controls at LD 0, 7 and 21 (LD 0: 272 g cf controls 288; LD 21: 299 g cf controls 321). At 30 ppm, bws were significantly less ($p \leq 0.05$) than that of controls at LD 7 (296 g cf 313). The study authors regarded the mean bw reductions at 10 and 30 ppm as treatment-related but mean bw gain over the entire lactation period was not depressed. Compared with the mean lactation bw gain in controls of 32.5 g, the mean bw gains at 1.0, 10 and 30 ppm were 26.3, 26.5 and 37.3 g respectively (statistical significance was not reported).

F_0/F_{1a} mean viable pup bw: The mean viable pup bws at various times during lactation are tabulated below. The study authors considered the decreases in pup bw at 10 and 30 ppm to be treatment-related. Over lactation the mean combined sex pup bw gain in controls was 46.0 g. The mean bw gains at 1.0, 10, 30 ppm were 42.2*, 40.7** and 32.9** g respectively (*/** significantly less than controls; *$p \leq 0.05$; ** $p \leq 0.01$). On this basis the decrease in mean pup bw at 1 ppm may also be treatment-related but statistical differences were not observed at 1.0 ppm with any of the other 3 litters. On this basis the significant differences at 1 ppm were not considered biologically significant.

F_0/F_{1a} mean viable pup bw for each dietary concentration consumed by dams during lactation

LD	F_0/F_{1a} mean viable pup bw (g) at each dose (ppm) for each sex : dose trend analysis												
	Control (C)			1.0 ppm (L)			10 ppm (M)			30 ppm (H)			T #
	M	F	S	M	F	S	M	F	S	M	F	S	
LD 0	7	6.7	6.8	7	6.6	6.8	7	6.6	6.8	6.8	6.3	6.6	U
LD 4[a]	10.8	10.3	10.5	9.8*	9.6	9.7*	10.3	10.0	10.2	8.5+	8.1+	8.3+	V
LD 4[b]	10.8	10.3	10.5	9.9*	9.6	9.7	10.3	10.0	10.2	8.5+	8.0+	8.3+	W
LD 7	16.7	16.0	16.3	15.4	15.2	15.2	16.0	15.7	15.7	12.3+	11.9+	12.1+	X
LD 14	31.6	30.6	31.1	31.1	29.9	30.5	30.7	30.1	30.4	25.2+	24.2+	24.8+	Y
LD 21	54.0	51.7	52.8	50.1*	47.8*	48.9*	48.2+	46.7+	47.4+	40.1+	38.9+	39.5+	Z

a = pre-cull, b = post-cull, M = male, F = female, S = combined sex
* or + ANOVA: statistically significantly different from controls, * ($p \leq 0.05$), + ($p \leq 0.01$)
# T = trend; statistically significant ($p \leq 0.05$) ANOVA trend analysis for CLMH, CLM or CL
U: CLMH (decreasing trend) in females; V, W, X & Y: CLMH (decreasing trend) in males, females and combined sex
Z: CLMH, CLM and CL (decreasing trend) in males, females and combined sex

2. F_0 adults/F_{1b} generation pups:

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F_0/F_{1b} male premating mean bw: At 30 ppm, bws were significantly lower ($p \leq 0.01$) than that of controls at wk 0, 1, 2 and 4 (wk 0: 423 g cf controls 454; wk 2: 431 g cf controls 462; wk 4: 422 g cf controls 463. The reduction in bw was considered treatment-related.

F_0/F_{1b} mean food consumption during gestation: At 30 ppm, maternal food consumption during GD 13-20 was significantly higher ($p \leq 0.05$) than that of controls (75 g/kg/d cf controls 70). Otherwise there were no differences between the groups. This difference was not considered treatment-related because there was no effect on food consumption during gestation at any of the other matings.

F_0/F_{1b} mean bw during gestation: There were no significant differences between treatment groups and controls.

F_0/F_{1b} mean food consumption during lactation: At 30 ppm, maternal food consumption during LD 14-21 was significantly lower ($p \leq 0.01$) than that of controls (60 g/rat/d cf controls 69). Otherwise there were no differences between the groups. The reduction in food consumption at 30 ppm was considered treatment-related.

F_0/F_{1b} mean maternal bw during lactation: At 1 ppm there were no significant differences between the groups. At 10 ppm, bws were significantly less ($p \leq 0.05$) than that of controls at LD 4, 14 and 21 (LD 4: 320 g cf controls 338; LD 21: 326 g cf controls 346). At 30 ppm, bws were significantly less ($p \leq 0.05$) than that of controls at LD 4 and 14 (LD 4: 322 g cf controls 338; LD 14: 341 g cf controls 363). The study authors regarded the mean bw deficits at 10 and 30 ppm as treatment-related but mean bw gain over the entire lactation period was not depressed. Compared with the mean lactation gain in controls of 26.4 g, the mean bw gains at 1.0, 10 and 30 ppm were 22.6, 16.2 and 26.1 g respectively (statistical significance was not reported).

F_0/F_{1b} mean viable pup bw: The mean viable pup bws at various times during lactation are tabulated below. There were no significant differences between treatment groups and controls and no dose-response trends at LD 0. The study authors considered that the decreases in mean bw at 10 ppm and 30 ppm were treatment-related. Over lactation the mean pup bw gain in controls was 46.0 g. The mean bw gains at 1.0, 10, 30 ppm were 45.5, 42.6* and 33.7** g respectively (*/** significantly less than controls; *$p \leq 0.05$; ** $p \leq 0.01$).

F_o/F_{1b} mean viable pup bw for each dietary concentration consumed by dams during lactation

LD	**F_o/F_{1b} mean viable pup bw (g) at each dose (ppm) for each sex: dose trend analysis**												
	Control (C)			**1.0 ppm (L)**			**10 ppm (M)**			**30 ppm (H)**			**T #**
	M	**F**	**S**	**M**	**F**	**S**	**M**	**F**	**S**	**M**	**F**	**S**	
LD 4[a]	11.0	10.5	10.7	11.3	10.5	10.9	10.6	10.1	10.4	8.9	8.6	8.7	V
LD 4[b]	11.0	10.4	10.7	11.2	10.5	10.8	10.6	10.0	10.3	8.9$^+$	8.5$^+$	8.6$^+$	W
LD 7	17.1	16.4	16.7	17.3	16.4	16.8	16.5	15.6	16.1	13.0$^+$	12.6$^+$	12.7$^+$	X
LD 14	32.9	31.6	32.3	32.6	31.5	32.0	31.4	30.4	31.0	25.4$^+$	25.3$^+$	24.9$^+$	Y
LD 21	54.0	51.5	52.9	53.8	51.2	52.4	50.3*	48.4	49.4*	41.0$^+$	39.8$^+$	40.4$^+$	Z

a = pre-cull, b = post-cull, M = male, F = female, S = combined sex

* or + ANOVA: statistically significantly different from controls, * ($p \leq 0.05$), + ($p \leq 0.01$)

T = trend; statistically significant ($p \leq 0.05$) ANOVA trend analysis for CLMH, CLM or CL

V, W, X & Y: CLMH (decreasing trend) in males, females and combined sex

Z: CLMH, CLM (decreasing trend) in males, CLMH (decreasing trend) in females and CLMH and CLM (decreasing trend) for combined sex

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3. F_1 adults/F_{2a} generation pups:

F_1/F_{2a} premating mean food consumption: There were no significant differences in males or females at 1.0 and 10 ppm. At 30 ppm, female food consumption was significantly higher ($p \leq 0.05$) than that of controls at wk 2, 3 and 5 (wk 2: 121 g/kg/d cf controls 111; wk 5: 93 g/kg/d cf controls 88.4). At 30 ppm, male weekly food consumption was significantly higher ($p \leq 0.05$ or ≤ 0.01) than that of controls from wk 1 to 21 (wk 1: 159 g/kg/d cf controls 137; wk 9: 68 g/kg/d cf controls 63; wk 17: 61 g/kg/d cf controls 55; wk 21: 61 g/kg/d cf controls 54.5). The increase in male food consumption at 30 ppm was considered treatment-related.

F_1/F_{2a} premating mean bw: At 10 and 30 ppm, female bws were significantly less ($P \leq 0.01$) than that of controls at d 0 (127 g and 123 g respectively cf controls 138). These deficits in bw (8 and 11% respectively) were considered treatment-related. Otherwise there were no significant differences between the groups in females or males at 1.0 ppm. At 10 ppm, male bws were significantly less ($p \leq 0.05$ or ≤ 0.01) than that of controls at wk 0-21 (wk 0: 150 g cf controls 167; wk 9: 358 g cf controls 391; wk 17: 411 g cf controls 442; wk 21: 405 g cf controls 460). At 30 ppm the same pattern ($p \leq 0.01$) occurred (wk 0: 138 g cf controls 167; wk 9: 347 g cf controls 390; wk 17: 389 g cf controls 442; wk 21: 405 g cf controls 460). The reductions in male bw at 10 ppm and 30 ppm were considered treatment-related.

In females, the respective mean bw gains in controls and at 1.0, 10 and 30 ppm over 10 wk were 105, 117, 107 and 111 g. In males over 21 wk they were 293, 321, 277 and 267 g respectively (statistically significant differences in mean bw gains were not determined). In males at 30 ppm a biologically significant reduction in food conversion efficiency occurred.

F_1/F_{2a} mean maternal food consumption during gestation: There were no significant differences between treatment groups and controls.

F_1/F_{2a} mean maternal bw during gestation: One significant difference ($p \leq 0.05$) occurred at 10 ppm at GD 13 (276 g cf controls 292). All other mean bws at 10 and 30 ppm on GD 0, 6, 13 and 20 were less than that of controls but not statistically different. The mean bws at 10 ppm were GD 0: 235 g cf controls 245; GD 6: 253 g cf controls 266; GD 20: 346 g cf controls 362. The mean bws at 30 ppm were GD 0: 239 g cf controls 245; GD 6: 255 g cf controls 266; GD 13: 278 g cf controls 291; GD 20: 347 g cf controls 362. Over gestation the mean bw gain in controls was 117 g. The mean bw gains at 1.0, 10 and 30 ppm were 132, 111 and 108 g respectively. These bw gains were not statistically different from controls.

F_1/F_{2a} mean maternal food consumption during lactation: At 30 ppm, consumption over LD 14-21 was significantly less ($p \leq 0.05$) than that of controls (61 g/d cf controls 67). The decrease was considered treatment-related.

F_1/F_{2a} mean maternal bw during lactation: At 10 ppm, bw was significantly less ($p \leq 0.05$) than that of controls at LD 7 and 21 (LD7: 289 g cf controls 307; LD21: 285 g cf controls 306). At 30 ppm, bw was significantly less ($p \leq 0.05$) at LD 7 (289 g cf controls 307). The decreases in bw at 10 and 30 ppm were considered treatment-related.

Compared with mean lactation bw gain in controls of 29.6 g, the mean bw gain at 1.0, 10 and 30 ppm was 24.8, 18.9 and 22.4 g respectively (statistical significance was not reported).

F_1/F_{2a} mean viable pup bw: The mean viable pup bws at various times during lactation are tabulated below. There were no significant differences between treatment groups and controls

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and no dose-response trends at LD 4 (pre-culling) or LD 4 (post-culling). The study authors considered the decreases in pup bw at 10 and 30 ppm to be treatment-related. Over lactation the mean pup bw gain in controls was 44.4 g. The mean bw gains at 1.0, 10, 30 ppm were 42.9, 40.7 and 37.3** g respectively (** significantly less than controls; $p \leq 0.01$). On this basis the decrease in mean bw at 10 ppm may not have been been treatment-related.

F_1/F_{2a} mean viable pup bw for each dietary concentration consumed by dams during lactation

LD	F_1/F_{2a} mean viable pup bw (g) at each dose (ppm) for each sex: dose trend analysis												
	Control (C)			1.0 ppm (L)			10 ppm (M)			30 ppm (H)			T #
	M	F	S	M	F	S	M	F	S	M	F	S	
LD 0	6.7	6.3	6.5	6.8	6.5	6.7	7.1	6.7	6.9	7.2	6.7	6.9	U
LD 7	16.7	16.5	16.5	16.3	15.7	16.0	16.8	16.4	16.6	15.5	14.5^{+}	15.0	X
LD 14	31.8	30.9	31.2	31.3	30.5	30.9	30.7	30.1	30.5	28.8^{+}	27.4^{+}	28.1^{+}	Y
LD 21	52.0	50.4	51.1	50.3	48.9	49.6	48.1^{*}	46.8	47.6	45.2^{+}	43.2^{+}	44.2^{+}	Z

a = post-cull, M = male, F = female, S = combined sex
* or + ANOVA: statistically significantly different from controls, * ($p \leq 0.05$), + ($p \leq 0.01$)
# T = trend; statistically significant ($p \leq 0.05$) ANOVA trend analysis for CLMH, CLM or CL
U: CLMH (increasing trend) in females and combined sex
X: CLMH (decreasing trend) in females; Y: CLMH (decreasing trend) in males, females and combined sex; Z: CLMH and CLM (decreasing trend) in males, females and combined sex

4. F_1 adults/F_{2b} generation pups:

F_1/F_{2b} male premating mean bw: At 10 ppm, bw was significantly less ($p \leq 0.05$) at wk 1, 2 and 3 (wk 1: 427 g cf controls 459; wk 3: 436 g cf controls 470). At 30 ppm, bw was significantly less ($p \leq 0.01$) at wk 1-4 (wk 1: 405 g cf controls 459; wk 4: 407 g cf controls 471). Over a 4 wk period, the mean bw gains for controls and at 1.0, 10 and 30 ppm were 11.8, 15.4, 33.6 and 2.0 g respectively (statistical significance was not reported). The decrease in bw at 30 ppm was considered treatment-related.

F_1/F_{2b} mean maternal food consumption and bw during gestation: There were no significant differences in food consumption between treatment groups and controls. Over gestation the mean bw gain in controls was 133 g. The mean bw gains at 1.0, 10 and 30 ppm were 127, 123 and 116* respectively (*significantly less than controls; $p \leq 0.05$).

F_1/F_{2b} mean maternal food consumption during lactation: There were no significant differences between treatment groups and controls.

F_1/F_{2b} mean maternal bw during lactation: At 1 ppm there were no significant differences from controls. At 10 ppm, mean bws were significantly less ($p \leq 0.05$) than that of controls at LD 4 and 7 (LD 4: 315 g cf controls 332; LD 7: 322 g cf controls 342). At 30 ppm, mean bws were significantly less ($p \leq 0.05$) than that of controls at LD 7 (321 g cf controls 342). The study authors considered the decreases in bw at 10 and 30 ppm to be treatment-related. Over lactation the mean bw gain in controls was 27.1 g. The mean bw gain at 1.0, 10 and 30 ppm was 21.1, 22.6 and 22.2 g respectively (statistical significance not reported). On this basis the decreased mean bw gain at 1 ppm may also be treatment-related.

F_1/F_{2b} mean viable pup bw: The mean viable pup bws at various times during lactation are tabulated below. There were no significant differences between treatment groups and controls and no dose-response trends at LD 0. The study authors considered the decreases in pup bw at 10 and 30 ppm to be treatment-related. Over lactation the mean pup bw gain in controls was

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47.0 g. The mean bw gains at 1.0, 10, 30 ppm were 46.3, 40.7** and 35.0** g respectively (** significantly less than controls; $p \leq 0.01$).

F_1/F_{2b} mean viable pup bw for each dietary concentration consumed by dams during lactation

LD	F_1/F_{2b} mean viable pup bw (g) at each dose (ppm) for each sex: dose trend analysis												
	Control (C)			1.0 ppm (L)			10 ppm (M)			30 ppm (H)			T #
	M	F	S	M	F	S	M	F	S	M	F	S	
LD 4[a]	10.6	10.2	10.4	11.0	10.5	10.7	9.8	9.5	9.7	7.2*	6.7*	7.0*	V
LD 4[b]	10.6	10.3	10.4	11.0	10.6	10.8	9.7	9.5	9.6	9.2*	8.8+	9.0*	W
LD 7	16.8	16.4	16.6	16.9	16.3	16.6	15.1	14.8	15.0	13.3+	12.7+	13.0+	X
LD 14	32.6	31.6	32.1	32.1	30.9	31.5	29.7*	28.2+	28.6+	26.4+	25.7+	26.0+	Y
LD 21	55.2	52.4	53.8	54.7	51.9	53.2	49.1*	46.8*	47.4*	42.7+	41.6+	42.0+	Z

a = pre-cull, b = post-cull, M = male, F = female, S = combined sex
* or + ANOVA: statistically significantly different from controls, * ($p \leq 0.05$), + ($p \leq 0.01$)
# T = trend; statistically significant ($p \leq 0.05$) ANOVA trend analysis for CLMH, CLM or CL
V & W: CLMH (decreasing trend) in males, females and combined sex
X, Y & Z: CLMH and CLM (decreasing trend) in males, females and combined sex

Reproductive data: At the F_0/F_{1a} and F_1/F_{2a} matings there were no differences between the groups in the number or length of oestrus cycles. Information about the other matings in this respect was not reported. For each mating there were no differences between the groups in cumulative % inseminated females at d 21 of mating, mean number of d to insemination, female mating index, female fertility index, gestation index or gestation length.

Litter data: For all matings there were no significant statistical differences between the groups in any generation in number of litters, total number pups born, total number of pups missing, litters with pups missing, total number pups found dead, litters with pups found dead, total number of pups cannibalised, litters with pups cannibilised, litter size, sex distribution at birth, number of stillborn pups, number of viable pups (birth, LD 4 pre-cull, 4 post-cull and 21), live birth index, or viability index.

Lactation indices and cannibalism: At 30 ppm, there were treatment-related decreases in lactation indices for the F_0/F_{1a}, F_0/F_{1b} and F_1/F_{2b} generations which was related to the degree of cannibalism occurring at that concentration. The observed lactation indices and the incidence of cannibalism together with 2 recalculated lactation indices are tabulated below. The number of pups counted as cannibalised were those missing and those for which remnants were observed.

Lactation indices and the incidence of cannibalism

Generation	Observed lactation index (%); twice recalculated lactation indices (%) and incidence (number and %) of dams with cannabilised pups			
	0 ppm	1.0 ppm	10 ppm	30 ppm
F_0/F_{1a}	96.0 (96#) (96##) 2/28 (7)	96.4 2/28 (7)	99.6 2/30 (6.6)	85.9 (91#) (89##) 7/24 (29)*
F_0/F_{1b}	99.6 (100#) (100##) 1/26 (4)	97.8 3/24 (12.5)	100 4/29 (13.8)	86.9 (98#) (88##) 5/22 (22.7)
F_1/F_{2a}	99.0 4/27 (14.8)	99.0 2/25 (8)	99.6 2/29 (6.8)	97.5 5/25 (20)
F_1/F_{2b}	98.4 (100#) (100##) 4/24 (16.6)	99.1 4/27 (14.8)	96.3 2/20 (10)	90.9 (97#) (94##) 10/25 (40)

* Statistically significant compared to control incidence, $p \leq 0.05$
# First recalculation; ## Second recalculation.

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On the basis that cannibalism was an effect on the dam (i.e. cannibalised pups may not otherwise have died) the lactation index for the 3 generations of concern was recalculated for controls and the 30 ppm group. The basis for the second recalculation was that pups which were weak or cold to the touch prior to being cannibalised, may have died anyway. On the basis of the 2nd calculation, the study authors concluded that at 30 ppm there was a treatment-related decrease in lactation index. The cause of the cannibalism was not determined.

Cholinesterase activity: The plasma, RBC and brain ChE activity and % inhibition for adults and pups are tabulated below.

Plasma, RBC and brain ChE activity in F_0 adults

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_0 adults at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At 8 wk: Plasma	0.57	0.71	0.36*	0.21*	2.05	1.89	1.03*	0.33*
	-	-	36.8	63.2	-	7.8	49.8	83.9
RBC	1.16	0.95	0.39*	0.25*	1.21	1.21	0.74*	0.32*
	-	18.0	66.4	78.4	-	-	38.8	73.6
At termination: Plasma	0.51	0.55	0.31*	0.15*	0.92	0.88	0.41*	0.14*
	-	7.8	39.2	70.6	-	4.3	55.4	84.8
RBC	1.28	1.01*	0.34*	0.20*	1.33	1.18	0.38*	0.16*
	-	21.1	73.4	84.4	-	11.3	71.4	88.0
Brain	12.9	13.7	7.1*	3.2*	12.1	11.3*	5.1*	2.9*
	-	6.2	45.0	76.0	-	6.6	57.9	76.0

* Statistically significant $p \leq 0.05$

At 10 and 30 ppm biologically significant inhibition (≥ 20% inhibition) of plasma and RBC ChE was observed in males and female F_0 adults at 8 wk and of plasma, RBC and brain ChE at termination. At 1 ppm ChE inhibition ≥ 20% was observed with RBC ChE in males at termination. There was also statistically significant ($p \leq 0.05$) inhibition of RBC ChE in the 1 ppm males at termination.

Plasma, RBC and brain ChE activity in F_{1a} pups

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_{1a} pups at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At LD 4: Plasma	0.70	0.74	0.69	0.50*	0.68	0.73	0.58*	0.49*
	-	-	1.4	28.6	-	-	14.7	27.9
RBC	1.65	1.21*	1.28	1.16*	1.25	1.42	1.26	1.16
	-	26.7	22.4	29.7	-	-	-	7.2
Brain	4.73	4.51	4.71	3.79*	4.47	4.38	4.45	3.55
	-	4.7	0.4	19.9	-	2.0	0.4	20.6
At LD 21: Plasma	0.64	0.66	0.42*	0.36*	0.63	0.65	0.44*	0.41*
	-	-	34.4	43.8	-	-	30.2	34.9
RBC	1.63	1.67	1.25	1.06*	1.9	1.53	1.31*	1.31*
	-	-	23.3	35.0	-	19.5	31.1	31.1
Brain	11.66	11.79	8.99*	7.04*	11.56	11.84	9.29*	7.68*
	-	-	22.9	39.6	-	2.5	19.6	33.6

* Statistically significant $p \leq 0.05$

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At 10 and 30 ppm biologically significant inhibition of plasma, RBC and brain ChE was observed in male and female F_{1a} pups at LD 21. At 1 ppm, ChE inhibition ≥ 20% was also observed in RBC ChE in males at LD 4 but was not considered biologically significant as inhibition at 1 ppm was not observed in F_{1b} pups.

At 10 ppm, biologically significant inhibition of plasma, RBC and brain ChE was observed in male and female F_{1b} pups LD 21. At 30 ppm, biologically significant inhibition of plasma, RBC and brain ChE was observed at LD 4 and 21.

Plasma, RBC and brain ChE activity in F_{1b} pups

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_{1b} pups at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At LD 4: Plasma	0.69	0.67	0.63	0.48*	0.69	0.67	0.65	0.53*
	-	2.9	8.7	30.4	-	2.9	5.8	23.2
RBC	1.32	1.46	1.21	0.90*	1.66	1.46	1.34	0.99*
	-	-	8.3	31.8	-	-	19.3	40.4
Brain	4.62	4.61	4.17	3.39*	4.54	4.48	4.29	3.69*
	-	0.2	9.7	26.6	-	1.3	5.5	18.7
At LD 21: Plasma	0.69	0.69	0.45*	0.42*	0.73	0.67	0.45*	0.41*
	-	-	34.8	39.1	-	8.2	38.4	43.8
RBC	1.70	1.57	1.12*	1.07*	1.74	1.43	1.14*	1.05*
	-	7.6	34.1	37.1	-	17.8	34.5	39.7
Brain	11.57	11.69	9.49*	7.98*	11.39	11.50	9.11*	7.37*
	-	-	18.0	31.0	-	1.0	20.0	35.3

* Statistically significant $p \leq 0.05$

At 10 and 30 ppm, biologically significant inhibition of plasma and RBC ChE was observed in male and female F_1 adults at 8 wk and of plasma, RBC and brain ChE at termination.

Plasma, RBC and brain ChE activity in F_1 adults

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_1 adults at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At 8 wk: Plasma	0.54	0.64	0.33*	0.20*	1.86	1.75	0.79*	0.33*
	-	-	38.9	63.0	-	5.9	57.5	82.3
RBC	0.88	0.77	0.22*	0.12*	1.12	0.92	0.46*	0.23*
	-	12.5	75.0	86.4	-	17.9	58.9	79.5
At termination: Plasma	0.54	0.61	0.31*	0.15*	0.91	0.87	0.35*	0.16*
	-	-	42.6	72.2	-	4.4	61.5	82.4
RBC	1.30	1.07*	0.37*	0.15*	1.43	1.25	0.42*	0.15*
	-	17.7	71.5	88.5	-	12.6	70.6	89.5
Brain	12.7	12.8	7.3*	4.0*	11.9	11.3	4.7*	2.3*
	-	-	42.5	68.5	-	5.0	60.5	80.7

* Statistically significantly different $p \leq 0.05$

At 10 and 30 ppm biologically significant inhibition (≥ 20% inhibition) of plasma, RBC and brain ChE was observed in male and female F_{2a} pups at LD 21. At 1 ppm, ChE inhibition ≥ 20% was observed in RBC ChE in males at LD 4 but was not considered biologically significant as inhibition at 1 ppm was not observed in F_{2b} pups.

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Plasma, RBC and brain ChE activity in F_{2a} pups

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_{2a} pups at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At LD 4: Plasma	0.72	0.70	0.65	0.52*	0.68	0.69	0.64	0.50*
	-	2.8	9.7	27.8	-	-	5.9	26.5
RBC	1.6	1.23*	1.59	1.09*	1.51	1.52	1.33	1.06*
	-	23.1	0.6	31.9	-	-	11.9	29.8
Brain	4.69	4.34	12.4	3.94	4.76	4.27*	4.59	3.88*
	-	7.5	1.59	16.0	-	10.3	3.6	18.5
At LD 21: Plasma	0.68	0.64	0.53*	0.25*	0.68	0.72	0.48*	0.24*
	-	5.9	63.2	63.2	-	-	29.4	64.7
RBC	1.72	1.61	1.39	0.62*	1.70	1.54	1.32*	0.52*
	-	6.4	19.2	64.0	-	9.4	22.4	69.4
Brain	11.87	11.68	9.41	5.88*	11.88	11.57	9.79*	5.56*
	-	1.6	20.7	50.5	-	2.6	17.6	53.2

* Statistically significantly different $p \leq 0.05$

At 10 and 30 ppm biologically significant inhibition (≥ 20% inhibition) of plasma, RBC and brain ChE was observed in male and female F_{2b} pups at LD 21.

Plasma, RBC and brain ChE activity in F_{2b} pups

Interval/ChE	ChE activity (IU/mL or IU/g) and % inhibition in F_{2b} pups at various concentrations of methamidophos in diet (ppm)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
At LD 4: Plasma	0.65	0.65	0.60	0.51*	0.65	0.67	0.57	0.56
	-	-	7.7	21.5	-	-	12.3	13.8
RBC	1.70	1.41	1.28*	0.94*	1.28	1.25	1.33	1.19
	-	17.1	24.7	44.7	-	2.3	3.9	7.0
Brain	4.51	4.42	4.21	3.58*	4.46	4.46	4.07*	3.66
	-	2.0	6.7	20.6	-	-	8.7	17.9
At LD 21: Plasma	0.67	0.64	0.42*	0.35*	0.64	0.63	0.44*	0.33*
	-	4.5	37.3	47.8	-	1.6	31.3	48.4
RBC	1.74	1.83	1.25*	0.95*	1.84	1.74	1.27*	1.04*
	-	-	28.2	45.4	-	5.4	31.0	43.5
Brain	11.09	10.79	8.44*	6.52*	11.46	11.18	8.76*	6.95*
	-	2.7	23.9	41.2	-	2.4	23.6	39.4

* Statistically significantly different $p \leq 0.05$

Gross pathology: There were no treatment-related necropsy observations in F_0 and F_1 adults. Apart from the effects of cannibalism there were no treatment-related necropsy observations in pups.

Terminal bw and organ wts: The terminal bws, absolute gonad wts and relative gonad wts are tabulated below. In F_0 or F_1 females there were no differences between the groups in terminal bw. Terminal bw in F_0 males at 30 ppm and in F_1 males at 10 and 30 ppm were significantly less ($p \leq 0.05$) than that of controls. These decreases were considered treatment-related. There were no differences between the groups in absolute gonad wt in F_0 or F_1 males or females. In F_0 females relative ovarian wts at 1.0 and 30 ppm were significantly higher ($p \leq 0.05$) than that of controls. In F_1 males relative testicular wts at 10 and 30 ppm were significantly higher

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($p \leq 0.05$) than that of controls. These increases in relative gonad wts were considered to be a consequence of decreased terminal bws at the specified concentrations.

Terminal bw, absolute gonad wt and relative gonad wt/terminal bw

Generation/Gonads	Terminal (d 175) bws (g), gonad wts (g) and relative gonad wts/ terminal bws (%)							
	Male				Female			
	0	1.0	10	30	0	1.0	10	30
F_0 terminal bw	458.5	451.6	455.8	429.2*	326.4	316.6	307.8	316.3
F_0 testes or ovaries wt	3.7	3.7	3.8	3.6	0.13	0.14	0.13	0.14
Testes or ovaries wt/bw	0.81	0.83	0.85	0.86	0.040	0.045*	0.044	0.045*
F_1 terminal bw	465.8	487.0	434.2*	407.4*	320.0	332.2	301.0	309.1
F_1 testes or ovaries wt	3.67	3.94	3.74	3.62	0.13	0.14	0.13	0.14
Testes or ovaries wt/bw	0.79	0.81	0.87*	0.89*	0.041	0.042	0.045	0.045

* Statistically significant compared to controls, $p \leq 0.05$

Histopathology: There were no treatment-related observations in F_0 or F_1 adults. Histopathological findings in pups were not reported.

Conclusions

1a. In adults, methamidophos caused a deficit in premating bw in F_0 males at 30 ppm and in F_1 males and females at 10 and 30 ppm. Maternal bw gain during gestation of the F_{2a} and F_{2b} litters was decreased at 30 ppm. During lactation, maternal food consumption was inhibited at 30 ppm and maternal bw was decreased relative to controls at 10 and 30 ppm.

1b. In adults, statistically or biologically significant inhibition of RBC ChE occurred at 1 ppm in F_0 and F_1 males at termination. At 10 and 30 ppm, plasma, RBC and brain ChE inhibition was observed in both sexes of F_0 and F_1 adults at 8 wk and study termination.

1c. Methamidophos caused decreases in lactation index for the F_0/F_{1a}, F_0/F_{1b} and F_1/F_{2b} generations at 30 ppm.

1d. In neonates, bw and/or bw gain were decreased at 10 and 30 ppm.

1e. In neonates, plasma, RBC and brain ChE were consistently inhibited to a statistically or biologically significant extent at 10 and 30 ppm.

2a. Thus a NOEL for parental toxicity was not established due to the RBC ChE inhibition in males at 1 ppm.

2b. The NOEL for effects on neonates was 1 ppm (equivalent to 0.2 mg/kg bw dam/d).

2c. The NOEL for reproductive toxicity was 10 ppm (equivalent to 2.4 mg/kg bw female/d or 2.5 mg/kg bw male/d).

8. DEVELOPMENTAL STUDIES

8.1 Rats

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Breeden BA, Hoss HE, Brewer GJ, Mallicoat DR & Lamb DW (1980): A pilot teratology study using technical methamidophos in CD rats. Mobay Report No. 68765. Study No. 80-611-01. Toxicology Report No. 96 (Revised). Lab: Stanley Research Centre, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 23 April 1980 - 16 May 1980. Report Date: 24 July 1980, revised 19 August 1980. Unpublished. (QA: Yes; GLP & Test Guidelines: not specified).

Test Substance:	Methamidophos technical. Batch Number: 77-297-149. Purity: 75%. Source: Mobay Chemical Corporation, Kansas City, MO, USA
Test Species:	Rats: CD outbred, males, females, 60 d old, of an unspecified bw obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts.
Dose:	0, 0.1, 0.3, 1.0, 3.3, 10.0 mg/kg bw po

Method

The rats were individually housed in suspended stainless steel cages and acclimatised for 7 d in standard environmental conditions. They were fed an unspecified diet and had access to water *ad libitum*. Four females were used at each test substance dose level. Proven male breeders were used on a one-to-one basis and pregnancy was confirmed the following morning by spermatozoa-positive vaginal smears. All doses were administered orally in water at a volume of 5 mL/kg. Females were observed twice daily for signs of toxicological reactions or mortality. Foetal observations were made by gross examination at caesarian section. Skeletal and soft tissue examinations were made after tissues had been prepared in an unspecified manner. All individual rat results were hand-written on report forms. It is apparent from these forms that each rat was dosed and weighed on GD 6-20 and terminated on GD 21. Statistical evaluation of the results was not performed.

Results and discussion

Clinical signs and mortalities: No mortalities occurred. Mild to severe signs were observed at 3.3 and 10 mg/kg bw. The signs ranged from slight to heavy fasciculations with salivation and lacrimation.

Reproductive data: The results of reproductive parameter determinations are tabulated below.

Reproductive indices

Index	Dose (mg/kg bw)					
	0	**0.1**	**0.3**	**1.0**	**3.3**	**10**
Mean no. foetuses/litter	11	13	15	13	15	14
Mean foetal bw	5.9	5.3	5.6	5.4	5.1	4.6
Mean no. implantation sites/dam	14	14	15	16	16	14.5
Mean no. corpora lutea/dam	14	15	16	17	16	16
No. resorptions/dose level	10	2	0	9	4	1
No. litters with resorptions	¾	1/3	0/4	4/4	2/4	1/4
% litters with resorption	0.75	0.33	0	1.0	0.5	0.25
Mean corrected bw gain of dams	114.8	91.7	83.9	87.0	62.5	54.0

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The mean foetal bw was substantially depressed at 10 mg/kg bw. There was a dose-related depression in the corrected bw gain of dams.

Gross necropsy: No treatment-related lesions or effects were observed in the dams during caesarian section. No external foetal anomalies were observed.

Conclusion

It was concluded that the highest dose level for a definitive teratology study involving methamidophos should be 10 mg/kg bw. As this was a dose-ranging study involving a limited number of animals and test parameters, a NOEL will not be set.

Hixson EJ, Mallicoat DR & Lamb DW (1984a): Embryonic and teratogenic effects of methamidophos (Monitor^{R}) in rats. Mobay Report No. 87480. Study No. 82-611-01. Toxicology Report No. 542. Lab: Environmental Health Research, Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Sponsors: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA & Agricultural Chemicals Division, Chevron Chemical Company, Richmond, California, USA. Study Duration: 14 April 1982 - 11 January 1984. Report Date: 15 October 1984. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: 40 CFR Part 160).

Test Substance:	Methamidophos technical Batch Number: 77-297-149 Purity: 70.5% Source: Mobay Chemical Corporation
Test Species:	Rats: CD, males, females, 50-55 d old, 175 to 200 g bw obtained from Charles River Laboratories Inc.
Dose:	0, 0.3, 1, 3 mg/kg bw/d po

Method

For mating female rats were co-housed with males overnight. Each morning vaginal smears were prepared and examined to determine the stage of oestrus and to verify the presence of spermatozoa. The d on which spermatozoa were found was designated GD 0. After mating females were randomly assigned to groups of 24 on the basis of bw.

The vehicle for test substance administration was distilled water. The test substance was administered at 0.5% bw on GD 6 to 15 inclusive at approximately the same time each d. The dosage volume was based on bw on GD 6 and was not adjusted for wt gain as pregnancy advanced. The vehicle control group received distilled water at 0.25% bw. The positive control group received hydroxyurea at 350 mg/kg bw in distilled water on GD 9, 10 and 11.

It was known from preliminary work that stock solutions of the test material were stable for 1 wk when stored refrigerated. Dosing solutions were prepared by dilution from a stock solution prepared weekly. Analysis of aliquots of dosing and stock solutions showed a recovery of 97.3 ± 9.5%.

Pregnant females were observed daily for toxicologic and pharmacologic signs and for signs of abortion and premature delivery. They were weighed on GD 0, 6, 13 and 21 (prior to termination). Food consumption was measured on GD 6, 13 and 21. Rats were killed on GD

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21 by carbon dioxide asphyxiation. The viscera of the dams were examined grossly. The uterus and ovaries were removed *in toto* and weighed. The uterus was opened and the number and position of live, dead and resorbed foetuses were recorded. The placentae were inspected for abnormalities and the number of corpora lutea was determined. The foetuses were counted, numbered, weighed and sexed. Foetuses were examined for viability and for gross external abnormalities.

One-half of the foetuses in each litter were fixed in 70% ethanol. They were then examined internally, eviscerated, partially skinned, macerated and stained with Alizarin Red for evaluation of skeletal formation. The remaining foetuses of each litter were fixed in Bouin's solution and subsequently examined for organ structure and integrity by the freehand razor blade method.

Maternal bw and food consumption was tested for significant difference using Duncan's Multiple Range Test. Litter data, gross observations, soft tissue observations and skeletal observations were tested for statistically significant differences (at the 95% level) by chi-square analysis. The positive control group was excluded from statistical analysis.

Results and discussion

Sensitivity of the test system: No clinical signs were observed in positive controls. However extensive effects on foetuses were observed. Gross, internal and skeletal effects were observed frequently. As a consequence the test system showed sufficient sensitivity to teratogenic agents.

Clinical signs and mortality in pregnant rats: At 3 mg/kg bw signs of toxicity typical of cholinesterase-inhibiting compounds occurred. They were fasciculations, hyperactivity, salivation, lacrimation and excessive urination. These signs were seen in all rats at this dose. They were initially observed on GD 6 to 8 and persisted until GD 20. One rat at this dose showed initial signs of delivery (bloody vaginal discharge) on GD 20 without actually delivering. No mortalities occurred in any of the groups. Two non-pregnant rats were identified in the vehicle control group, 1 at 3 mg/kg bw and 4 in the positive control group. These non-pregnancies were considered incidental. Alopecia was observed in 1 vehicle control dam and in 1 dam at 1 mg/kg bw.

Food consumption: At 3 mg/kg bw mean dam food consumption at GD 13 and 21 was statistically significantly less ($p \leq 0.05$) than vehicle controls (125 g cf control 176 g at GD 13; 185 g cf control 223 g at GD 21).

Body wt: At 3 mg/kg the following parameters were statistically significantly less ($p \leq 0.05$) than that of vehicle controls:-

a. mean dam bw on GD 13 and 21 (265 g cf control 296 on GD 13; 356 g cf control 401 g on GD 21), and,
b. mean dam bw gain (mean bw GD 21 - mean bw GD 0); 119 g cf control 160 g, and.
c. mean dam bw gain corrected for uterine contents (mean bw gain - wt of gravid uterus); 33 g cf control 58 g.
d. mean live foetus bw (4.6 g cf control 4.9 g).

Cumulative litter data: There were no statistically significant differences between methamidophos-treated groups and vehicle controls in number of implants, number of early

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resorptions (no late resorptions occurred in any group) number of live or dead foetuses or foetuses bearing anomalies.

Gross foetal abnormalities: There were no statistically significant differences between methamidophos-treated groups and vehicle controls in the incidence of gross abnormalities. The % mean abnormal foetuses/litter for vehicle controls, 0.3, 1 and 3 mg/kg bw dose groups and positive controls were 60, 57, 59, 54, and 84 respectively.

Foetal soft tissue examinations: The soft tissue findings were classified as variations or as spontaneous abnormalities. There were no statistically significant differences between methamidophos-treated groups and vehicle controls in the incidence of variations or abnormalities. A total of 7 foetuses from 3 litters in the vehicle control group and a single foetus from the 1 mg/kg bw group exhibited spontaneous abnormalities.

Foetal skeletal examinations: The incidence of incomplete ossification of the interparietal and of enlargement of the occipital fontanel were statistically significantly greater ($p \leq 0.05$) in vehicle controls than in methamidophos-treated groups. The incidences of incomplete ossification of the interparietal for vehicle controls, 0.3, 1 and 3 mg/kg bw dose groups and positive controls were 9/170, 1/181, 3/191, 1/186 and 8/166 respectively. The incidences of occipital fontanel enlargement for vehicle controls, 0.3, 1 and 3 mg/kg bw dose groups and positive controls were 6/170, 0/181, 7/191, 0/186 and 18/166 respectively. The differences between the methamidophos-treated groups and the vehicle controls were not considered biologically significant.

Conclusion

(1) No embryotoxic or teratogenic effects were observed.

(2) At 3 mg/kg bw, significant maternal and foetal toxicity occurred. The maternal toxicity was manifest as lowered food consumption and bw gain (effects consistent with ChE inhibition) and foetal toxicity as reduced bw.

(3) Under the conditions of this study and on the basis of maternal toxicity and foetal toxicity a NOEL of 1 mg/kg bw was established.

Guo L, Chen Y, Lu R, Liu Y, Xia L, Chen J, Zhang X & Mao J (1986g): Toxicity studies of methamidophos (II). Teratogenicity and mutagenicity tests. Zhonghua Laodong-Weisheng Zhiyebing Zazhi, 4(5):261-263.

[Note: The results of the genotoxicity tests reported in this study have been added to the tabulated summary of genotoxicity studies in Section 9].

Test Substance:	Methamidophos crystalline Purity: 90.4% No further details provided.
Test Species:	Rat: Wistar, females of an unspecified bw, age or source.
Dose:	0.1, 0.5, 2.0 mg/kg bw/d po, GD 6-15.

Method

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Eighty pregnant rats were randomly divided into 3 treatment, 1 negative control and 1 positive control groups of unspecified size. The positive control group received 1 mg/kg of a suspension of N,N'-bis(1,3,4-thaidiazol-2-yl)methanediamine in sesame oil. It was not clear whether this was the actual dose of the positive control agent or of the suspension. Methamidophos was administered to rats by daily oral drip feeding on GD 6-15. The vehicle for methamidophos was not specified nor was the treatment that the negative control group received. The rats were observed for clinical signs. They were killed by dislocation of the cervical vertebrae on d 20. Foetal appearance, state of growth and development and survival were examined conventionally. Two thirds of the foetuses from each litter were skinned, the viscera and cervicodorsal fatty tissue cleared away and the skeletons were stained with alcian blue and alizarin red. The remaining foetuses were fixed in Bouin's solution and used for examination of visceral soft tissue sections. Unspecified statistical analyses were performed.

Results and discussion

Clinical signs of an unspecified nature were observed in an unspecified small number of rats at 2.0 mg/kg bw/d. The same group showed an unquantified depression of foetal body length, foetal wt and placental wt with retarded ossification of the occipital bone, cervical vertebrae and vertebral bodies ($p < 0.01$). Otherwise there were no statistically significant differences between the methamidophos treatment groups and the negative controls in respect of litter average of live foetuses, foetal mortality rate, embryonic resorption rate, foetal appearance and visceral tissue section findings. The positive control group showed an unquantified high embryonic resorption rate, and a marked depression ($p < 0.01$) of foetal growth and development. Observations of foetal appearance and skeletal and visceral tissue sections indicated "typical malformations", ($p < 0.01$), of an unspecified nature.

Comment

Given the absence of quantitative data, a NOEL will not be assigned.

Hanafy MS, Atta AH & Hashim MM (1986a): Studies on the teratogenic effects of Tamaron^{TM} (an organophosphate pesticide). Vet Med J, 34:357-363.

Test Substance:	Methamidophos. Obtained as 60% water miscible concentrate. Source: not specified.
Test Species:	Rats: mature, no further details provided.
Dose:	0, 1.0, 2.0 mg/kg bw/d for 10 d po.

Method

Sixty mature rats were maintained on a commercial ration and allowed free access to water. Females in oestrus were kept overnight with mature fertile males. The 1st d of pregnancy was taken to be the d of detecting spermatozoa in vaginal smears. A solution of 1.0 mg methamidophos/mL saline was freshly prepared for use. Pregnant rats were divided into 3 groups of 20. The 1st group received saline only. The 2nd and 3rd group received 1.0 and 2.0 mg methamidophos/kg bw respectively. These doses were chosen as being 1/20th and 1/10th respectively of the oral LD_{50} in rats (Robinson & Beiergrohslein, 1980). The doses were administered orally daily from GD 6-15. All rats were killed by cervical dislocation on GD 19. Uteri were opened. Foetuses were weighed and examined for morphological

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abnormalities. Skeletal and visceral examinations were performed according to the methods of (Staples & Schnell, 1964; Wilson, 1965). Uteri were soaked in 10% ammonium sulfate for 20 min after which implantation sites were counted. Mean viable foetal bw results were subjected to the student "t" test.

Results and discussion

No observations on the health or behaviour of dams were provided.

Reproductive and teratological parameters: The findings are tabulated below. There was a dose-related increase in the incidence of embryo lethality, malformed foetuses, anencephaly and anotia and a dose-related decrease in the number of foetuses and viable foetuses. A definition of the term "malformed foetuses" was not provided. Mean foetal bw was depressed in both treated groups.

Teratological effects following oral treatment with methamidophos

Parameters	**Dose of methamidophos (mg/kg bw/d)**		
	0	**1.0**	**2.0**
No. of pregant dams	20	20	15
No. of implantation sites	170	150	101
Total no. foetuses	170	140	92
No. of live foetuses	165	120	42
No. implantation sites/dam	8.5	7.5	6.7
No foetuses/dam	8.5	7	6
No. live foetuses/dam	8.3	6	2.8
% embryo lethality	2.9	14.3	54.4
% malformed foetuses	0	11	83
% anencephaly	0	9	41
% of anotia	0	4	21
#Mean foetal wt (g)	3.5	2.2*	2.2*

* Statistically significant ($p \leq 0.005$) cf controls
# Excluded dead foetuses; no further details were provided

Gross pathology: Cyanosis and congestion were observed in foetuses of dams at 2.0 mg/kg. The study authors stated that "in foetuses with retardation of growth, all skeletal structures were present and had normal appearance but were smaller in size". No gross abnormalities were observed in viscera.

Conclusions

Methamidophos caused significant embryo lethality, retardation of foetal growth, anencephaly and anotia. A NOEL for these effects was not established.

Comment

The study appears to have demonstrated dose - and/or treatment-related decreases in foetal survival and bw and increases in the incidence of anencephaly and anotia, that were present at both 1 and 2 mg/kg bw/d. However, the study reported insufficient detail to permit independent evaluation. Of particular concern is the fact that at 2 mg/kg bw/d, the % of "malformed foetuses" exceeds the sum of the incidences of anencephaly and anotia. The study

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authors did not define their use of the term "malformed" and it is possible that there were additional undescribed foetal abnormalities in the treated groups.

The study authors statement that "all skeletal structures were present and had normal appearance but were smaller in size" in foetuses with retarded growth is difficult to comprehend, given that anencephaly involves the absence of cranial bones. It is possible that they were referring only to viable foetuses.

The findings of embryolethality and malformation in this study are in contrast to the results of (Hixson *et al.*, 1984a) who observed reduced foetal bw and delayed ossification of cranial bones at 3 mg/kg bw/d in the presence of maternal toxicity, but demonstrated a NOEL of 1 mg/kg bw/d. The discrepancy may arise from the different test materials employed. Hixson *et al.*, (1984a), administered technical grade methamidophos of 70.5% purity obtained from Mobay Chemical Corporation whereas (Hanafy *et al.*, 1986a) utilised a commercial product containing 60% methamidophos and other unidentified constituents.

Astroff BA, Phipps DE, Stuart BP, Landes GK, Mueller RE & Sangha GK (1996a). A dose range-finding developmental toxicity study with MonitorTM technical in the Sprague-Dawley rat. Study No. 94-612-ZA. Toxicology, Agriculture Division, Bayer Corporation, Stilwell, Kansas, USA. Report No. 107406, Dated 15 February 1996. Unpublished. (QA: Yes: GLP and Test Guidelines: FIFRA Guideline 83-3, US-EPA-TSCA 40 CFR Section 798.4900, OECD 1981, Japanese MAFF 59 NohSan No. 4200).

Test Substance:	Methamidophos technical. Batch No. 0067009 Purity: 75.6% Source: Bayer Corporation, Kansas City, Missouri, USA
Test Species:	Rat: Sprague-Dawley, 12-15 wk of age, of an unspecified bw, obtained from Sasco Inc. Omaha, NE, USA
Dose:	0, 0.04, 0.1, 1.0, 4.0 mg/kg bw/d po nominal

Method

The doses selected for this study were based on other studies (Christenson *et al.*, 1991) and (Hixson *et al.*, 1984a) evaluated in this Section. Once found to be spermatozoa positive following mating (GD 0), 30 females were assigned to 1 of 5 treatment groups at 6 females/group. Doses were administered by oral gavage in deionised water at a dosage volume of 5 mL/kg bw on GD 6-20. The dosing volume was adjusted daily on the basis of dam bw during the dosing period. Control females were administered the deionised water vehicle at 5 mL/kg bw.

Inseminated females were observed for moribundity, mortality, behavioural changes and other signs of toxicity twice/d on GD 0-19. Dam bws were measured on GD 0, 2, 4 and 6-20. Food consumption was recorded on GD 2, 4, and 6-20.

On GD 20, 90 min after administration of the final dose, blood was collected via the retroorbital sinus for the determination of plasma and RBC ChE activities. The rats were then terminated by CO_2 asphyxiation and subjected to gross examination. Gravid rats were encephalectomised for subsequent brain ChE activity determination. ChE activities were determined using a modified version of the Ellman method (Ellman *et al.*, 1961; Hackathorn

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et al., 1983). The ovaries were excised, corpora lutea counted and pregnancy determined. Livers, thyroids and intact uteri were removed and weighed. The uterus was opened and resorptions observed were characterised. Foetuses were removed from the uterine wall and each implant was noted. The placentas were trimmed of extraneous material, blotted and weighed. Foetuses were terminated by intracranial injection of 0.01-0.04 mL Fatal Plus (Vortech Pharmaceuticals, Ml, USA). All foetuses were sexed, weighed, externally examined and discarded.

The data was analysed with the litter as the experimental unit. Parametric data were analysed using a univariate ANOVA and then to Dunnett's Test if significant differences were observed. Non-parametric data were first analysed by the Kruskal-Wallis Test and then subjected to Dunn's Test if significant differences were identified. Non-parametric dichotomous data were initially analysed by the Chi-Square Test and if significance was observed between groups by the Fisher's Exact Test with the Bonferroni adjustment. The reproductive and developmental indices used are defined in Appendix IV. All ChE activity/inhibition data was subjected to regression analysis to enable interpolative prediction of the dose that would induce biologically significant inhibition (≥ 20% inhibition) of ChE activity.

Results and discussion

Dose analysis: The homogeneity and 5 wk stability of the test substance was confirmed. The analytical doses used were 0, 0.038, 0.113, 1.07 and 4.58 mg methamidophos/kg bw/d.

Mortalities and clinical signs: No mortalities were observed. Treatment-related clinical signs (muscle fasciculation, tremors) were seen only at 4 mg/kg bw/d in 5/6 rats.

Maternal bw: At 4 mg/kg bw/d, there was a significant ($p \leq 0.01$) mean bw loss of 12 g (cf gain in controls of 5.4 g) over GD 6-7 and a non-statistically significant bw loss over GD 7-8 of 2.5 g (cf gain in controls of 2.6 g). At 4 mg/kg bw/d, bw gain over GD 6-20 and GD 0-20 was significantly less ($p \leq 0.05$) than that of controls (GD 6-20, 55.1 g; GD 0-20, 76 g cf controls 99.9 and 115.9 g respectively). There were no differences between the other 3 treatment groups and controls. The mean bw data included only those dams found pregnant at GD 20.

Gravid uterus wts and net maternal bw gain: At GD 20 following termination, there were no statistically significant differences between the groups in mean gravid uterus wt. At each dose used, 0, 0.04, 0.1, 1 and 4 mg/kg bw/d, the respective mean gravid uterus wts were 72.4, 66.5, 77.4, 77.2 and 56.6 g. The mean gravid uterus wt at 4 mg/kg bw/d was however notably lower than that of the controls. At 4 mg/kg bw/d the mean net maternal bw on GD 20 was significantly less ($p \leq 0.05$) than that of controls (253.4 g cf controls 285.3 g) and the mean gain in net maternal bw over GD 0-20 was significantly less ($p \leq 0.01$) than that of controls (19.4 g cf controls 43.5 g). There were no differences in maternal bw at GD 20 and maternal net bw gain over GD 0-20 between the 3 other treatment groups and controls.

Litter and placental wts: At 4 mg/kg bw/d the mean male and combined sex litter wt on GD 20, was significantly less ($p \leq 0.05$) than that of controls (3.2 and 3.1 g cf controls 4.1 and 3.9 respectively). The mean female litter wt was 3.0 g (cf controls 3.8). There were no statistical diferences between the groups in placental wts. However the mean placental wt at 4 mg/kg

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bw/d was 0.43 g (cf controls 0.55 g). There were no differences in litter and placental wts between the 3 other treatment groups and controls.

Food consumption: At 4 mg/kg bw/d, mean maternal food consumption was significantly less that that of controls over GD 6-7, 7-8, 8-9 ($p \leq 0.01$) and GD 10-11 and 12-13 ($p \leq 0.05$). Over the 5 intervals the respective food intakes were 47.4, 45.3, 53.9, 59.1 and 64.4 g/kg bw/d cf controls 81.6, 76, 75.8, 80.3 and 77.4 g/kg bw/d. There were no differences between the other 3 treatment groups and controls. The mean food consumption data includes only those dams found pregnant at GD 20.

ChE: The mean ChE activities determined are tabulated below. The statistical significance for the plasma ChE determination was considered by the study authors to be due in part to an unusually low plasma ChE activity (0.93 IU/mL) in 1 rat. Without this value the mean plasma ChE activity was 1.83 IU/mL (16% inhibition). A linear interpolation of activity/inhibition data for plasma ChE indicated that the dose predicted to induce 20% inhibition was 0.15 mg/kg bw/d. On these bases, inhibition of plasma ChE activity at 0.04 mg/kg bw/d was not considered statistically or biologically (≥ 20% inhibition) significant. The inhibition of plasma, RBC and brain ChE was dose-dependent and therefore treatment-related with significant inhibition of ChE observed at 0.1 mg/kg bw/d and above.

Mean ChE activity and % inhibition

Origin of ChE	**Mean ChE activity (IU/mL) and % inhibition at each dose (mg/kg bw/d)**				
	0	**0.04**	**0.1**	**1.0**	**4.0**
Plasma	2.17 -	1.65* 24.0	1.61* 26.0	0.68* 69.0	0.15* 93.0
RBC	1.68 -	1.53 9.0	1.51 10.0	0.45* 73.0	0.14* 92.0
Brain	15.0 -	14.3 5.0	13.3* 11.0	7.9* 47.0	3.7* 75.0

* Statistically significantly different from controls (p level not specified; assumed $p \leq 0.05$)

Embryo implantation/resorption: No statistically significant differences between treatment groups and controls were seen in the number of corpora lutea, the number of implantation sites, resorptions or pre- and post-implantation losses.

Litter effects: No statistically significant differences between treatment groups and controls were observed in litter size and the number and proportion of live foetuses/litter

Reproductive parameters: No treatment-related effects on the fertility index, gestation index or mating index were observed.

Foetal external malformations and variations: No malformations or variations were observed in any litter.

Maternal gross necropsy: There were no treatment-related effects on absolute or relative liver or thyroid wts or in the gross appearance of these and other organs.

Conclusion

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(1) The doses proposed for the definitive developmental toxicity study were 0, 0.04, 0.1 and 4 mg/kg bw/d. On the basis of this study, the proposed dose range was expected to produce maternal toxicity at the high dose and no maternal, embryo or foetal effects at the low dose.

Astroff BA, Phipps DE, Stuart BP, Landes GK, Mueller RE, Moore KD & Sangha GK (1996b): A developmental toxicity study with Monitor^{R} technical in the Sprague-Dawley rat. Study No. 95-612-EM. Report No. 107178. Lab: Toxicology, Agriculture Division, Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Bayer Corporation, Kansas City, Missouri, USA. Study Duration: 24 April 1995 - 5 June 1995. Report Date: 17 January 1996. Unpublished. (QA: Yes: GLP and Test Guidelines: FIFRA Guideline 83-3, US-EPA-TSCA 40 CFR Section 798.4900, OECD 1981, Japanese MAFF 59 NohSan No. 4200).

Test Substance:	Methamidophos technical. Batch No. 0067009 Purity: 75.9% Source: Bayer Corporation, Kansas City, Missouri, USA
Test Species:	Rat: Sprague-Dawley, 12-15 wk of age, of an unspecified bw, obtained from Sasco Inc. Omaha, NE, USA)
Dose:	0, 0.04, 0.1, 4.0 mg/kg bw/d po nominal

Method

The doses selected for this study were based on the dose-ranging study (Astroff *et al.*, 1996a) evaluated in this Section. The doses selected were 0, 0.04, 0.1 and 4 mg/kg bw/d. The selected dose range was expected to produce maternal toxicity at the high dose and no maternal, embryo or foetal effects at the low dose. Once found to be spermatozoa positive following mating (GD 0), 144 females were assigned to 1 of 4 treatment groups at 36 females/group. Doses were administered by oral gavage in deionised water at a dosage volume of 5 mL/kg bw on GD 6-15. The dosing volume was adjusted daily on the basis of dam bw during the dosing period. Control females were administered the deionised water vehicle at 5 mL/kg bw.

Inseminated females were observed for moribundity, mortality, behavioural changes and other signs of toxicity twice/d on GD 0-15 or GD 0-19 at 90 min following dosing. Peak behavioural effects were expected at 90 min after dosing (Sheets *et al.*, 1993). Dam bws were measured on GD 0, 2, 4, 6-16, 18 and 20. Food consumption was recorded on GD 2, 4, and 6-16, 18 and 20.

From the 1st 6 pregnant dams at each dose level, 90 min after dosing, blood was collected via the retroorbital sinus for the determination of plasma and RBC ChE activities. Then the dams were terminated by CO_2 asphyxiation. The abdomen was opened and the uterus examined for pregnancy. Gravid dams underwent gross external and internal examination and were encephalectomised for subsequent brain ChE activity determination. All ChE determinations were performed using the methods of (Ellman *et al.*, 1961; Hackathorn *et al.*, 1983). ChE activity was not determined for non-pregnant dams.

On GD 20, the remaining dams were terminated by CO_2 asphyxiation and subjected to gross examination. The abdominal and thoracic cavities were opened and a gross necropsy performed. The ovaries were excised, corpora lutea counted and pregnancy determined. The intact uterus was removed and weighed. The uterus was opened and resorptions observed

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were characterised. Foetuses were removed from the uterine wall and each implant was noted. The placentas were trimmed of extraneous material, blotted and weighed. Foetuses were terminated by intracranial injection of 0.01-0.04 mL Fatal Plus (Vortech Pharmaceuticals, Ml, USA). All foetuses were sexed, weighed and externally examined. One half of all foetuses from each litter were fixed *in toto* in 70% alcohol, eviscerated and evaluated for general skeletal development. The remaining foetuses were subjected to gross visceral examination and placed in Bouin's solution. Prior to foetal cranial examination, the foetuses were transferred to 70% alcohol. Sectioning of the head was performed using the method of (Wilson, 1965).

External, visceral and skeletal findings were considered either malformations or variations. Malformations were defined as defective or abnormal development that may be life-threatening or life-limiting. Spontaneous malformations could occur in 1 to several individuals within a given litter but would not typically occur in several litters within one treatment group unless induced. Variations were defined as alterations in development that are commonly observed in a given strain of rat. These were not considered meaningful unless they occurred in a treatment group, in a dose-dependent fashion and at significantly higher rates than observed in the control group (Khera, 1981).

In addition to dams terminated as scheduled, those found moribund, showing signs of abortion, or delivering prematurely were also terminated and a gross necropsy conducted. Dams scheduled to be terminated and suspected not to be pregnant were terminated and examined for pregnancy. No further examinations were conducted on those not pregnant.

The data was analysed with the litter as the experimental unit. Parametric data were analysed using a univariate ANOVA and then Dunnett's Test if significant differences were observed. Non-parametric data were first analysed by the Kruskal-Wallis Test and then subjected to Dunn's Test if significant differences were identified. Non-parametric dichotomous data were initially analysed by the Chi-Square Test and if significance was observed between groups by the Fisher's Exact Test with the Bonferroni adjustment. The reproductive and developmental indices used are defined in Appendix IV.

Results and discussion

Dose analysis: The homogeneity and 5 wk stability of the test substance was confirmed. The analytical doses were 0, 0.05, 0.14 and 5.49 mg methamidophos/kg bw/d.

Mortalities and clinical signs: No mortalities were observed. Treatment-related muscle fasciculation and tremors were seen in all dams at 4 mg/kg bw/d. Two dams at this dose showed salivation after dosing, 1 on GD 8 and the 2nd on GD 8 and 13. The signs were noted from the onset of dosing to the last d of dosing. Without exception, the signs seen following morning dosing had reversed at the afternoon observation. No signs were seen from GD 16-20. Treatment-related clinical signs were not seen in the other 2 treatment groups or in controls.

Maternal bw: At 4 mg/kg bw/d, there were significant ($p \leq 0.01$) mean bw losses of 6.2, 5.2 and 0.3 g over GD 6-7, 7-8 and 8-9 (cf gain in controls of 2.8, 3.0 and 2.6 g respectively). At 4 mg/kg bw/d, bw gain over GD 6-15 and GD 0-20 was significantly less ($p \leq 0.05$, $P \leq 0.01$ respectively) than that of controls (GD 6-15, 15.7 g; GD 0-20, 120.5 cf controls 38.9 and

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143.1 g respectively). There were no differences between the other 2 treatment groups and controls. The mean bw data included only those dams found pregnant at GD 20.

Gravid uterus wts and net maternal bw gain: At GD 20 following termination, there were no statistically significant differences between the groups in mean gravid uterus wt. At each dose used, 0, 0.04, 0.1 and 4 mg/kg bw/d, the respective mean gravid uterus wts were 78.0, 73.8, 72.9 and 70.9 g. At 4 mg/kg bw/d the mean net maternal bw on GD 20 was significantly less ($p \leq 0.05$) than that of controls (264.0 g cf controls 281.1 g) and the mean gain in net maternal bw over GD 0-20 was significantly less ($p \leq 0.01$) than that of controls (49.6 g cf controls 65.1 g). There were no differences in maternal bw at GD 20 and maternal net bw gain over GD 0-20 between the 2 other treatment groups and controls.

Litter and placental wts: At 4 mg/kg bw/d the mean male and female litter wt on GD 20, was significantly less ($p \leq 0.05$) than that of controls (3.8 and 3.6 g cf controls 4.1 and 3.9 respectively). The mean combined sex litter wt was 3.6 g (cf controls 3.9). The mean placental wt at 4 mg/kg bw/d was significantly less ($p \leq 0.01$) than that of controls (0.52 g cf controls 0.59 g). There were no differences in litter and placental wts between the 2 other treatment groups and controls.

Food consumption: Throughout the dosing period, mean maternal food consumption was significantly depressed at 4 mg/kg bw/d ($p \leq 0.01$), mean maternal food consumption was significantly less that that of controls over GD 6-7, 7-8, 8-9, 10-11, 11-12, 12-13, 13-14, 14-15, 16-18 and 18-20. Over the 10 intervals respective consumption was 69.6, 56.3, 70.9, 85.9, 87.9, 87.6, 84.9, 81.0, 103.9 and 96.8 g /kg bw/d cf controls 93.1, 92.7, 91.3, 95.5, 95.5, 93.9, 90.2, 90.3, 97.2 and 87.6 g/kg bw/d. There were no differences between the other 3 treatment groups and controls. The mean food consumption data includes only those dams found pregnant at GD 20.

ChE: The mean ChE activities determined are tabulated below. Statistically ($p \leq 0.05$) and biologically significant inhibition ($\geq 20\%$) was observed only at 4 mg/kg bw/d.

Mean ChE activity and % inhibition

Origin of ChE	**Mean ChE activity (IU/mL) and % inhibition at each dose (mg/kg bw/d)**			
	0	**0.04**	**0.1**	**4.0**
Plasma	2.66 -	2.71 -	2.30 13.5	0.24* 91.0
RBC	1.10 -	1.16 -	1.08 1.8	0.20* 81.8
Brain	14.5 -	14.4 0.7	13.9 4.1	3.1* 78.6

* Statistically significantly different from controls ($p \leq 0.05$)

Embryo implantation/resorption: No statistically significant differences between treatment groups and controls were seen in the number of corpora lutea, the number of implantation sites, resorptions or pre- and post-implantation losses.

Litter effects: No statistically significant differences between treatment groups and controls were observed in litter size and the number and proportion of live foetuses/litter

Reproductive parameters: No treatment-related effects on the fertility index, gestation index or mating index were observed.

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Foetal external malformations and variations: No treatment-related malformations or variations were observed in any litter.

Foetal visceral malformations and variations: No treatment-related effects on either the foetal or litter incidence of visceral malformations or variations were observed.

Foetal skeletal malformations and variations: No treatment-related malformations were observed. Statistically significantly ($p \leq 0.05$) increased skeletal variations were seen only at 4 mg/kg bw/d. They were incompletely ossified frontal bones, incompletely ossified sacral arches, unossified sacral arches, unossified metacarpals, incompletely ossified sternebrae (3rd, 4th and xiphoid) and unossified 5th sternebra. The incidences of incompletely ossified frontal bones, unossified metacarpals, unossified 5th sternebra and incompletely ossified xiphoid were within the historical control ranges. The incidences of the remaining findings, incompletely ossified sacral arches and incompletely ossified 3rd and 4th sternebrae were outside of the historical control ranges. These changes whether inside or outside historical control ranges were not unexpected because of the reduced mean foetal bw. The study authors considered all of them to be the consequence of foetal immaturity.

Maternal gross necropsy: There were no treatment-related effects observed at necropsy.

Conclusion

(1) Under the conditions of this study, methamidophos was not a primary developmental toxicant.

(2) The maternal and developmental NOEL were both 0.14 mg/kg bw/d.

8.2 Rabbits

Machemer L & Lorke D (1979): SRA 5172 (Methamidophos). Studies of embryotoxic and teratogenic effects on rabbits following oral administration. Mobay Report No. 67990. Study No. 79-T-095. Report No. 8410. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration; 1 June 1978 - 30 September 1978. Report Date: 31 May 1979. Unpublished. (QA: No; GLP & Test Guidelines not specified).

Test Substance:	Methamidophos technical Purity: 62% No further details provided.
Test Species:	Rabbit: Himalayan, sexually mature males and females, of an unspecified age, bw approximately 2-2.5 kg obtained from Dr Karl Thomas, GmbH, Biberach.
Dose:	0, 0.1, 0.5 and 2.5 mg/kg bw po

Method

The day on which copulation occurred twice was specified as GD 0. Male rabbits were not treated. The test substance was administered daily to inseminated females via an oral

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intubation tube from GD 6 to 18 (a total of 13 times). Cremophor emulsion (0.5%) was used as a vehicle for the test substance. All rabbits received their doses in 5 mL vehicle/kg bw. Control rabbits received 5 mL/kg bw of Cremophor emulsion daily.

Observation of the appearance, behaviour and mortality among the dams occurred daily. They were weighed on GD 0, daily during the treatment period and on GD 29. Caesarean section was conducted on GD 29. The method of Caesarean section or the subsequent fate of the dams was not specified. The following parameters were determined: number of implantations, number of living and dead foetuses or embryos, sex, wt of each litter and mean foetus wt/litter, number of stunted foetuses (bw < 25 g), mean placenta wt/litter, and prevalence of external malformations and alterations.

All foetuses were necropsied and the thoracic and abdominal organs evaluated. The method of evaluation was not specified. Brains were extracted whole, cross sectioned with a razor blade, fixed in a solution of alcohol (70%), formalin and glacial acetic acid (80:20:5; v/v) and examined for malformations. The foetuses were cleared with dilute potassium hydrozide. Their skeletal systems were stained with alizarin red S and evaluated. For each dose level the numbers of foetuses examined were 80, 69, 91 and 82 respectively.

The statistical methods used to evaluate results were:-

a. non-parametric ranking method (Wilcoxon, 1947) for wt gain, number of implantations, number of foetuses, number of resorptions, foetus wt and placenta wt,
b. chi-square test for number of foetuses with skeletal alterations or malformations and number stunted, and
c. chi-square test (correction of Yates) or the exact test of Fischer for fertilised and pregnant rabbits.

A difference from control was regarded as statistically significant at a probability of error of less than 5% ($p < 0.05$).

Results and discussion

Clinical signs and mortality: Signs seen were not treatment-related. They were keratoconjunctivitis in 1 control rabbit, temporary diarrhea and constipation in 2 rabbits respectively at 0.1 mg/kg bw and alopecia in the abdominal area of 1 rabbit at 2.5 mg/kg bw. Rabbits at 0.5 mg/kg bw showed no signs. One rabbit of the control group died from pulmonary intubation of the vehicle. A rabbit at 2.5 mg/kg bw died on gestation d 9 from suppurative cystitis.

Copulation and fertilization: The fertilization and pregnancy rates achieved are tabulated below. The methodology for achieving the estimates was not described.

The outcomes of copulation in the dams

Dose Group (mg/kg bw)	Inseminated females	Fertilized females		Pregnant females	
		Total	as % Inseminated	Total	as % Fertilized
0	15	14*	93	13	93
0.1	15	15	100	15	100
0.5	15	15	100	15	100
2.5	15	14*	93	14	100

* 1 dam from each group died and were not included in these determinations

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Body weight gains: The bw gains for each treatment group are tabulated below. Compared to the control group all treatment groups showed lower bw gains. The bw gains of the 2.5 mg/kg/bw group were statistically significantly less than those of the control group. It is apparent that the effect on bw gain over both periods is treatment-related.

Mean bw gain (g) in the dams

Period	Dose Group (mg/kg bw)			
	0[#] ^	**0.1**	**0.5**	**2.5**[#]
Treatment	96.5	35.3	45.3	40.4*
Gestation	231.2	110.7*	156.0	108.6**

* $p < 0.05$ ** $p < 0.01$ [cf control group]
# bw of the 2 dams that died[#] or the 1 that aborted^ at gestation d 26 were not included

Foetal development: There were no statistical differences between the groups with respect to the number of implantations, the number of resorptions, the sex distribution of the foetuses, foetal wt, placental wt and the number of stunted foetuses. Similarly, there were no treatment-related effects on foetal development, including skeletal and visceral abberations or malformations.

Conclusion

Methamidophos technical at doses of 0.1, 0.5 or 2.5 mg/kg po depressed maternal bw gain but did not induce other clinical signs or mortality in the dams. Therefore, there was no NOEL for maternotoxicity. Under the conditions of this study, these doses had no embryotoxic effect or effects on foetal development.

Hoberman AM, Tellone CI, Barnett JF, Lai JC & Christian MS (1996): Oral (stomach tube) developmental toxicity study of Monitor[R] technical in Rabbits. Study No. Argus 222-001 & Valent VP-10143. Report No. not specified. Lab: Argus Research Laboratories, Horsham, Pennsylvania, USA & Lancaster Laboratories Inc, Lancaster, Pennsylvania, USA & Bayer Corporation, Stilwell, Kansas, USA. Sponsor: Valent USA Corporation, Walnut Creek, California, USA. Study Duration: 21 July 1995 - 8 March 1996. Report Date: 8 March 1996. Unpublished. (QA: Yes; GLP and Test Guidelines: FIFRA (40 CFR Part 160), Japanese MAFF (59 NohSan No. 3850) & OECD).

Test Substance:	Methamidophos technical. Lot No. 0067009. Purity: 76%. Source: Bayer Corporation, Stilwell, Kansas, USA.
Test Species:	Rabbit: New Zealand White [Hra:(NZW)SPF], female, 5.5 mo of age, 2.6-4.1 kg bw, obtained from HRP Inc, Denver, Pennsylvania.
Dose:	0, 0.1, 0.5, 2.5 mg/kg bw/d ig nominal; 0, 0.2, 0.65, 2.47 mg/kg bw/d ig analytical.

Method

Mated female rabbits (23/group) were administered the test substance orally via stomach tube daily from GD 6-18. Nominal dosages of 0 (vehicle), 0.1, 0.5 and 2.5 mg/kg bw/d were administered at a dosage volume of 10 mL/kg, adjusted daily on the basis of the individual bws recorded before intubation. They were dosed at the same time each d.

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All rabbits were observed twice/d for general appearance and clinical signs. Additional examinations for abortions, premature deliveries and maternal deaths were conducted immediately before and 60 min after each daily intubation from GD 6-18. These observations were also made once/d during the post-intubation period, GD 19-29. Body wts were measured on GD 0 and daily on GD 6-29. Food consumption values were recorded daily.

All surviving rabbits were terminated by Beuthanasia-D euthanasia solution iv on GD 29. The thoracic, abdominal and pelvic viscera of each rabbit were examined for gross lesions. Gross lesions, except for parovarian cysts which were common spontaneous lesions in rabbits, were preserved in neutral buffered 10% formalin for possible future evaluation. The number of corpora lutea in each ovary was recorded. The uterus was removed, weighed and examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead foetuses. Uteri appearing non-pregnant were stained with 10% ammonium sulfide to confirm the absence of implantation sites. An early resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live foetus was defined as a term foetus that responded to mechanical stimuli. Non-responding term foetuses were considered dead. Dead foetuses and late resorptions were differentiated by the degree of autolysis present. Marked to extreme autolysis indicated that the foetus was a late resorption.

Each Caesarean-delivered foetus was placed in an individual container, weighed, examined for gross external alterations and individually identified with a tag noting litter number, study number and uterine distribution. Live foetuses were terminated with ip Beuthanasia-D euthanasia solution. All foetuses were examined were examined internally to determine sex and for visceral alterations. Cavitated organs including the brain were evaluated by dissection. The brain was cross-sectioned and examined *in situ*. Foetal gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation. All foetuses were eviscerated, stained with alizarin red S and evaluated for skeletal alterations. All skeletal preparations were stored in 80% glycerin with thymol crystals added to retard fungal growth. Late resorptions were examined and retained for future evaluation.

Rabbits that aborted were terminated and examined for the cause of the abortion on the d it occurred. Pregnancy status and uterine contents were observed. Aborted foetuses and late resorptions were examined.

Statistical analyses were performed. Bartlett's test was used to identify homogeneity of variance. Parametric data were subject to ANOVA and Dunnett's test and non-parametric to Kruskal-Wallis test and Dunn's test. All tests were evaluated at $p \leq 0.05$ to $p \leq 0.01$.

Results and discussion

Dosage and test substance stability: Analytical results showed that the actual dosage administered was 0.2, 0.65 or 2.47 mg/kg bw/d. The mean recovery of methamidophos from the 85 d storage stability samples was 94.4%.

Clinical signs: Hyperactivity (thumping of the cage with hindlimbs) was seen in 14/23 rabbits at 2.5 mg/kg bw/d at 2-4 h after treatment and was regarded as treatment-related. It occurred on 1 or 2 d in 9/14 rabbits and on 3-8 d in 5/14.

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Mortality, abortions and premature delivery: No moribund or dead rabbits were observed. One vehicle control group rabbit aborted on GD 28 and 1 rabbit at 0.5 mg/kg bw/d aborted on GD 26. These abortions were not regarded as treatment-related. All other rabbits survived to the scheduled Caesarean-sectioning on GD 29.

Maternal bw changes and gravid uterine wts: The maternal bw gains or losses which were statisitically significantly different ($p \leq 0.01$) from that of controls are tabulated below. The bw losses at 2.5 mg/kg bw/d and the lower bw gains at 0.5 mg/kg bw/d were considered treatment-related. There was no treatment-related effect on mean gravid uterine wts. The mean gravid uterine wts for the controls, 0.1, 0.5 and 2.5 mg/kg bw/d were 443.3, 477.1, 445.8 and 502.7 g respectively. The mean gravid uterine wts for any dose group were not statistically significantly different from controls.

Maternal bw gains or losses during selected intervals

Period (GD)	Changes in maternal bw (kg) from GD 0 to 29 for each dose (mg/kg bw/d)			
	0	**0.1**	**0.5**	**2.5**
0-6	0.06	0.08	0.06	0.06
6-9	0.07	0.06	0.05	-0.01*
6-19	0.32	0.31	0.28	0.2*
19-29	0.23	0.25	0.19	0.24
6-29	0.55 (0.1)	0.56 (0.08)	0.47 (0.03)	0.44 (-0.07*)
0-29	0.61 (0.17)	0.64 (0.16)	0.53 (0.09)	0.49 (-0.01)

( ) Figures in brackets are the gains or losses in maternal bw corrected for gravid uterine wt
* Statistically significantly different ($p \leq 0.01$)

Maternal food consumption: There were no statistically significant differences between the groups in mean absolute (g/rabbit/d) or mean relative (g/kg bw/d) food consumption. The post-intubation (GD 19-29) absolute food consumption was lower at 0.5 and 2.5 mg/kg bw/d (148.8 and 148.5 g/d respectively cf controls 159.5 g/d). The relative food consumption for the same interval at these dose levels was 39.7 and 39.0 g/kg bw/d respectively (cf controls 42.3 g/kg bw/d). The study authors stated that the relative food consumption at 0.5 and 2.5 mg/kg bw/d for other intervals was generally lower than that of controls (GD 6-19; 50.6 and 48.4 g/kg bw/d respectively cf controls 51.4: GD 6-29; 45.7 and 44.1 g/kg bw/d cf controls 47.1). They considered this lower relative food consumption to be treatment-related.

Caesarean-sectioning and litter observations: In the high dose group, the pregnancy rate observed at termination on GD 29 was 17/23 (73.9%) compared to controls 22/23 (95.6%). This was the only pregancy rate in any group which was notably lower than that of controls. This lower rate was considered unrelated to treatment because it was not statistically significantly less than that of controls, it was within historical limits for the facility and the pregnancy rate was unaffected in the dose range finding study at the same dose or at a higher dose (7.5 mg/kg bw/d). The incidence of placental abnormalities, number of corpora lutea, implantations, live foetuses, early and late resorptions, % resorbed conceptuses, % male foetuses, foetal bws and the number of litters with viable foetuses were comparable among the groups and were within the ranges observed historically at the facility. There were therefore no treatment-related effects on these reproductive indices.

Foetal alterations: In the controls, 0.1, 0.5 and 2.5 mg/kg bw/d groups there were 7 (35%), 10 (50%, 14 (70%) and 12 (70.6%) litters respectively which had foetuses with alterations. The total number of foetuses with alterations in each group were 8 (5.4%), 18* (12.2%), 21* (14.6%) and 22* (16.2%) respectively (*significantly different from controls, $p \leq 0.01$). These

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significant differences in foetal incidence were not considered treatment-related because the incidence in the control group was exceptionally low compared to control group incidences of 9-35% in 12 other studies where the same vehicle was used and because there were no statistically significant differences between the groups in litter incidences. Significantly different ($p \leq 0.01$) incidences of intranasal variation (5 foetuses (3.7%), 1 in each of 5 litters (29.4%) cf controls with no occurrence) and misaligned caudal vertebrae (3 foetuses (2.2%), 1 in each of 3 litters (17.6%) cf controls with no occurrence) were observed at 2.5 mg/kg bw/d. These variations were not considered treatment-related because each was a common variation in this strain of rabbit and their occurence was not dose-dependent. The study authors described all foetal alterations in detail. There were no other dose-dependent or statistically different alterations.

Gross necropsy: No treatment-related gross differences were observed in the dams.

Conclusion

(1) Methamidophos administered daily by stomach tube to pregnant rabbits on GD 6-18 caused lower bw gains at 0.6 mg/kg bw/d, clinical signs and bw losses at 2.5 mg/kg bw/d and lowered absolute and relative food consumption at both doses.

(2) There were no treatment-related effects on embryonic or foetal development at any of the doses used.

(3) A maternal NOEL of 0.2 mg/kg bw/d was therefore established by this study.

9. GENOTOXICITY STUDIES

A summary of submitted and published findings of genotoxicity studies is tabulated below.

Summary of *in vitro* Genotoxicity Studies

Assay	Bacterial strain or Cell type	Conc. or Dose	Batch/ Purity	Metab. Act.	Result	Reference
Gene Mutation						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA 1535 TA 1537	Main study 0, 20, 100, 500, 2500, 12500 µg/plate; Repeat tests 0, 100, 500, 2500 µg/plate	ns/ 62.6%	+, - +, - +, - +, -	-, - -, - -, - -, -	(Herbold & Lorke, 1980a)
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA 1535 TA 1537 TA 1538	0.1, 0.5, 1.0, 5.0, 10.0 mg/plate	77-297-149/ns	+, - +, - +, - +, - +.-	-, - -, - -, - -, - -, -	(Machado *et al.*, 1982) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100	1, 100, 10000 µg/plate	ns/90.4%	+, - +, -	-, - -, -	(Guo *et al.*, 1986g)
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA 1535 TA 1537	0, 16, 50, 158, 500, 1581, 5000 µg/plate	2781670 52/73.4%	+, - +, - +, - +, -	-, - -, - -, - -, -	(Herbold, 1994) [GLP]
HGPRT locus forward mutation assay	Chinese hamster ovary cells (CHO-K_1-BH_4)	3.5, 2.0, 1.0, 0.5 and 0.2 µL/mL	77-297-149/ns	+, -	-, -	(Harbell *et al.*, 1990) [GLP]

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HGPRT locus forward mutation assay	Chinese hamster ovary cells (CHO-K_1-BH_4))	1000, 2000, 3000, 4000, 5000 µg/mL	0-06-7009/ 75.6%	+, -	-, -	(Bigger *et al.*, 1993) [GLP]
DNA Damage and Repair						
Pol test	*E coli* (K12)p 3478 W 3110	625, 1250, 2500, 5000, 10000 µg	8083191 01/ 71.2%	+, - +, -	-, - -, -	(Herbold & Lorke, 1983)
Rec assay						
Unscheduled DNA synthesis	Primary rat hepatocytes from male SD rats	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0 µL/mL	77-297-149/ 71.2%	-	-	(Curren *et al.*, 1988) [GLP]
In vivo methylation of guanine	Swiss mice males 20-30 g bw, 3-4 mo old	1.6, 4.4 and 5.3 mg/kg bw	98% (^{14}C methamidophos)	na	+ (7-methyl guanine formed)	(Zayed & Mahdi, 1987)
Chromosomal Effect Assays						
Chromosomal aberration	Red muntjac (*Muntiacus muntjak*) lung fibroblast line KIZ-7901	4.2, 14.1, 42.3 µg/mL	ns/90.4%	ns	+/- *	(Guo *et al.*, 1986g)
Chromosomal aberration	White Swiss mouse spleen cells	0.25, 0.5, 1.0, 2.0 µg/mL	ns/ns (Lab. grade)	-	+	(Amer & Sayed, 1987)
Chromosomal Aberration	Chinese hamster ovary cells (CHO-WBL)	Without activation: 1870, 2500 µg/mL 10 h assay; 2570, 3850, 5140 µg/mL 20 h assay; 3150, 4200, 5250 µg/mL 20 h repeated assay. With activation: 1250, 2500, 3750, 4990 µg/mL 10 h assay	2339903 79/ 74.5%	+,-	-,+/-	(Murli & Spicer, 1990) [GLP]
Sister Chromatid Exchange	Chinese hamster cell line V79	0, 10, 20, 40, 80 µg/mL	ns/99.8%	+	-	(Chen *et al.*, 1982a)
Sister Chromatid Exchange	Chinese hamster cell line V79	0, 10, 20, 40, 80 µg/mL	ns/99.8%	-	+/-[#]	(Chen *et al.*, 1982b)
Sister Chromatid Exchnage	Red muntjac (*Muntiacus muntjak*) diploid cells	4.2, 14.1, 42.3 µg/mL	ns/97%	-	Weak +	(He *et al.*, 1982; Guo *et al.*, 1986g)
Sister Chromatid Exchange	White Swiss mouse spleen cells	0.25, 0.5, 1.0, 2.0 µg/mL	ns/ns (Lab. grade)	-	Weak +	(Amer & Sayed, 1987)

Abbreviations used: HGPRT= Hypoxanthine-guanine phosporibosyl transferase; SD = Sprague-Dawley; ns = not specified; Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation. *No effect on chromosome aberration rate but the number of aberrant types increased - chromatid interchanges, multicentric fragments and disintegration fragments appearing in addition to

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simple fragments and fragment pairs. #No increase in SCE/cell but cell-cycle delay at all doses after 27 h of treatment.

Methamidophos was negative in *in vitro* tests for gene mutation in bacterial (*S. typhimurium*) and mammalian cells and in most tests for DNA damage and repair and in *in vivo* tests for gene mutation and chromosomal effects. It was weakly positive in *in vitro* and *in vivo* tests for sister chromatid exchange (SCE) and a mouse micronucleus test. In tests for DNA damage, 7-methyl guanine was formed following administration of ^{14}C-methamidophos (Zayed & Mahdi, 1987). Some *in vitro* tests for SCE were weakly positive (He *et al*, 1982, reported in Guo *et al*, 1986g; Amer & Sayed, 1987); in these tests, there was no increase in the rate of SCE but the frequency was slightly increased. In the study by Amer & Sayed (1987), there was an increase (approximately 2 x controls) in the mean percentage of metaphases with chromosomal aberrations (tetraploid cells and rings) in cultured spleen cells incubated with up to 2 µg/mL methamidophos.

Summary of *in vivo* Genotoxicity Studies

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Gene Mutation					
Dominant lethal mutation	Mouse (NMRI/ORIG Kissleg)	5 mg/kg bw po	Batch not specified /62.6%	-	(Herbold & Lorke, 1980b)
Dominant lethal mutation	Mouse (CD-1) (ICR derived)	Methamidophos intake averaged 0.45, 4.6, 8.9 mg/kg bw/d po for 5 d	77-297-149/ 74.3%	-	(Eisenlord *et al.*, 1984) [GLP]
Dominant lethal mutation	Mouse (Kunming)	0, 0.2, 2.0 mg/kg bw/d po to males for 7 d pre-mating	ns/90.4 %	-	(Guo *et al.*, 1986g)
Chromosomal Effect Assays					
Micronucleus (marrow cells)	Mouse (NMRI/W 77)	5/sex 5, 10 mg/kg bw po	Batch not specified 62.6%	-	(Herbold & Lorke, 1981)
Micronucleus (marrow cells)	Mouse (Outbred CD-1)	Main: 0.6, 2.0, 6.0 mg/kg bw po (4/sex per dose per obs times 6, 24 & 48 h) Supplemental: 6.0, 9.0, 12.0 mg/kg bw po (4/sex per dose per obs time 24 h)	77-297-149 73.5%	-	(Esber & Carver, 1983) [GLP]
Micronucleus (marrow cells)	Mouse (Kunming)	0, 5.0 mg/kg sc at 24 h intervals	ns/90.4 %	-	(Guo *et al.*, 1986g)
Micronucleus (marrow cells)	Mouse (White Swiss)	ip 6 mg/kg bw once ip 4.5 mg/kg bw 2/wk for 2 wk po 50, 100 ppm in diet Dermal 12, 24 mg/kg bw 2/wk for 2 wk	ns/ns (Lab. grade)	+ + + +	(Amer & Sayed, 1987)
Micronucleaus (marrow cells)	Mouse (Hsd/Win: NMRI)	8 mg/kg bw ip	2784670 30/ 75.1%	-	(Herbold, 1996)

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Sister Chromatid Exchange	Mouse (White Swiss)	4, 6, 8 mg/kg bw ip	ns/ns (Lab. grade)	+	(Amer & Sayed, 1987)
Nucleated marrow cell chromosome aberration	Mouse (Kunming)	0, 1.5, 3.0 mg/kg bw po x 2 and 1.25, 2.5, 5.0 mg/kg sc x 2 at 24 h intervals	ns/90.4 %	-	(Guo *et al*., 1986g)
Nucleated marrow cell chromosome aberration	Rat (ns)	0, 10, 20 ppm in diet for 12 wk	ns/90.4 %	-	(Guo *et al*., 1986g)
Methylation of guanine	Swiss mice males 20-30 g bw, 3-4 mo old	1.6, 4.4 and 5.3 mg/kg bw ip	98% (^{14}C methamidophos)	+ (7-methyl guanine formed)	(Zayed & Mahdi, 1987)

Abbreviations used: obs=observation; Results are expressed as +, positive; -, negative; +/-, equivocal.

Some *in vivo* tests were positive following administration to mice of single doses of 6 mg/kg bw ip or 3 ip injections of 4.5 mg/kg bw over 3 wks, or administration of 50 or 100 ppm in the diet. At these doses, there were significant increases in the percentage of PCEs and slight increases in the numbers of PCEs with micronuclei (about 2 times control), which had returned to normal by 2 weeks after cessation of treatment. A slight but significant increase in the number of SCEs was noted at ip doses of 6 and 8 mg/kg methamidophos (Amer & Sayed, 1987). Taken together, the genotoxicity studies indicate that methamidophos is weakly clastogenic.

10. NEUROTOXICITY STUDIES

10.1 Hens

Kruckenberg SM, Fenwick BW, Brown SM, Kassebaum LJ & Nilson RK (1979): Acute delayed neurotoxicity study on Monitor^R technical. Mobay Report No. 68037. Study No. 79 ANH01. Report No. 68037. Lab: Department of Pathology, Veterinary Medical Centre, Kansas, USA. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: 1 May 1979 - 29 July 1979. Report Date: 29 July 1979. Unpublished. (QA: Yes; GLP: US EPA/FIFRA; Test Guidelines: US Federal Register, paras 58.120 & 163.81-7 (1978)).

[This study had two parts; an acute toxicity (LD_{50}) study and a delayed neurotoxicity study. The results of the acute toxicity study are shown in the acute toxicity tables of Section 3.1.1. The delayed neurotoxicity study is evaluated here]

Test Substance: Methamidophos technical.
Batch Number: 9030005.
Purity: 74%.
Source: Mobay Chemical Corporation, Stilwell, Kansas, USA

Test Species: White Leghorn Chicken: KSU females, 9-18 mo of age, bw not specified, obtained from the Department of Poultry Science, Kansas State University (KSU).

Dose: 0, 30, 34 and 51 mg/kg bw on d 0 and 21; 50 mg/kg bw im atropine simultaneously.

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Method

The hens were kept individually in laying cages (Harford Metal Company, Maryland) under standard environmental conditions. They were fed laying mash and water *ad libitum* prior to and during the 42 d of the study. The food was free of chemical contamination. The hens were randomly divided into treatment groups. There were 10 hens at 30 mg/kg, 6 at 34 mg/kg and 12 at 51 mg/kg. The positive and negative control groups contained 10 and 8 hens respectively. The positive control substance was triorthocresylphosphate (TOCP) (Eastman Organic Chemicals, New York). The dose used was 500 mg/kg on d 0 with atropine simultaneously at 50 mg/kg im. The doses of the test substance equalled or exceeded its LD_{50} in this strain of hen (30 mg/kg po) as established in the first part of this study. The test substance was administered diluted with an unspecified vehicle so that the "total volume was equal to 1% of the hens' wt". It was administered by gastric intubation without fasting.

Daily observations were made for behavioural abnormalities, morbidity, locomotor ataxia, paralysis or other pharmacological or toxicological sign. Food consumption and bw were measured every 3 d.

Test and control hens were subjected to gross necropsy at their death or when killed. All hens were killed at d 42 except for 4 negative control and 8 positive control hens killed on d 37. Killing was by exsanguination following anaesthesia with sodium pentobarbitone iv. Twenty nine of the hens killed were prepared for histopathological examination. They consisted of 7, 9, 6, 3 and 4 hens from the negative control, positive control, 30, 34 and 51 mg/kg groups respectively. Preparation for histological examination involved *post-mortem* whole-body perfusion of warm 10% buffered neutral formalin until the musculature stiffened. The vertebral column was dissected out and divided at the thoracic lumbar junction. The distal end of the sciatic nerve with the proximal portions of the peroneal and tibial nerves were dissected from the medial aspect. These were left attached to the surrounding muscle and fixed in formalin. The vertebral column was then immersed in 10% buffered neutral formalin for 7 d. Then the spinal cord was dissected free. Six transverse sections and 3 longitudinal sections of the cervical region, 2 transverse sections and 1 longitudinal section of the thoracic region, 2 transverse sections of the lumbo-sacral region and a longitudinal section of peripheral nerve at the most distal end were made. Ten micron sections were cut, mounted and stained with haematoxylin and eosin and luxol-fast-blue.

Statistical procedures were not used in the evaluation of results.

Results and discussion

Clinical signs and mortality: The negative control hens showed no clinical signs. Among the positive control hens, 7/10 developed unsteadiness (leg weakness). The leg weakness progressed to paresis resulting in immobility. One hen was killed moribund on d 34. Six/9 developed signs of delayed neurotoxicity. Three had not shown signs of delayed neurotoxicity by d 36. Among the test groups no signs of delayed neurotoxicity were observed. Signs of acute toxicity occurred "in most cases" within the test groups but disappeared within 24 h. A "few" hens vomited following treatment. The signs were not specified.

The occurrence of acute mortality is tabulated below. The incidence of mortality was positively correlated with dose, while the latency of death decreased with increasing dose.

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The incidence of treatment related mortality*

Dose Group (mg/kg bw)	Mortality
0	0/8
30	1/10 on d 1, 1/9 on d 25
34	3/6 on d 1
51	2/12 on d 1, 1/10 on d 5 and 1/9 on d 15
TOCP 500	1/10 on d 25 (preceeded by several days of moribundity)

* Antidotal protection provided by simultaneously administered atropine at 50 mg/kg bw im

Feed consumption and bw: Food consumption in the negative control group was not affected. In the positive control group it decreased from a mean of 183.8 g on d 1 to a mean of 151.4 g on d 34 - a difference of 32.4 g. The change in mean food consumption from d 1 to 42 for the 30, 34 and 51 mg/kg groups was + 0.65, - 48.0 and + 6 g respectively. No conclusions can be drawn about the effect of the test substance on food consumption. There were no differences between the groups in mean bw.

Histopathology of the nervous system: Clinical delayed neurotoxicity was not observed in the negative control or test groups and there was no evidence of neuronal degeneration more significant than that normally found in hens of this strain and age. The relationship between the occurrence of clinical delayed neurotoxicity and the severity of histopathological findings in the positive control group is tabulated below. Spinal cord tissue from 5 of the TOCP treated hens showed marked Wallerian degeneration of neurons.

The occurrence of clinical delayed neurotoxicity and histopathological findings in nervous system in the positive control group

Dose Group (mg/kg bw)	No. hens	Clinical delayed neurotoxicity	Severity of lesions observed (No. hens)			
			N	S	M	Mk
TOCP 500	9	Yes 6	-	-	1	5
		No 3	-	1	2	-

N = no significant lesions, S = slight lesions, M = moderate lesions, Mk = marked lesions

No other pathological findings were reported.

Conclusion

Under the conditions of this study, methamidophos-related clinical delayed neurotoxicity or histological neuronal changes were not observed.

Thyssen JH, Eben A & Lorke D (1982): Methamidophos (Tamaron active ingredient) and Tamaron, Sri Lanka formulation. Special study for neurotoxic effects on the chicken. Mobay Report No. 87437. Study No. T 8005724. Report No. 10815. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 20 April 1982. Unpublished. (QA: No; GLP & Test Guidelines not specified).

This study followed the publication:-
Senanayake N & Johnson MK (1982): Acute polyneuropathy after poisoning by a new organophosphate insecticide. The New England Journal of Medicine, 306(3):155-157.
The evaluation of this article is presented in section 11.4 of this review.

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Test Substances:	Methamidophos technical (Tamaron™ technical BR) Batch Number: 808018244. Purity: 74.6% methamidophos technical. Tamaron™ Fl Identification Code: 1376/476, Sri Lanka formulation. Concentration: 52.4% methamidophos technical in glycol monomethyl ether.
Test Species:	White Leghorn: VALO leghorn SPF, female, approximately 1 yr of age, bw 1.2-2 kg, obtained from Lohmann Co., Cuxhaven.
Doses/Study:	Pilot study: 30 mg/kg bw/d for 5 d po Tamaron™ technical BR. Clinical and NTE determinations Tamaron™ technical BR: 25 mg/kg bw/d for 5 d po. Clinical determinations Tamaron™ technical BR: 30 mg/kg bw/d for 5 d po, repeated after 10 d; 35 mg/kg bw/d for 5 d po, repeated after 10 d. Clinical and NTE determinations Tamaron™ F1: 35.7 mg/kg bw/d for 5 d po (equivalent to 25 mg/kg bw/d for 5 d po of pure methamidophos technical)

Method

The hens were kept in a heated run with access to an open air run with a natural floor. They were acclimatised for 1 wk prior to the study. They were fed poultry grain (Hoeveler Co.) and water *ad libitum*.

The test substances were formulated in distilled water and administered to hens orally by stomach tube at a volume of 2.5 mL/kg bw. Atropine sulfate was simultaneously administered with each treatment as antidote, at 50 mg/kg bw im. The post-treatment observation period was at least 6 wk. Four separate studies were carried out. They were a pilot study using Tamaron™ technical BR (5 hens), clinical and neurotoxic esterase (NTE) determinations using Tamaron™ technical BR (26 hens), clinical determinations using Tamaron™ technical BR (2 groups of 30 and 20 hens respectively) and clinical and NTE determinations using Tamaron™ Fl (25 hens). The studies were conducted in the sequence shown. Control hens also received atropine sulfate at the same dose as the treated hens.

Determinations (Johnson, 1977) of NTE activity in brain, spinal cord and sciatic nerve were carried out. For every 2 test hens, 2 atropine treated controls were used for comparison. NTE determinations were conducted in that sequence throughout the studies. The NTE determinations for control hens were not provided. Hens were killed at the appropriate interval by decapitation and exsanguination. The nerve tissue was removed and immediately placed in vessels cooled with iced water.

The need for the second and third study was determined by the outcome of the first and second respectively. Statistical evaluation of results was not conducted. Pathological examination of nerve tissue was not performed.

Results and discussion

(1) Pilot Study using Tamaron™ technical BR.

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Three of 5 hens died 4-6 d after the start of treatment showing acute cholinergic signs. One hen was killed after the 5th treatment because it was moribund. The fifth hen was observed for 63 d. It showed non-specific disturbed behaviour (lassitude, ungroomed plumage) for 15 d and dyspnoea. After sedation these signs ceased. The hen killed and another which died showed the following NTE % inhibition (cf atropine treated controls). The hen killed: brain 85%, spinal cord 70% and sciatic nerve 100%. The hen which died: brain 94%, spinal cord 85% and sciatic nerve 63%.

The high mortality rate in this study precluded an assessment of the potential neurotoxic effect. The next study was performed at lower doses.

(2) Clinical and neurotoxic esterase (NTE) determinations using Tamaron™ technical BR.

Preliminary note: The study authors indicated that 25 hens were used in this study. They indicated that 4 died at the 4th to 5th treatment d. They then specified 8 moribund hens (2/test date) as being killed to determine NTE inhibition. Since there were 5 treatment d, 2/test date should have equalled 10 hens. The term "moribund" was not defined but if the general meaning of the word is accepted it is difficult to understand how such hens survived for as long as 38 d post-treatment. Thirteen surviving hens were observed for 42 d post-treatment. They then tabled NTE results for 10 hens (2/post-treatment date).

Four of 26 hens died 4-5 d after the start of treatment showing acute cholinergic signs. Eight moribund hens (2/test date) were killed to determine NTE inhibition at 1, 2, 3, 5 and 38 d after the end of treatment. Eight control hens were used to establish NTE activity but the results obtained were not specified. The 13 surviving hens were observed for 42 d after treatment. The % NTE inhibition (cf atropine treated controls) in the 8 (10) birds is tabulated below. Very severe to severe inhibition was noted in the hens' brains and spinal cords for 5 d after the end of treatment. At 1 d the % NTE inhibition was very severe in both hens. At 2 and 3 d, 1 hen still exhibited severe inhibition. From d 5 onwards no NTE inhibition in sciatic nerve was observed.

% NTE inhibition in hens following repeated doses of Tamaron™ technical BR over 5 d

	Days after last administration									
	1		**2**		**3**		**5**		**38**	
Hen No.	**7**	**25**	**12**	**27**	**9**	**13**	**14**	**28**	**6**	**16**
Brain	90.9	90.9	86.0	81.4	70.8	75.0	51.3	61.5	17.9	16.1
Spinal cord	80.0	80.0	80.0	80.0	70.4	70.4	68.2	63.6	0	0
Sciatic nerve	89.0	100	14.3	71.4	80.0	10.0	0	0	0	0

The surviving hens showed disturbed behaviour, sedation and recumbancy on stomach and side for up to 9 d after treatment ceased. After this time the signs subsided. There were no signs of neurotoxic delayed paralysis.

Because the moribund birds killed for % NTE inhibition determinations failed to show neurotoxic effects in the face of distinct inhibition of NTE, it was not clear if signs of delayed neurotoxicity could have developed. As a result the next study using the maximum possible doses was developed.

(3) Clinical determinations using Tamaron™ technical BR.

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After the first series of treatments at 30 mg/kg bw/d, 10/30 hens died at 3-8 d. Two of the 20 surviving hens died at 4-5 d after the start of the second series of treatments at the same dose. The rest showed acute cholinergic signs which persisted for 10 d after the last treatment. They were observed for a total of 51 d. There were no signs of delayed neurotoxicity.

After the two series of treatments at 35 mg/kg bw/d, 14/20 hens died after showing acute cholinergic signs. Survivors were observed for a total of 42 d. This second group did not show signs of delayed neurotoxicity.

(4) Clinical and NTE determinations using Tamaron™ F1.

Eight of 25 hens died on the 3rd to 5th treatment d showing acute cholinergic signs. Eight (10) "moribund" hens were examined for brain, spinal cord and sciatic nerve NTE activity. Two "moribund" hens were examined at 1, 2, 3, 5 and 38 d after the end of treatment. The % NTE inhibition (cf atropine treated controls) is tabulated below. Very severe to severe NTE inhibition occurred in all tissues for 3 d after the end of treatment.

% NTE inhibition in hens following repeated doses of Tamaron™ F1 over 5 d

	Days after last administration									
	1		**2**		**3**		**5**		**38**	
Hen No.	**5**	**24**	**13**	**14**	**7**	**12**	**10**	**21**	**22**	**23**
Brain	76.9	87.2	63.2	78.9	72.5	74.4	46.2	59.0	0	0
Spinal cord	57.1	78.6	72.7	100	62.5	62.5	28.6	57.1	0	0
Sciatic nerve	80.8	60.0	100	75.0	66.7	100	0	50.0	0	16.7

The remaining 9 hens were observed for 42 d after the end of treatment. Their acute cholinergic signs subsided after 10 d. No indications of delayed neurotoxic effect were noted.

Both test compounds caused largely similar inhibition of NTE activity in brain, spinal cord and sciatic nerve. The inhibition was most pronounced in the first 3 d after the end of treatment. It then subsided during the rest of the observation periods. Those hens which died did so after showing acute cholinergic signs. Surviving hens showed signs of acute poisoning (disturbed behaviour, apathy and sedation) for up to 14 d. There were no indications of a delayed neurotoxic effect.

Compared with an equivalent dose of methamidophos technical, Tamaron™ Fl caused slightly less brain and spinal cord NTE inhibition and a shorter duration of brain NTE inhibition which had regained control levels by post-treatment at d 38. However NTE inhibition in the sciatic nerve was present 38 d after treatment with Tamaron™ Fl, whereas it was not observed for more than 3 d after administration of methamidophos technical. Given the small group size (n = 2) for NTE measurement, it is not clear whether these differences are caused by random variation, or are biologically significant

Conclusion

This study showed that neither methamidophos technical nor Tamaron™ Fl caused symptoms of delayed neurotoxic effects in chickens and failed to confirm earlier observations made in humans in Sri Lanka (Senanayake & Johnson, 1982). It did show however NTE inhibition in brain at 38 d after treatment with methamidophos technical and in sciatic nerve 38 d after treatment with Tamaron™ Fl.

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Comment

(1) The achieved doses of methamidophos in the human poisoning incidents in Sri Lanka (Senanayake & Johnson, 1982) ranged from single oral 150 mg/kg bw to single oral 800 mg/kg. The person receiving the latter dose was given within 24 h, 270 mg atropine as protection. The highest dose regime administered in this study (4th part) involved single oral doses of 30 mg/kg bw/d for 5 d repeated after 10 d. Each administration was accompanied by a simultaneously administered 50 mg/kg bw atropine. Hens in this study received 300 mg/kg bw over 20 d with atropine protection. The degree of NTE inhibition in hens showed the potential for delayed neurotoxicity and the lack of associated histopathological studies was a significant limitation on the conclusions possible.

(2) These findings also contrast with the unpublished work of Johnson (1980), conducted following the Sri Lankan episodes. It was established then that the Sri Lankan formulation produced more marked NTE activity inhibition than pure methamidophos given in equivalent doses in hens.

Flucke W, Kaliner G & Machemer L (1985b): SRA 5172 TA (Tamaron TA) (c.n. methamidophos). Study for acute neurotoxicity to the chicken after dermal administration. Study No. T 8019936. Report No. 13852. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 1 May 1985 - 31 July 1985. Report Date: 1 October 1985. Unpublished. (QA: Yes; GLP: OECD Bundesanzeiger 35:3-16 (1983); Test Guidelines: US EPA).

Test Substance:	Methamidophos technical. Batch Number: 233490699. Purity: 73.3%. Physical state: not specified. Source: Bayer AG, Leverkusen, Germany
Test Species:	White Leghorn Chickens: female, 7-10 mo old, 1.45 to 1.7 kg, obtained from breeder Brinkschulte, Senden.
Dose:	200 mg/kg bw repeated at 3 wk; applied dermally.

Method

The hens were acclimatised for 8 wk prior to the start of the study. They were conventionally kept under standard environmental conditions. They were fed LA Eierblitz Type 7.4 for laying hens (Rheinkrone-Kraftfutterwerke Co, Gebr. Huebers, Wesel), and drinking water *ad libitum*. The hens were weighed and randomly allocated to the various groups which consisted of negative control, test substance and positive control groups containing 6, 30 and 5 hens respectively. The test-substance was administered in concentrated form. The positive control substance was triorthocresylphosphate (isomer mixture) (TOCP, batch number 25 E 5268, purity 99%). TOCP was formulated at 7.5% in demineralised water with Cremophor EL (2% v/v) and applied at a rate of 5 mL/kg bw. Atropine sulfate was dissolved at 10% or 6% demineralised water and applied at 0.5 mL/kg bw.

Each 200 mg of test substance was divided into 4 equal portions of 50 mg which were applied via microscopic slides to the concave protected side of the comb at 1.5 h intervals. A further

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200 mg was applied in the same way 3 wk later. The treatment area was not covered and the test substance was not removed. Atropine sulfate was given at 50 mg/kg bw im immediately before the first 50 mg of test substance and at 50 mg/kg bw sc immediately before the 4th 50 mg increment of test-substance. Atropine sulfate was also given at 30 mg/kg bw sc twice/d on the two following days. This pattern of atropine sulfate administration was repeated at the second administration of test substance. TOCP was administered once po (stomach tube) at 375 mg/kg bw.

The appearance and behaviour of all hens were recorded several times/d during treatments and then at least every d between treatments. Observations continued every d for 21 d after the 2nd treatment. Motor co-ordination of individual hens was checked twice/wk. Each hen was placed on the floor and chased for 2-3 min around an area of 100 m^2. Movement was graded on the following scale:-

0 = normal.
1 = slightly abnormal gait.
2 = ataxia/disturbed co-ordination of movement.
3 = severe ataxia/paresis (frequent collapse of legs).
4 = complete paralysis.

Body wt was recorded before the first application and then once/wk. All the hens that died were necropsied. At termination survivors and negative controls were anaesthetised with sodium hexabarbitone, grossly appraised, exsanguinated by perfusing with physiological saline solution and finally fixed by perfusion with 10% aqueous formaldehyde solution. Positive control hens were subjected to the same procedures on d 20 after exhibiting severe signs and moribundity. Nerve tissue (proximal sciatic nerve and distal branches; brain *in toto* and spinal cord *in toto*) was removed from all negative and positive control hens and 6 terminated test substance treated hens and placed in 10% aqueous formaldehyde. Sections of the nerve tissue were treated with haemalum eosin, luxol blue and axon silver (Novotny & Novotny, 1974).

Statistical evaluations of results were not performed.

Results and discussion

Clinical signs and mortality: The signs seen in test substance-treated hens were staggering gait, lassitude (drooping wings), reduced mobility extending to prostration, fluffed plumage, dyspnoea and apathy. Signs persisted for 10 min to 7 d. After the 1st administration of test-substance all hens showed signs. Ten died between 6 h and 12 d - a mortality rate of 33% (5 died at 6 h, 4 on d 1 and 1 on d 12 - this last death was the result of cannibalism). After the 2nd administration all remaining hens showed signs. One hen died on d 2 - a mortality rate of 5%. Signs of delayed neurotoxicity were not observed. TOCP-treated hens showed a progressive disturbance of movement co-ordination (ataxia to paresis) from d 13-20. On d 20 they were terminated. The negative controls showed no signs or mortalities.

Forced movement signs: None of the negative control or test substance-treated hens rated more than 0. Up to and including d 10 all TOCP-treated hens rated 0. On d 13 all rated 1 and on d 14, 15 and 17 all rated 3.

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Body wt: Mean bw measurements are tabulated below. There was a continuous decline in bw in the positive controls. A decline in bw at wk 1 and 4 in the test substance treated hens suggested that a treatment-related effect occurred.

Mean bw measurements*

Group	Mean bw (kg) for each group at the times shown					
	d 0	wk 1	wk 2	wk 3	wk 4	wk 6
Negative control	1.60	1.54	1.59	1.62	1.67	1.63
Test substance treated	1.60	1.40	1.49	1.47	1.31	1.49
Positive control	1.57	1.46	1.32	1.14	-	-

* The measurement for wk 5 is not shown

Gross necropsy: There were no differences in gross findings between the negative controls and the test substance-treated hens. There were no organ alterations in the TOCP-treated hens which could be attributed to TOCP.

Histopathology: In the TOCP-treated hens, there were signs of fibre damage in the sciatic nerve and in the cervical, thoracic and lumbar areas of the spinal cord. The myelin was missing or fragmented in the fibre segments and the axon was swollen or lytic. The Schwann cells of the fibres were activated. These changes were not observed in the other 2 groups. In all 3 groups there were no findings apart from minimal alterations in the medulla oblongata and cerebellum of the brain. There were no histological differences in nerve tissue beween the negative controls and the test substance-treated hens. A test substance-related effect on the brain or nerve tissue was not observed.

Conclusion

Under the conditions of this study the dermal administration of 200 mg/kg bw on 2 occasions 21 d apart to female White Leghorn Chickens did not induce delayed neurotoxicity.

Guo L, Chen Y, Lu R, Liu Y, Xia L, Chen J, Zhang X & Mao J (1986e): Acute, cumulative and sub-chronic toxicity, delayed neurotoxicity and experimental treatment of intoxication. Part 4: Delayed neurotoxicity. Zhonghua Laodong-Weisheng Zhiyebing Zazhi, 4(5):257-260.

Test Substance:	Methamidophos "direct isomerisation crystal". Purity: 90.4% Source: Zhejiang Research Institute.
Test Species:	White Leghorn hen: 1-1.7 kg bw, 8-12 mo old, obtained from an unspecified source.
Dose:	50 mg/kg bw po

Method

The housing, environmental conditions and diet of the hens were not described. A group of 11 chickens were each drip fed a single oral dose of an aqueous solution of methamidophos. They were protected with 5 mg/kg bw atropine sulfate im and 25 mg/kg bw pralidoxime iodide im 15 min before and 3 and 6 h after dosing with methamidophos. Positive control hens were given 0.8 mL/kg (7 hens) or 0.3 mL/kg (1 hen) po of an unspecified concentration of TOCP and 8 untreated hens were designated negative controls. The neurotoxic signs were

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assessed using previously established criteria (Abou-Donia, 1979). Food consumption and bw were measured at an unspecified frequency. The post-dosing observation period was not specified.

At termination, the hens were killed by exsanguination via the carotid artery. Three sections of cervical, thoracic and lumbar spinal cord and 3 sections of ischial nerve were removed from an unspecified number of carcasses, fixed and prepared for histological examination using haematotoxylin-eosin and luxol fast blue, silver or crystal violet staining.

Results

Clinical signs and mortalities: Signs of delayed neurotoxicity were not observed in methamidophos treated hens. Signs of acute toxicity or mortality were not discussed.

The positive control group experienced an unspecified decrease in food consumption, an unspecified loss of bw within "several" d of dosing and developed neurotoxic signs. The study authors indicated that the rate of onset and severity of the signs in TOCP treated hens were dose-related. Ataxia appeared 8-11 d after dosing with TOCP. Ataxia progressed to motor paralysis at 12-32 d with concurrent dyspnoea and dysphagia. No abnormalities were observed in the negative control group.

Neurohistological observations: No abnormalities were observed in the treratment or negative control goups. Varying degrees of degeneration were observed in spinal cord and nerve of all positive control hens. No further details were provided.

Conclusion

Delayed neurotoxicity was not observed in hens following the oral administration of 50 mg/kg bw methamidophos.

Comment

The reliability of the study is reduced by the limited detail in which the methods and results were described.

Sachsse K, Oharek A, Zbinden K, Madoerin K, Luetkemeier H, Wilson J, Terrier C, Vogel W & Machemer L (1987): 3-month subchronic delayed neurotoxicity study with SRA 5172 (c.n. methamidophos) in the hen. Mobay Report No. 94213. RCC Project No. 064293, Bayer Project No. T 0022114. Report No. 94213. Lab: KFM Kleintierfarm Madoerin AG CH 4414 Fuellinsdorf, Switzerland, RCC Research and Consulting Company AG CH 4452 Itingen, Switzerland & RCC Umweltchemie AG CH 4452 Itingen, Switzerland. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 31 March 1986 - 14 July 1986. Report Date: 15 May 1987. Unpublished. (QA: Yes; GLP: not specified; Test Guidelines: US EPA/FIFRA/Pesticide Assessment Guidelines/OECD/UK Pesticide Safety Scheme/Swiss IKS).

Test Substance: Methamidophos technical.
Batch Number: 808 525 298
Purity: 76.0%
Source: not specified

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Test Species: White Leghorn Chicken: SPF derived, females, 12 mo of age, 1.4 - 2.2 kg bw, obtained from Lohmann Tierzucht GmbH, am Seedeich 9, D 2190 Cuxhaven, FRG.
Dose: 0, 0.3, 1, 3 mg/kg bw/d for 5 d/wk for 3 mo po.

Method

The hens were acclimatised for 1 wk under test conditions and subjected to a health examination. Those chosen were free from apparent viral diseases and were unmedicated. They had no abnormalities of gait. The hens were kept under standard environmental conditions and were housed 4/cage. They were fed Kliba (Klingentalmeehle AG, 4303 Kaiser-augst/Switzerland) Legehennen Starterfutter (Mehl) Nr 565 and tap water *ad libitum*. The hens were randomly allocated to two experimental units; A, hens for delayed neurotoxicity and ChE determination (10/dose group) and B, hens for determination of neuropathy target esterase (NTE) activity (6/dose group).

Preparations of the test substance were made immediately prior to each dosing. The vehicle used was distilled water. The test substance was administered at a volume of 2 mL/kg bw. The stability of the test substance dilution was determined once at study commencment. The purity of the test article was determined by HPLC on a monthly basis.

Each hen was observed twice/d for changes in appearance and behaviour over the study period. All abnormalities were recorded. Body wt was recorded weekly. Motor co-ordination of individual hens was checked twice/wk. Each hen was removed from its cage and placed on the floor. Locomotion was induced for 2-3 min to ascertain any abnormality of gait (specifically for locomotor ataxia and/or paralysis). The hens were assessed on the following scale:-
0 = no sign of ataxia; motor activity and normal co-ordinated locomotion.
1 = motor activity slightly reduced; occasionally unco-ordinated, particularly after phases of increased motor activity.
2 = motor activity markedly reduced; stilted gait as well as occasional stumbling, clumsy landing (with the help of the tail) after jumping from a height of 0.5 - 1 m.
3 = motor activity considerably reduced; hen sitting on hocks, only shuffling gait possible.
4 = hen unable to stand; total paralysis, lying on abdomen or side with spread wings.

The schedule for the determinations of plasma butyryl ChE (BuChE) and neuropathy target esterase (NTE) was as follows. Blood was collected from the vena cutanea ulnaris from A unit hens at pretest and at 4, 8 and 12 wk. Brain and spinal cord was collected at termination from B unit hens. The method used to determine NTE has been described (Johnson, 1977; Johnson & Richardson, 1983). The study authors regarded the threshold of NTE-inhibition initiating severe neuropathy in hens as 45-65% inhibition (Johnson, 1982).

At termination, A and B hens were anaesthetised by iv injection of sodium pentobarbitone. Unit A hens were then flushed with Ringer's solution and perfused with neutral buffered 4% formaldehyde solution. After perfusion, brain (cerebrum, cerebellum and medulla oblongata), spinal cord (upper cervical bulb, mid-thoracic and lumbo-sacral levels), sciatic and tibial nerves were dissected and placed in neutral buffered 4% formaldehyde solution for 48 h. Tissue samples were embedded in paraffin wax transversely (cerebrum, medulla oblongata, 3 levels of spinal cord, sciatic and tibial nerves) or longitudinally (cerebellum, 3 levels of spinal cord, sciatic and tibial nerves). The paraffin blocks were cut at 5 µ and stained with

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haematoxylin and eosin, luxol fast blue for myelin sheaths and Bodian's silver method for axons. Brains and spinal cords were removed from unit B hens for NTE determination.

Univariate one-way analysis of variance was used to assess the signifance of intergroup differences. If the variables could be assumed to follow a normal distribution, the Dunnett-test based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups. The Steel-test was applied when the data could not be assumed to follow a normal distribution. Group means were calculated for continuous data and medians were calculated for discrete data. Test statistics were calculated for exact values for means and pooled variances and then rounded off to two decimal places. It was therefore possible to record different statistic values for similar means.

Results and discussion

Clinical signs and mortality: No treatment-related signs were noted in control hens or hens receiving 0.3 and 1 mg/kg. Hens at 3 mg/kg showed somnolence and slight emaciation from d 10 onwards. There were 4 deaths, 2 in control hens on d 36 and 57 respectively and 2 at 3 mg/kg both on d 75. The mortalities were not treatment-related. The forced locomotion response test showed that all hens rated 0. There was no sign of ataxia. Motor activity with co-ordinated locomotion was observed.

Body wt at selected times for control hens and hens at 3 mg/kg bw. At 3 mg/kg the weekly mean bw of hens was statistically significantly lower than that of controls each wk from wk 3 to wk 14 ($p \leq 0.01$). This was most likely a treatment-related response. Otherwise there were no differences that could be attributed to the test substance.

Treatment-related effects on mean weekly bw*

Dose mg/kg bw	**Mean weekly bw (g) at selected wk for control and 3 mg/kg bw hens**				
	1	**3**	**7**	**11**	**14**
0	1738	1763	1858	1871	1833
3	1808	1533	1528	1396	1473

* Statistically significant differences between the groups at all times except wk 1 ($p \leq 0.01$)

Gross necropsy and histopathological findings: The gross examination results of the 4 hens that died were presented. There were no gross findings implicating the test substance. Histopathological findings in brain and nerve tissue in hens terminated as scheduled occurred in all groups at a low incidence and severity. Treatment-related effects were not observed.

Plasma BuChE and NTE activity: Plasma BuChE and brain and nerve tissue NTE determinations are tabulated below. At 1 and 3 mg/kg there was a treatment-related and dose-dependent inhibition of plasma BuChE. Spinal cord NTE showed the same pattern of inhibition. Brain NTE activity was statistically significantly less than controls at 3 mg/kg.

The effect of methamidophos on plasma BuChE and brain and nerve tissue NTE activity

Time	**Mean BuChE and NTE activity and % of controls in hens at each dose level (mg/kg bw) at specified times**			
	0	**0.3**	**1**	**3**
Plasma BuChE (µmol-SH/mL/min)				
Pretest	2.38 100	2.84 119	2.59 109	2.36 99

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4 wk	2.26 100	2.20 97	1.74 77	1.18* 52
8 wk	2.42 100	2.26 93	1.76* 73	1.19* 49
12 wk	2.13 100	2.21 103	1.76 83	1.19* 56
NTE (µg Phenol/mL reaction mixture)				
12 wk brain	5.3 100	5.8 110	5.2 98	4.4* 83
12 wk spinal cord	4.1 100	3.8 94	3.2* 78	2.4* 58

* Statistically significantly different from control ($p \leq 0.05$)

Conclusion

(1) Under the conditions of this study, doses of methamidophos of up to 3 mg/kg bw/d 5 d/wk for 3 mo po did not induce delayed neuropathy in hens. The highest degree of NTE inhibition recorded was 42% in spinal cord at 12 wk at 3 mg/kg bw/d.

(2) On the basis of bw depression, clinical signs, biologically significant inhibition (≥ 20% inhibition) of plasma BuChE activity and NTE inhibition at 1 mg/kg bw/d and above, this study established a NOEL of 0.3 mg/kg bw/d.

Vilanova E, Johnson MK & Vicedo JL (1987): Interaction of some unsubstituted phosphoramidate analogs of methamidophos (O,S-Dimethyl phosphorothioamidate) with acetylcholinesterase and neuropathy target esterase in hen brain. Pesticide Biochemistry and Physiology, 28(2):224-238.

Test Chemicals: S-Me series analogs:
Methamidophos laboratory grade.
(*O*-methyl *S*-methyl phorothioamidate)
Purity: 99%.
Other *O*-alkyl *S*-methyl phosphorothioamidates; alkyl = ethyl, purity 95%, *n*-propyl, 90%, *iso*-propyl, 99%, *n*-butyl, 99%, *n*-hexyl, 99%.
DCP series analogs:
O-alkyl *O*-2,5-dichlorophenyl phosphoramidates; alkyl = methyl, purity 95%, ethyl, 95-99%, *n*-butyl, 90-95%, *n*-hexyl 95%.
Source: Bayer Chemical Company, Wuppertal, Germany.

Test Species: Hen: no further details provided.

Method

Stock solutions of test substances were made as required in dry acetone or ethanol and stored refrigerated for 1 wk.

Inhibition, aging, reactivation and assay of NTE: The study authors referred the reader to (Johnson *et al.*, 1985) for details of the methodology used. They described some refinements of their own designed to improve dispersion of sedimented particles prior to assay. In the determination of time-course of inhibition, paired unspecified tissue samples, routinely called "P" and "M" were prepared and used exactly as described in (Clothier & Johnson, 1979;

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Johnson *et al.*, 1985). The samples were identical except that "P" contained active NTE while "M" did not. Inhibitors were added from concentrated stock solutions. The final solvent concentration was not more than 1% (v/v). In the determination of spontaneous reactivation and aging the improved procedure of (Johnson *et al.*, 1985) was used.

Inhibition, aging, reactivation and assay of AChE: The procedures of (Johnson *et al.*, 1985) were used. Manipulation of tissue for aging and reactivation was exactly as for NTE except that "P" and "M" were not needed. The reactivation procedure used potassium fluoride (KF) - 200 mM final at pH 5.2 for 30 min at 37^{o}C.

Calculation of rate constants for inhibition: The slopes (k') of each semi-log plot were calculated by a linear regression procedure applied to each set of data points. These values of k' were then plotted against inhibitor concentration [I] and the slope (k") of the line was derived by linear regression as above. Then the 2nd-order rate constant (k_a) was calculated from the equation k_a = 2.303 k'/[I] = 2.303 x k". Concentrations of inhibitor giving 50% giving progressive inhibition in 20 min (I_{50}) were calculated from k_a.

A substantial part of this study were also concerned with the influence of various refinements of technique designed to minimise artifactual errors in the measurement of spontaneous reactivation of inhibited enzyme or induced reactivation following treatment of aged enzyme-inhibitor complex with KF or KCl. A description of the refinements or a report of the results of their influence will not be reproduced here.

Results and discussion

The k_a and I_{50} values obtained are tabulated below. The capacity of S-Me compounds to inhibit AChE increased progressively as the length of the alkyl chain increased from methyl to *n*-hexyl except for *n*-butyl. Their capacity to inhibit NTE increased progressively over the whole range. The capacity of DCP to inhibit AChE and NTE increased progressively with increasing chain length. The greater inhibitory effect of the DCP esters on NTE was attributed to their greater lipophilicity.

When AChE was inhibited by methyl, ethyl, *n*-butyl or *n*-hexyl compounds from either series, there was marked spontaneous reactivation (75-100%) and no apparent aging at 19 h at 37^{o}C. With inhibition of AChE by *O-iso*-propyl SMe some spontaneous reactivation with complete aging of all remaining inhibited AChE occurred during 19 h. This latter effect was considered structure-related and was not unexpected by the study authors in the case of branched-chain OP inhibitors where the aging process proceeds by S_Nl cabonium ion mechanism (Aldridge & Reiner, 1972). There was no spontaneous reactivation or aging of NTE.

Inhibition of AChE and NTE by methamidophos and selected analogs

Methamidophos analogs	**Second-order rate constants (k_a: M^{-1} min^{-1}) for inhibition of AChE and NTE and derived I_{50}s (µM)**			
	AChE		**NTE**	
	k_a	**I_{50}**	**k_a**	**I_{50}**
S-Me series				
Methyl[#]	7.2 x 10^2	48	1.6 x 10^1	2150
Ethyl	6.4 x 10^3	5.4	6.3 x 10^1	547
iso-Propyl	2.5 x 10^4	1.4	5.5 x 10^1	630

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n-Propyl	7.8×10^4	0.44	6.4×10^2	55
n-Butyl	5.0×10^4	0.69	7.6×10^3	4.6
n-Hexyl	1.0×10^5	0.34	7.9×10^4	0.44
DCP series				
Methyl	-	4.4*	-	2.9*
Ethyl	9.3×10^3	3.7	9.7×10^4	0.36
n-Butyl	5.0×10^5	0.049	1.4×10^6	0.025
n-Hexyl	8.9×10^5	0.039	7.8×10^6	0.0044

* Values of I_{50} refer to 20 min incubation and are derived from k_a except these marked values which are approximate being derived from fixed-time (20 min) incubations. Studies to obtain rate constants for methyl-DCP were vitiated by decomposition of the stored sample
# Methamidophos

Conclusions

(1) At 37°C and pH 7.4-8.0, 5 higher *O*-alkyl analogs of methamidophos and 4 *O*-alkyl *O*-2,5-dichlorophenyl phosphoramidates were more potent inhibitors of hen brain AChE and NTE than was methamidophos. For AChE, k_a increased from 7.2×10^2 to 1.0×10^5 M^{-1} min^{-1} between methyl and *n*-hexyl *S*-methyl esters and from 9.3×10^3 to 8.9×10^5 M^{-1} min^{-1} between ethyl and *n*-hexyl dichlorophenyl analogs. For NTE the ranges were from 16 to 7.9×10^4 for *S*-methyl esters and were 9.7×10^4 to 7.8×10^6 M^{-1} min^{-1} for dichlorophenyl analogs.

(2) *S*-methyl esters were up to 450 times more active against AChE than against NTE and the dichlorophenyl esters were up to 10 times more active against NTE than against AChE.

(3) Spontaneous reactivation of 75-100% activity without aging of AChE was found after 19 h incubation at 37°C after inhibition by all 9 straight-chain alkyl analogs. After inhibition by *O*-*iso*-propyl *S*-methyl phosphorothioamidate, some spontaneous reactivation with complete aging of all remaining inhibited AChE occurred during 19 h.

(4) The inhibition of NTE by the analogs tested did not result in spontaneous reactivation or aging of NTE .

Comment

The failure to observe aging of inhibited NTE in this study raised questions about the mechanism of the OPIDP attributed to methamidophos since aging was considered to be an essential second step in its initiation (Johnson, 1974; Clothier & Johnson, 1979). The study authors discussed possibilities after first observing that failure to age *in vitro* did not rule out aging *in vivo* since failure to age *in vitro* might reflect a change in tertiary structure brought about by homogenisation of tissue. Inhibition of NTE *in vivo* may be caused by a metabolite of methamidophos (Eto *et al.*, 1977). Stereoselective "disposal" of isomers may occur *in vivo*. The inhibition of NTE *in vivo* which initiates OPIDP may involve an isomer other than that which inhibits *in vitro*. OPIDP had not been detected in hens dosed with methamidophos but only in man (WHO, 1977; Senanayake & Johnson, 1982). It was possible that human NTE inhibited by methamidophos differed in its capacity to age. No studies of aging of human NTE had been reported. Later work (Flucke *et al.*, 1990b; Flucke *et al.*, 1990c) evaluated in this Section, reported that the optically pure enantiomers of methamidophos differed distinctly in their effects on NTE and in their neuropathic potential. Another study (Bertolazzi *et al.*, 1991), evaluated in Section 10.2, reports on the *in vitro* interaction of resolved optical enantiomers of methamidophos with hen and human AChE and NTE.

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Flucke W, Eben A, Stegh R, Lobbecke EA & Machemer L (1988): SRA 5172 TA (Tamaron TA) (c.n. methamidophos). Study for the effect on the neurotoxic esterase (NTE) after dermal administration to chickens. Study No. T 4022127 Report No. 17268. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: not specified. Report Date: 20 October 1988. Unpublished. (QA: Yes; GLP: OECD (Bundesanzeiger No 42a (1983); Test Guidelines: not specified).

[Note: This study was conducted to supplement *Flucke W, Kaliner G & Machemer L (1985b) SRA 5172 TA (Tamaron TA) (c.n. methamidophos). Study for acute neurotoxicity to the chicken after dermal administration. Study No. T 8019936. Bayer AG Institut fuer Toxikologie Fried-Ebert-Str. 217 D 5600 Wuppertal, Germany. Report No. 13852, Dated 1 October 1985* - evaluated above in section 10.1]

Test Substance:	Methamidophos technical. Batch Number: 808 526 309. Purity: 73.0%. Source: not specified.
Test Species:	White Leghorn Chicken: Lohmann Selected Leghorn strain, females, 11 mo old, bw 1.35-1.65 kg obtained from breeder Brinkschulte, Senden.
Dose:	200 mg/kg bw applied dermally

Method

The hens were acclimatised for 14 d prior to the start of the study during which time their states of health were monitored. Only healthy, unvaccinated and untreated hens were selected. They were conventionally kept under standard environmental conditions. They were fed pelletised whole grain ssniff-LVK laying hen food (Ssniff Co.) and drinking water *ad libitum*. The composition of the diet and water were recorded. Twenty seven hens (9/group) were weighed and randomly allocated to the vehicle control, test substance and positive control groups. The test-substance was administered in concentrated form. The positive control substance was triorthocresylphosphate (isomer mixture) (TOCP, batch number 25 E 5268, purity 99%). TOCP was formulated at 2% in demineralised water with Cremophor EL (2% v/v) and applied at a rate of 5 mL/kg bw. The homegeneity and stability of TOCP was confirmed and recorded. Atropine sulfate (batch number 1575, Merck, Darmstadt) was dissolved at 10% (50 mg/kg bw) or 6% (30 mg/kg bw) in demineralised water and applied at 0.5 mL/kg bw.

The total quantity of test substance to be adminstered was divided into 4 equal portions which were applied via microscopic slides to the concave protected side of the comb at 1.5 h intervals. The treatment area was not covered and the test substance was not removed. Atropine sulfate was given at 50 mg/kg bw im 10 min after the 1st portion of test substance and at 50 mg/kg bw sc with the 4th portion. The surviving hens and those not used for neurotoxic esterase (NTE) determinations were also given atropine at 30 mg/kg bw sc at 24 and 30 h after the administration of the 1st portion of test substance. TOCP was administered once po (stomach tube) at 100 mg/kg bw in a volume of 5 mL/kg bw. The vehicle control group hens received 5 mL/kg bw Cremophor EL (2% v/v) in demineralised water. TOCP and the vehicle were administered in the same period as the 1st portion of the test substance.

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The hens were weighed prior to treatment and on d 1, 2 and 7 following. At 24 and 48 h and 7 d after treatment, 3 hens from each group were killed by decapitation. Those hens most affected were selected first for termination in order to avoid unscheduled mortalities. The brain and spinal cord were removed and cooled with ice. NTE was then determined (Johnson, 1977; Johnson & Richardson, 1983). Phenylvalerate was used as a substrate. The phenol that arose during enzymatic cleavage was photometrically determined. A statistical evaluation of the results was not performed.

Results and discussion

Clinical signs and mortality: Observations were not reported.

Body wt: There were no differences between the groups in mean bw that could be attributed to the test substance.

NTE determinations: The results of the NTE determinations are tabulated below. TOCP caused 93 and 85% brain NTE inhibition at 24 and 48 h. The test substance caused a 57, 61 and 44% inhibition of brain NTE at 24 and 48 h and 7 d after treatment, and a 46 and 44% inhibition of spinal cord NTE at 24 h and 7 d.

Mean NTE activity in brain and spinal cord of chickens

Group	Activity (µg phenol) and % inhibition NTE at three times after dosing					
	24 h		48 h		7 d	
	Brain	Spinal Cord	Brain	Spinal Cord	Brain	Spinal Cord
Vehicle	5.9 -	3.6 -	6.6 -	*	6.3 -	3.6 -
Test Sub.	2.5 57	1.9 46	2.6 61	*	3.5 44	2.3 36
TOCP	0.4 93	0.3 91	1.0 85	*	2.8 56	2.0 44

* Evaluation errors; results not recorded

On the basis of knowledge of the initial biochemical process of delayed neuropathy induced by organic phosphoric acid esters (Johnson, 1982), the study authors assumed that a 70-80% inhibition of NTE within 1-40 h of treatment is a necessary prelude to delayed neurotoxicity. These degrees of inhibition were not reached in this study in response to methamidophos. Previously the study authors (Flucke *et al.*, 1985b) had determined the dermal LD_{50} in chickens as 50 mg/kg bw. The difference between 50 mg/kg bw and a potentially neurotoxic dose (> 200 mg/kg bw applied dermally) was therefore greater than 1:4. The neurotoxic potential for the test substance applied dermally to chickens is therefore low. The study authors concluded that the risk of delayed neurotoxicity via the dermal route for humans was most likely negligible.

Conclusion

In hens a dermal dose of 200 mg/kg bw methamidophos caused biologically significant inhibition of brain and spinal cord NTE activity that did not reverse fully within 7 d of treatment. However the degree of inhibition observed (up to 61% in brain and 46% in spinal cord) appears to be insufficient to cause delayed neurotoxicity (Flucke *et al.*, 1985b).

Flucke W, Kruger J, Lobbecke EA & Machemer L (1990b): Methamidophos (Racemate and Enantiomers). Study for OPIDP (Organophosphorus Ester-Induced Delayed Polyneuropathy). Exploratory studies on hens. Study Nos T 3029958, T 2029722, T

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2029957, and T 1029956. Report No. 18987 (100281). Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 6 July 1988 - 13 September 1988. Report Date: 10 April 1990. Unpublished. (QA: Yes; GLP: OECD and FIFRA (40 CFR 160); Test Guidelines: not specified).

Test Substances: Methamidophos (racemate and enantiomers)
Batch Numbers:-
T 3029958: (+)-isomer: GSS 307-3
T 2029722: (+)-isomer: GSS 307-2; GSS 307-1
(-)-isomer: GSS 306-2; GSS 306-1
T 2029957: (-)-isomer: GSS 306-4
T 1029956: straight racemic methamidophos (95.5%)
Source: Bayer AG, Leverkusen

Test Species: White Leghorn Chickens: Lohmann Selected Leghorn strain, females, 8 mo old, bw 1.27-1.73 kg, obtained from Brinkschulte Breeders, Senden.

Dose: 100, 200 and 400 mg/kg bw po

Method

The hens were acclimatised for 5 d during which time their health was monitored. Healthy symptom-free, unvaccinated and untreated hens were used. The hens were kept in floor based cages in a standard chicken coop except for the 1st 24 h after dosing when they were kept in individual cages. After this time the hens had access to open-air areas with sand floors. Low-dust wood shavings were used as litter. The shavings were checked for contaminants. The hens were kept under standard environmental conditions except that the relative humidity varied from 25-95%. The hens were provided with pelletised Ssniff-LVK whole wheat food for laying hens (Ssniff Spezial-diaten GmbH, D-4770 Soest) and tap water *ad libitum*. Food and water analyses were conducted.

The hens were weighed and assigned to groups:-

Study Number	Test Substance	Dosage (mg/kg bw)	Total animals
T 3029958	(+)-methamidophos	100	5
		200	5
T 2029722	Vehicle control		11
	(+)-methamidophos	400	13
	(-)-methamidophos	400	13
	Tricresyl phosphate	100	2
T 2029957	(-)-methamidophos	400	10
T 1029956	Racemic	200	10
	methamidophos	400	10

The tricresyl phosphate (TOCP) hens were used for neuropathy target esterase (NTE) activity determination at an unspecified time and in an unspecified manner. Six hens from each other group were killed in an unspecified manner on 4 d after treatment for NTE determination either in numerical order or on the basis of severity of toxic effects. The study authors did not indicate whether hens killed on the latter basis were classed as terminations or deaths. Findings on NTE are discussed in the next study (Flucke *et al.*, 1990c).

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The test substances were dissolved in demineralised water prior to treatment and given po to unfasted hens (gavage). The administration volume was 5 mL/kg bw. The vehicle control hens were given 5 mL/kg demineralised water po (gavage). Atropine sulfate (Batch no. 1575, Merck, Darmstadt) and a combination of atropine sulfate and pralidoxime chloride (2-PAM) (Batch no. 32231, Aldrich-Chemie) were dissolved in demineralised water. The administration volume was 0.5 mL/kg bw sc. All preparations were made immediately prior to use. A comprehensive regime of antidote administration was provided and is shown in an appendix to this evaluation. Under the antidote regime used the LD_{50} for each test-substance (Flucke, 1990d) was as follows:-

a. (±)-methamidophos: oral LD_{50} hens = 25 mg/kg bw
b. (+)-methamidophos: oral LD_{50} hens = 43 mg/kg bw
c. (-)-methamidophos: oral LD_{50} hens = 82 mg/kg bw

The appearance and behaviour were determined several times on the day of treatment and at least once/d thereafter for the observation periods: 21 d (T 2029722) or 28 d. The nature, duration and intensity of signs were recorded. In the case of mortalities the time of death was recorded.

The assessment of ataxia or paresis was by enforced driving of the hens around a100 m^2 enclosure for 2-3 min twice/wk. The results were scored as follows:-

0 = normal
1 = slightly abnormal gait
2 = ataxia/disturbance in motor co-ordination
3 = pronounced ataxia/paresis
4 = complete paralysis

The bw of the hens were determined prior to treatment (d 1) and at weekly intervals thereafter. The hens of study 2029722 were also weighed on study d 2 and 3.

Results and discussion

Clinical signs and mortality: The acute clinical signs observed were:-

(+)-Methamidophos: apathy, ruffled feathers, staggering gait, diarrhoea, rapid shallow breathing, flat/lateral prostration and spasms.
(-)-Methamidophos: apathy, ruffled feathers, staggering gait, diarrhoea, rapid shallow breathing, flat/lateration prostration, spasms, salivation, labored breathing, dry and limp comb and occasional random wingbeats.
(±)-Methamidophos: apathy, ruffled feathers, staggering gait, diarrhoea, flat/lateral prostration, spasms, rapid shallow breathing and a dry limp comb.

No changes in appearance or behaviour were observed in the vehicle control group.

The study authors reported on the duration and intensity of each sign observed for each enantiomer and the racemate at each dose. The variation between antidote regimes chosen for each treatment group, preclude a meaningful intergroup comparison of the duration and intensity of the signs observed.

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The effects of treatment are tabulated below. The effects are presented as number died/number showing signs/number in group. The onset of treatment-related signs was not determinable precisely because they overlapped those induced by antidotes (ruffled feathers, staggering gait). The time to 1st death in hens treated with either enantiomer of methamidophos was the least at 400 mg/kg bw.

The acute effects of methamidophos enantiomers and racemate

Study Number	The effects of methamidophos enantiomers and racemate			
	Dose mg/kg bw	**Effects**	**Duration of signs**	**Time of death**
T 3029958	(+)- 100	0/5/5	20 min - 4 d	-
	(+)- 200	0/5/5	25 min - 13 d	-
T 2029722	Vehicle control	0/0/11	-	-
	(+)- 400	3/13/13	35 min - 22 d	7 h - 5 d
	(-)- 400	6/13/13	30 min - 17 d	2 h - 5 d
T 2029957	(-)- 400	9/10/10	45 min - 10 d	2 d - 6 d
T 2029756	(±)- 200	1/10/10	20 min - 10 d	3 d
	(±)- 400	7/10/10	1 h - 13 d	1 d - 4 d

Body wt: The effects of treatment on bw are tabulated below. All (+)-methamidophos groups lost bw. Their bw was minimal at d 8-9. The hens at 400 mg/kg bw did not fully recover their initial bw at termination. The hens treated with (-)-methamidophos at 400 mg/kg bw also lost bw but returned to their initial bw at termination. The racemate caused bw loss at d 8. Hens treated with racemate showed a return to their initial bw at termination.

The effect of treatment on mean bw

Study Number	The effects on bw (kg) of methamidophos enantiomers and racemate at intervals after treatment (d)								
	Dose mg/kg bw	**Initial**	**2**	**3**	**8**	**9**	**15**	**22**	**29**
T 3029958	(+)- 100	1.5			1.2		1.4	1.5	1.5
	(+)- 200	1.3			1.2		1.5	1.5	1.5
T 2029722	Vehicle control	1.5	1.6	1.5		1.5	1.5	1.5	
	(+)- 400	1.5	1.4	1.3		1.2	1.3	1.3	
	(-)- 400	1.5	1.4	1.3		1.3	1.3	1.5	
T 2029957	(-)- 400	1.5			1.1		1.4	1.7	1.6
T 2029756	(±)- 200	1.4			1.0		1.3	1.4	1.4
	(±)- 400	1.4			1.0		1.3	1.4	1.4

Clinical neurotoxicity results: The findings of the clinical delayed neurotoxicity examinations are tabulated below. The (+)- enantiomer caused reversible delayed neurotoxicity at 200 mg/kg bw and irreversible delayed neurotoxicity at 400 mg/kg bw. Racemate methamisophos also induced delayed neurotoxicity at 400 mg/kg bw.

The capacity of the treatments to cause organophosphorus ester-induced delayed polyneuropathy (OPIDP)

Study Number	The delayed neurotoxic effects of methamidophos enantiomers and racemate	
	Dose mg/kg bw	**Clinical neurotoxicity findings**
T 3029958	(+)- 100	No evidence of OPIDP.

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	(+)- 200	4/5 hens showed a slightly abnormal gait at 25 d. This was reversible and was not seen at d 28.
T 2029722	Vehicle control	No evidence of OPIDP.
	(+)- 400	Distinct signs of OPIDP appeared progressively from d 8-18. 2/4 remaining hens were totally paralysed at 18 d. Another hen showed marked ataxia and collapse. The remaining hen showed ataxia and other unspecified motor co-ordination disturbances.
	(-)- 400	No evidence of OPIDP.
T 2029957	(-)- 400	No evidence of OPIDP.
T 2029756	(±)- 200	No evidence of OPIDP.
	(±)- 400	Developing OPIDP was observed starting on d 18. One of 3 surviving hens showed distinct ataxia and unspecified disturbances of motor co-ordination on d 25. The other 2 hens showed slightly abnormal gait.

Gross pathology: There were no gross lesions at termination in vehicle control hens. Nor were there any gross lesions in terminated hens of any other group. The study authors provided a description of abnormalities observed in treated hens that died during the study. These were apparently treatment-related findings (test-substance or antidotes) of a general pathological nature which did not differ substantially in incidence or type between treatment groups. Typical changes were those listed for (-)-methamidophos:- spleen pale and patchy, lung distended, liver pale, lobulated or friable, kidneys dark or pale, crop distended, egg concretions in abdominal cavity, duodenal mucosa reddened.

OPIDP and oral LD_{50}: The results obtained in this study and those those of another study (Flucke, 1990d) on the oral LD_{50} of the enantiomers and racemate in hens, are tabulated below.

The OPIDP/Oral LD_{50} ratio for methamidophos

Study Number	**OPIDP/LD_{50} (mg/kg bw) ratio for each enantiomer and racemate**				
	Dose mg/kg bw	**No hens***	**OPIDP#**	**LD_{50}**	**OPIDP/LD_{50}**
T 3029958	(+)- 100	5/5	0	43	> 2.5
	(+)- 200	5/5	(+)		> 5
T 2029722 & T 2029957	(+)- 400	4/13	++		10
	(-)- 400	2/23	0	82	> 5
T 2029756	(±)- 200	9/10	0	25	> 8
	(±)- 400	3/10	+		16

* n_1/n_2: n_1 = no. of hens observed after treatment; n_2 = no. of animals treated
# Degree of intensity of OPIDP: 0 = negative, (+) = low, reversible, + = low to moderate, ++ = high

The dose of (+)-methamidophos enantiomer inducing high intensity OPIDP is 10 times its LD_{50}. The racemate induced a low to moderate OPIDP at 400 mg/kg bw at 16 times its LD_{50}.

Conclusions

(1) Dextrorotary methamidophos: induced a distinct neuropathic effect at a dose of 400 mg/kg bw, equivalent to 10 times its oral LD_{50} in hens of 43 mg/kg bw. The chronology and

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intensity of occurrence paralleled the occurrence of typical OPIDP. Low intensity reversible neurotoxic signs were also seen at 200 mg/kg bw, equivalent to 5 times its oral LD_{50}.

(2) Levorotary methamidophos: no evidence of OPIDP occurred at 400 mg/kg bw, equivalent to 5 times its oral LD_{50} in hens of 82 mg/kg bw.

(3) Racemic methamidophos: low to moderate intensity neurotoxic signs typical of OPIDP occurred at 400 mg/kg bw, equivalent to 8 times its oral LD_{50} in hens of 25 mg/kg bw.

Appendix

The antidote regime applied to each treatment group was as follows. The unlabelled figures represent dosage in mg/kg bw of single or combination antidote. The %'s given are presumed to refer to the total concentration of antidote. The administration volume used to deliver each subcutaneous injection was 0.5 mL/kg bw.

Study 3029958:
Enantiomer (+)-methamidophos 100 mg/kg bw.
atropine, 20, 4%, 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, about 6, 22 and 30 h after.
Enantiomer (+)-methamidophos 200 mg/kg bw.
atropine, 20, 4%, at 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, at 6 and 22 h after;
atropine and 2-PAM, 10, 2%, at 30 h after administration

Study Number 2029722:
Vehicle control group. No antidote used.
Enantiomer (+)-methamidophos 400 mg/kg bw.
atropine, 20, 4%, 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, at 7, 24, 31, 48 and 54 h after administration
Enantiomer (-)-methamidophos 400 mg/kg bw.
atropine, 50, 10%, 1 h after administration of test substance;
atropine, 30, 6%, at 8, 24, 31, 48 and 55 h after administration

Study Number 2029957:
Enantiomer (-)-methamidophos 400 mg/kg bw.
atropine, 50, 10%, 10 min after administration of test substance;
atropine, 30, 6%, at 6, 23 h after; atropine and 2-PAM, 25, 5%, 30 h after;
atropine, 30, 6%, 47 h after administration

Study Number 1029956:
Racemic methamidophos 200 mg/kg bw.
atropine, 20, 4%, 30 min after administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, 5, 22, 30, 46, 54 h after administration
Racemic methamidophos 400 mg/kg bw.
atropine, 20, 10%, 30 min after administration of test substance;

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atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, 7, 23, 31, and 47 h after administration

Flucke W, Eben A & Machemer L (1990c): Methamidophos (Racemate and Enantiomers). Study for the effect on NTE (Neuropathy Target Esterase) in hens following oral administration. Study No.'s T 3027743, T 3029543, T 2029722, T 4030335 and T 6032001 Report No. 19092. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 9 February 1988 - 21 February 1989. Report Date: 29 May 1990. Unpublished. (QA: Yes; GLP: Yes: OECD (Bundesanzeiger No 42a of 2 March 1983); FIFRA; 40 CFR Part 160; Test Guidelines: not specified).

Test Substances:	Methamidophos technical (racemate and enantiomers) Batch Numbers:- T 3027743: pure racemic methamidophos (95.5%) T 3029543: (+)-isomer: Fr. II (-)-isomer: Fr. I T 2029722: (+)-isomer: GSS 307-2; GSS 307-1 (-)-isomer: GSS 306-2; GSS 306-1 T 4030335: (+)-isomer: GSS 307-5 T 6032001: (+)-isomer: GSS 307-5; GSS 307-6 (-)-isomer: GSS 306-6 Source: Bayer AG, Leverkusen
Test Species:	White Leghorn Chickens: Lohmann Selected Leghorn strain, females, 8 mo old, bw 1.26-1.88 kg, obtained from Brinkschulte Breeders, Senden.
Dose:	50, 100, 200, 300 and 400 mg/kg bw po

Method

The hens were acclimatised for 5 d during which time their health was monitored. Healthy symptom-free, unvaccinated and untreated hens were used. The hens were kept in floor based cages in a standard chicken coop except for the 1st 24 h after dosing when they were kept in individual cages. Then the hens had access to open-air areas with sand floors. Low-dust wood shavings were used as litter. They shavings were checked for contaminants. The hens were kept under standard environmental conditions except that the relative humidity varied from 25-95%. The hens were provided with pelletised Ssniff-LVK whole wheat food for laying hens (Ssniff Spezial-diaten GmbH, D-4770 Soest) and tap water *ad libitum*. Food and water analyses were conducted. The hens were weighed and assigned to groups for neuropathy target esterase (NTE) determination (see below). The hens not used for NTE determinations in study T 2029722 were the subject of OPIDP studies which were evaluated previously in this Section (Flucke *et al.*, 1990b).

Study Number	**Test Substance**	**Dosage (mg/kg bw)**	**No hens**	**No hens for NTE determination**		
				24 h	**48 h**	**7 d**
T 3027743	Vehicle control	0	6	3	3	-
	(±)-methamidophos	50	6	3	3	-
	TOCP	300	4	2	2	-
T 3029543	Vehicle control	0	6	3	3	-

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	(+)-methamidophos	50	6	3	3	-
	(-)-methamidophos	50	6	3	3	-
	TOCP	100	2	1	1	-
T 2029722	Vehicle control	0	11	3	3	-
	(+)-methamidophos	400	13	3	3	-
	(-)-methamidophos	400	13	3	3	-
	TOCP	300	2	1	1	-
T 4030335	Vehicle control	0	3	3	-	-
	(+)-methamidophos	400	6	6	-	-
T 6032001	Vehicle control	0	9	3	3	3
	(+)-methamidophos	100	9	3	3	3
	(-)-methamidophos	200	9	3	3	3

The test substances were dissolved in demineralised water prior to treatment and given po to unfasted hens (gavage). The administration volume was 5 mL/kg bw. The vehicle control hens were given 5 mL/kg demineralised water po (gavage). Atropine sulfate (Batch no. 1575, Merck, Darmstadt) and a combination of atropine sulfate and pralidoxime chloride (2-PAM) (Batch no. 32231, Aldrich-Chemie) were dissolved in demineralised water. The administration volume was 0.5 mL/kg bw sc. Tricresyl phosphate (TOCP) (Batch no.25 E 1057, Bayer AG) was the positive control substance. It was dissolved in demineralised water containing 2% v/v Cremophor EL. The administration volume was also 5 mL/kg bw. The solutions were formulated immediately prior to use. The bw of the hens were determined prior to treatment and before termination. A comprehensive regime of antidote administration was provided and is shown in appendix 2 to this evaluation report. Under the antidote regime used the LD_{50} for each test substance (Flucke, 1990d) was as follows:-

a. (±)-methamidophos: oral LD_{50} hens = 25 mg/kg bw
b. (+)-methamidophos: oral LD_{50} hens = 43 mg/kg bw
c. (-)-methamidophos: oral LD_{50} hens = 82 mg/kg bw

The hens for (NTE) determination were selected from the groups in numerical order except when severely affected birds were selected as a matter of priority. NTE was determined in brain, nerve tissue and lymphocytes at the following times:-

Study number	**Test organ, tissue or cells**	**Time after administration**			
		d 0	**24 h**	**48 h**	**7 d**
T 3027743	Lymphocytes	+	+	+	
	Brain, spinal cord, sciatic nerve		+	+	
T 3029543	Brain		+	+	
T 2029722	Brain		+	+	
T 4030335	Brain		+		
T 6032001	Lymphocytes	+	+	+	+
	Brain, spinal cord, sciatic nerve		+	+	+

Five mL blood was withdrawn from the wing vein for pre-dosing lymphocyte NTE activity. For other NTE determinations, the hens were killed by decapitation and exsanguinated. The brain, spinal cord and sciatic nerve were removed and cooled in ice. NTE determinations were also conducted in vehicle control and TOCP hens as appropriate. Phenyl valerate was the substrate when NTE was determined (Johnson, 1977). A modified form of a previously established method (Johnson & Read, 1987) was used for reactivation of inhibited NTE in the brain tissue and determination of UI (unmodified inhibited - unaged) and MI (modified

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inhibited - aged) NTE. Reactivation was carried out by incubating the brain homogenate with potassium fluoride. Incubation with potassium chloride was used as a control. The study authors presumed that the induction of organophosphorus ester-induced delayed polyneuropathy (OPIDP) required 70-80% NTE inhibition in brain and nerve tissue and ageing of the enzyme complex

Results and discussion

Body wt: Mean bw of the vehicle and positive control groups were not affected. On the 1st or 2nd d post-dosing, bw depression (of up to 0.2 kg) was observed in hens treated with racemic, (+)- and (-)-methamidophos at 50 or 400 mg/kg. In study T 6032001 hens receiving 100 mg/kg (+)-methamidophos and 200 mg/kg (-)-methamidophos lost 0.2 and 0.5 kg respectively over 7 d following treatment.

NTE activity determinations: The data for the mean NTE activity and % inhibition in brain, spinal cord, sciatic nerve and lymphocytes together with the results of NTE reactivation are presented in a series of tables in appendix 1 to this evaluation report. These tables constitute the basis for the ensuing discussion.

TOCP at 100 and 300 mg/kg bw:

At 100 mg/kg bw TOCP induced 90-87% NTE inhibition in brain over 24-48 h, the inhibited brain NTE being capable of 19 and 23% reactivation. At 24 and 48 h respectively 81 and 77% inhibited NTE was present as aged NTE.

At 300 mg/kg bw TOCP induced 91-99% NTE inhibition in brain over 24-48 h, the inhibited brain NTE being capable of 7-10 and 18% reactivation. At 24 and 48 h respectively 90 and 82% inhibited NTE was present as aged NTE.

TOCP has a high potential for causing OPIDP in hens.

Pure racemic methamidophos, (+)-methamidophos and (-)-methamidophos at 50 mg/kg bw - 2, 1.2 and 0.6 times their respective LD_{50}s :

Pure racemic methamidophos induced 58-85% NTE inhibition in brain and nerve tissue over 24-48 h, the inhibited brain NTE being capable of 87 and 89% reactivation at those times. At 24 and 48 h respectively 13 and 11% inhibited NTE was present as aged NTE.

(+)-methamidophos induced 54-79% NTE inhibition in brain over 24-48 h, the inhibited NTE being capable of 88 and 93% reactivation at those times. At 24 and 48 h respectively 12 and 7% inhibited NTE was present as aged NTE.

(-)-methamidophos induced 23-18% NTE inhibition in brain over 24-48 h, the inhibited NTE being capable of 91 and 78% reactivation. At 24 and 48 h respectively 9 and 22% inhibited NTE was present as aged NTE.

These parameters suggest a low potential for OPIDP to occur in response to a 50 mg/kg dose of the three test compounds.

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The NTE inhibitory effects of pure racemic and (+)-methamidophos were similar and greater than that produced by (-)-methamidophos. Both enantiomers were present in racemic methamidophos at a 50:50 ratio and adding the expected effects on NTE inhibition for the individual components at 25 mg/kg suggests a NTE inhibition of 38-48% compared to that for racemic methamidophis of 58-85%. An approximately 1.6-fold factor potentiation of the combined effects of each enantiomer has occurred in the racemate.

(+)-methamidophos and (-)-methamidophos at toxicologically equivalent doses (100 and 200 mg/kg bw respectively) - 2.3 and 2.4 times their respective LD_{50}s:

(+)-methamidophos induced 83-91% NTE inhibition in brain and nerve tissue over 24-48 h, the inhibited brain NTE being capable of 84 and 100% reactivation. At 24 and 48 h respectively 16 and 0% respectively inhibited NTE was present as aged NTE. At 7 d this dose of (+)-methamidophos induced 52% inhibition of NTE, 99% of the inhibited brain NTE being capable of reactivation. At 7 d 1% inhibited NTE was present as aged NTE.

(-)-methamidophos induced 36-55% inhibition in brain and nerve tissue over 24-48 h, the inhibited brain NTE being capable of 67 and 63% reactivation. At 24 and 48 h, 33 and 37% respectively inhibited NTE was present as aged NTE. At 7 d this dose of (-)-methamidophos induced 43% inhibition of NTE, 63% of the inhibited brain NTE being capable of reactivation. At 7 d 37% inhibited NTE was present as aged NTE.

On the basis of the hypothesis that a potential for OPIDP is indicated by a 70-80% inhibition of NTE and ageing of the enzyme complex, a potential for OPIDP cannot be unequivocally ascribed to either (+)-methamidophos at 100 mg/kg or (-)-methamidophos at 200 mg/kg. It is to be noted however that (Flucke *et al.*, 1990b) showed the occurrence of a suggestive reversible slightly abnormal gait at d 25-28 (after acute intoxication signs had subsided) with (+)-methamidophos at 200 mg/kg bw.

(+)-methamidophos and (-)-methamidophos at 400 mg/kg bw respectively - 10 and 5 times their respective LD_{50}s:

(+)-methamidophos induced 97-98% NTE inhibition in brain at 24-48 h, the inhibited NTE being capable of 86 and 82% reactivation. At 24 and 48 h respectively 14 and 18% was present as aged NTE.

On the basis of these parameters a potential for OPIDP cannot be unequivocally ascribed to (+)-methamidophos at 400 mg/kg bw. It is to be noted however that (Flucke *et al.*, 1990b) showed that at this dose distinct signs of OPIDP appeared progressively from d 8-18 with 2/4 hens totally paralysed at d 18.

(-)-methamidophos induced 58-84% NTE inhibition in brain at 24-48 h, the inhibited brain NTE being capable of 49 and 27% reactivation. At 24 and 48 h respectively 51 and 73% NTE was present as aged NTE.

This combination of % NTE inhibition and proportion of aged NTE lies in a threshold range for the potential to induce OPIDP yet (Flucke *et al.*, 1990b) showed that (-)-methamidophos at 400 mg/kg bw did not induce OPIDP.

Lymphocyte NTE inhibition was completely reversible after 7 d.

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Conclusion

The effects of the optically pure enantiomers of methamidophos on NTE differed distinctly. The inhibition of NTE by dextrorotary methamidophos was subtantially greater than that caused by levorotary methamidophos. In the case of (+)-methamidophos reactivation of inhibited NTE occurred to a greater extent and was independent of the extent of inhibition and the time after dosing. The contrary applied in the case of (-)-methamidophos which was a less potent inhibitor of NTE but induced ageing of NTE to a greater extent than (+)-methamidophos.

Appendix 1

The data obtained in the determinations of NTE inhibition and NTE reactivation are tabulated below.

Mean activity and % inhibition of NTE in brain

Study Number	**Test substance and dose (mg/kg bw)**	**NTE activity and % inhibition at 24 & 48 h**			
		24 h		**48 h**	
		Activity (*)	% Inhibition	Activity (*)	% Inhibition
T 3027743	Vehicle control (±)-methamidophos 50 TOCP 300	2994 917 8.5	- 66.0 99.7	2677 918 242	- 65.7 91.0
T 3029543	Vehicle control (+)-methamidophos 50 (-)-methamidophos 50 TOCP 100	3034 1411 2322 317	- 53.5 23.5 89.6	2967 622 2433 383	- 79.0 18.0 87.0
T 2029722	Vehicle control (+)-methamidophos 400 (-)-methamidophos 400 TOCP 300	2445 65 1039 66.8	- 97.3 57.6 97.3	2353 41 383 209	- 98.2 83.7 91.0
T 4030335	Vehicle control (+)-methamidophos 400	2149 73	- 96.6	- -	- -
T 6032001[#]	Vehicle control (+)-methamidophos 100 (-)-methamidophos 200	2965 512 1898	- 82.7 36.0	2861 267 1570	- 90.7 45.0

(*) nmol phenylvalerate/min/g tissue

The 7 d NTE activity for study T 6032001 was 2939 (0% inhibition), 1408 (52.0%) and 1676 (43.0 %) for vehicle control, (+)-methamidophos (100 mg/kg bw) and (-)-methamidophos (200 mg/kg bw) respectively

Mean activity and % inhibition of NTE in spinal cord

Study Number	**Test Substance and dose (mg/kg bw)**	**NTE activity and % inhibition at 24 & 48 h**			
		24 h		**48 h**	
		Activity (*)	% Inhibition	Activity (*)	% Inhibition
T 3027743	Vehicle control (±)-methamidophos 50 TOCP 300	1150 489 24	- 57.5 97.9	615 246 77	- 60.0 87.5

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T 6032001[#]	Vehicle control (+)-methamidophos 100 (-)-methamidophos 200	556 139 447	- 75.0 19.6	679 74 360	- 89.0 47.0

* nmol phenylvalerate/min/g tissue

The 7 d NTE activity for this study was 590 (0% inhibition), 322 (45.4%) and 366 (38.0 %) for vehicle control, (+)-methamidophos (100 mg/kg bw) and (-)-methamidophos (200 mg/kg bw) respectively

Mean activity and % inhibition of NTE in sciatic nerve

Study Number	**Test Substance and dose (mg/kg bw)**	**NTE activity and % inhibition at 24 & 48 h**			
		24 h		**48 h**	
		Activity (*)	% Inhibition	Activity (*)	% Inhibition
T 3027743	Vehicle control (±)-methamidophos 50 TOCP 300	124 19 0	- 84.7 100.0	108 31 5	- 71.3 95.4
T 6032001[#]	Vehicle control (+)-methamidophos 100 (-)-methamidophos 200	84 18 79	- 78.6 6.0	119 6 53	- 95.0 55.5

* nmol phenylvalerate/min/g tissue

The 7 d NTE activity for this study was 107 (0% inhibition), 58 (45.8%) and 87 (21.8 %) for vehicle control, (+)-methamidophos (100 mg/kg bw) and (-)-methamidophos (200 mg/kg bw) respectively

Mean activity and % inhibition of NTE in lymphocytes

Study Number	**Test Substance and dose (mg/kg bw)**	**NTE activity and % inhibition at 24 & 48 h**			
		24 h		**48 h**	
		Activity (*)	% Inhibition	Activity (*)	% Inhibition
T 3027743	Vehicle control (±)-methamidophos 50 TOCP 300	7.4 2.9 0.09	- 60.8 98.8	6.2 4.4 2.9	- 29.0 53.2
T 6032001[#]	Vehicle control (+)-methamidophos 100 (-)-methamidophos 200	5.6 1.5 4.6	- 73.2 17.9	5.6 1.4 3.8	- 75.0 32.1

* nmol phenylvalerate/min/g tissue

The 7 d NTE activity for this study was 5.6 (0% inhibition), 5.6 (0%) and 6.1 (0%) for vehicle control, (+)-methamidophos (100 mg/kg bw) and (-)-methamidophos (200 mg/kg bw) respectively

Mean % modified inhibited and unmodified inhibited NTE in brain at 24 h

Study Number	**Test Substance and dose (mg/kg bw)**	**Modified and unmodified inhibited NTE at 24 h**			
		[+ KCl] % activity* (a)	[+ KF] % activity* (b)	Modified inhibited (aged) NTE (100 - b)%	Unmodified inhibited (unaged) NTE (b-a)%
T 3027743	(±)-methamidophos 50 TOCP 300	30.6 6.4	86.7 7.3	13.3 92.8	56.1 0.9
T 3029543	(+)-methamidophos 50 (-)-methamidophos 50 TOCP 100	41.8 87.2 13.0	87.9 90.7 19.2	12.1 9.3 80.8	46.1 3.5 6.2
T 2029722	(+)-methamidophos 400 (-)-methamidophos 400 TOCP 300	2.9 40.3 7.6	86.4 48.7 9.9	13.6 51.3 90.1	83.5 8.4 2.3

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T 4030335	(+)-methamidophos 400	3.7	86.0	14.0	82.3
T 6032001[#]	(+)-methamidophos 100 (-)-methamidophos 200	16.3 59.7	84.3 67.4	15.7 32.6	68.0 7.7

* nmol phenylvalerate/min/g tissue
(a) and (b): % activities calculation based on brain activities in control hens

Mean % modified inhibited and unmodified inhibited NTE in brain at 48 h

Study Number	**Test Substance and dose (mg/kg bw)**	**Modified and unmodified inhibited NTE at 48 h**			
		[+ KCl] % activity* (a)	[+ KF] % activity* (b)	Modified inhibited (aged) NTE (100 - b)%	Unmodified inhibited (unaged) NTE (b-a)%
T 3027743	(±)-methamidophos 50 TOCP 300	32.1 14.9	89.3 17.9	10.7 82.2	57.2 3.0
T 3029543	(+)-methamidophos 50 (-)-methamidophos 50 TOCP 100	22.0 84.8 22.7	93.4 78.2 23.3	6.6 21.8 76.7	71.4 0 0.6
T 2029722	(+)-methamidophos 400 (-)-methamidophos 400 TOCP 300	1.4 15.9 15.2	82.4 26.9 17.8	17.6 73.1 82.2	81.0 3.3 2.6
T 6032001[#]	(+)-methamidophos 100 (-)-methamidophos 200	9.5 54.6	100 62.9	0 37.1	90.5 8.3

* nmol phenylvalerate/min/g tissue
(a) and (b): % activities calculation based on brain activities in control hens

Mean % modified inhibited and unmodified inhibited NTE in brain at 7 d

Study Number	**Test Substance and dose (mg/kg bw)**	**Modified and unmodified inhibited NTE at 7 d**			
		[+ KCl] % activity* (a)	[+ KF] % activity* (b)	Modified inhibited (aged) NTE (100 - b)%	Unmodified inhibited (unaged) NTE (b-a)%
T 6032001[#]	(+)-methamidophos 100 (-)-methamidophos 200	51.2 55.9	99.0 62.7	1.0 37.3	47.8 6.8

* nmol phenylvalerate/min/g tissue
(a) and (b): % activities calculation based on brain activities in control hens

Appendix 2

The antidote regime applied to each treatment group was as follows. The unlabelled figures represent dosage in mg/kg bw of single or combination antidote. The %'s given are presumed to refer to the total concentration of antidote. The administration volume used to deliver each subcutaneous injection was 0.5 mL/kg bw.

Study 3027743:
Racemic methamidophos 50 mg/kg bw.
atropine, 20, 4%, 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, at 7, 24 and 31 h after administration.

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Study T 3029543:
(+)-methamidophos or (-)-methamidophos 50 mg/kg bw.
atropine, 20, 4%, at 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, at 7 nd 24 h after administration.

Study Number 2029722:
Enantiomer (+)-methamidophos 400 mg/kg bw.
atropine, 20, 4%, 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, at 7, 24, 31, 48 and 54 h after administration.
Enantiomer (-)-methamidophos 400 mg/kg bw.
atropine, 50, 10%, 1 h after administration of test substance;
atropine, 30, 6%, at 8, 24, 31, 48 and 55 h after administration.

Study Number 4030335:
Enantiomer (+)-methamidophos 400 mg/kg bw.
atropine, 20, 4%, 30 min before administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, 3, 8, 23 h after administration.

Study Number 6032001:
Enantiomer (+)-methamidophos 100 mg/kg bw.
atropine, 20, 4%, 30 min after administration of test substance;
atropine and 2-PAM, 50, 10%, immediately before;
atropine and 2-PAM, 25, 5%, 7, 26, 32 h after administration.
Enantiomer (-)-methamidophos 200 mg/kg bw.
atropine, 50, 10%, 10 min before administration of test substance;
atropine, 30, 6%, at 8 and 26 h after administration

Bomann W, Kalinger G, Mager H, Neal B & Flucke W (1992): SRA 5172 (c.n.Methamidophos): Subchronic dermal neurotoxicity study (Ninety-day hen study). Study No. T 1033771. Report No. 21428. Lab: Fachbereich Toxikologie, Bayer AG, Friedrich-Ebert-Strasse, Wuppertal-Elberfeld. Sponsor: Bayer AG, Leverkusen, Germany. Study Duration: 22 January 1990 - 21 May 1990. Report Date: 1 June 1992. Unpublished. (QA: Yes; GLP: OECD (Bundesanzeiger No. 42a 1983, Bundesgesetzblatt, Part 1, 1990; Test Guidelines: US EPA "Pesticide Assessment Guidelines" Section 82-5, 1984).

Test Substances:	Methamidophos technical (racemate). Formula Number: 011550. Analytical Number: 31192, FS 23280. Purity: 76.3%. TOCP (tri-orthocresyl phosphate) (mixture of isomers). Batch Number: 25 E 1057. Purity: 98.1%.
Test Species:	White Leghorn Hen: Lohmann Selected strain, 8 mo of age, 1.35-1.75 kg bw, obtained from Brinkschulte Breeders, Senden.
Dose:	0, 0.5, 1.5, 4.5 mg methamidophos/kg bw applied dermally 1/d 5 times/wk; 15 mg TOCP/kg bw applied dermally 1/d 5 times/wk.

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Method

The hens were adapted to the experimental environment over 18 d. Their state of health was monitored. Only unvaccinated and untreated hens without signs of illness or abnormalities were used. Initially the hens were kept under standard environmental conditions in a standard chicken coop with natural and artificial lighting. Later the experimental groups were kept separately. The floor was covered in low-dust wood shavings free of contaminants likely to affect the study. The hens were provided with a diet consisting of "Ssniff-LVK Whole Wheat Feed for Laying Hens" (Ssniff Spezial-diaten GmbH, D-4770 Soest) and tap water *ad libitum*. The composition of the diet and drinking water were determined. The hens were weighed prior to the first exposure and randomly allocated to individual groups. Twenty six hens were allocated to the vehicle control and 4.5 mg/kg groups. Sixteen hens were allocated to the 2 lower dose treatment groups and the TOCP group. Ten hens/group were scheduled for blood sampling to determine ChE activity and 3 hens/group were scheduled for two NTE assays.

Methamidophos and TOCP were applied to the concave side of the comb 1/d, 5 times/wk, using a 0.01-0.1 mL capacity Varipette. Formulations of 2.5, 7.5 and 22.5% methamidophos and 75% TOCP in 2-propanol were freshly prepared each d. The treatment volume was 0.02 mL/kg bw. The stability and homogeneity of the test substance over a storage period of 24 h were verified for concentrations of 0.1 and 50% and those of TOCP at 10 and 75%. The levels of methamidophos and TOCP in the prepared formulations were verified 3 and 4 times respectively.

The hens were monitored for appearance, behaviour and clinical signs during treatment. Hens in a moribund condition were terminated. Ten of the vehicle control group hens and 9 of the high dose treatment group were also observed for 4 wk after the cessation of exposure. Hens were weighed weekly after the initial pre-exposure weighing. Food consumption was not measured.

The hens were subjected to forced activity and ladder climbing tests. The former was designed to assess motor co-ordination. The hens of all groups were individually driven around a stall area of 18 m^2 for 2-3 min at least 1/wk and scored according to the following scale:-

0 = normal
1 = slightly abnormal gait
2 = ataxia/disturbance in motor co-ordination
3 = severe ataxia/paresis
4 = complete paralysis

Once weekly the hens were also required to mount a ladder, 2 m long and set at 45 degrees, twice in succession. The ladder was enclosed in a structure which ensured that hens could only re-emerge into the open at the top of the ladder. The time taken by each hen was measured.

In the selection of hens for NTE determination, priority was given to those most seriously affected. The hens were decapitated 24 h after the 17th (wk 4) and 62nd (wk 13) treatment. The brain and spinal cord were removed, chilled in ice and forwarded for assay. The NTE was assayed using the method of (Johnson, 1977) as modified by (Johnson & Richardson, 1983).

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Phenyl valerate was used as the substrate in the assay. NTE activity was determined by means of differential determination. One sample was pre-incubated with Paraoxon and another with Paraoxon and Mipafox. The difference in residual activities determined in the 2 samples represented the NTE activity.

Assays of the plasma ChE activity were performed using a modified method of (Herbold & Lorke, 1981) with 10 hens/group 1 wk prior to the 1st treatment, wk 3, 6 and 13 during treatment and in wk 17 for groups 1 and 4. Blood samples were taken 24 h after the previous treatment. Blood was taken from the wing vein and clotting was prevented with lithium heparin.

Birds which died were necropsied and assessed for gross pathology. Hens in moribund condition were terminated with 20% Evipan Natrium^{TM} solution and assessed grossly. All other hens were anaesthetised with the same solution at the end of the treatment or recovery period, assessed grossly, perfused with phosphate buffer to exsanguinate them and with 10% buffered aqueous formaldehyde solution to fix tissues. Sciatic nerve with branches into the tibial and lateral popliteal nerves, brain and spinal cord were removed and divided into 3 parts for further fixation.

The sciatic, tibial and lateral popliteal nerves, the cervical, thoracic and lumbar segments of the spinal cord and the metencephalon and medulla oblongata of the brain taken from hens terminated at the end of treatment in the high-dose, vehicle and TOCP groups were histologically examined.

Statistical analysis of bw, plasma ChE activity and ataxia scores was performed.

Results and discussion

Clinical signs: Treatment-related signs were observed at 4.5 mg/kg bw. All hens exhibited apathy, ruffled feathers and staggering gait from 5 h after the 1st treatment to termination. Reduced food consumption and disturbances of the gastrointestinal tract (discoloured faeces, diarrhoea) were also noted at unspecified incidences at variable times during treatment. All other signs in all groups were attributable to infectious or genetic causes.

Mortality: One mortality occurred in each of the treatment groups and in the TOCP group. One moribund hen from each of the vehicle control and 0.5 mg/kg groups were terminated. The mortalities were not attributable to treatment.

Body weights: Mean bws over the treatment and recovery periods at selected intervals are tabulated below. The mean bws for the TOCP group were not presented or discussed. The mean bw of the 0.5 mg/kg group was significantly less ($p \leq 0.05$) than that of controls at wk 12 and 13 (wk 12: 1.6 kg cf controls 1.7; wk 13: 1.6 kg cf controls 1.7). At 1.5 mg/kg there were no significant differences from controls. The negative effect on bw of the 4.5 mg/kg group fully reversed during the recovery period and was thus treatment-related.

Effect of methamidophos on bw

Dose mg/ kg	**Body wts (kg) of hens at selected intervals**									
	Initial	**Treatment period**						**Recovery period**		
	wk 0	wk 2	wk 4	wk 6	wk 8	wk 10	wk 13	wk 1	wk 3	wk 4
0	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.7	1.7	1.7

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4.5	1.5	1.2*	1.4*	1.4*	1.5*	1.6*	1.6	1.6	1.7	1.7

* Statistically significantly less ($p < 0.05$) cf controls (Dunnett test)

Clinical neurotoxicity tests: The forced activity tests produced no signs of delayed neurotoxicity or neuropathy in the treatment or vehicle control groups. The TOCP group developed delayed neurotoxicity from wk 2 onwards. Progressive deterioration of muscular co-ordination was observed. There were no significant differences between the treatment and vehicle control groups in ladder climbing times. In the TOCP group, an unspecified number of hens occasionally lost their footing on the ladder. However there were no significant differences in climbing times between this and other groups.

Neuropathy target esterase NTE activity: The determined NTE activities and inhibition at 4 and 13 wk are tabulated below. There was no inhibition of NTE at 0.5 or 1.5 mg/kg. The % inhibition observed at 4.5 mg/kg was below the levels of inhibition considered necessary (45-65%) to provoke a delayed neurotoxic effect in cases of repeated exposure to neurotoxic substances (Johnson, 1982). The inhibition of NTE caused by TOCP ranged from 50.5-75.9%

Activity and inhibition of NTE in brain and spinal cord

Treatment	**Interval (wk)**	**Activity (nmoles phenyl valerate/min/g) and % inhibition of NTE in brain and spinal cord**			
		Brain		**Spinal cord**	
		Activity	**Inhibition**	**Activity**	**Inhibition**
0 mg/kg bw methamidophos	4	2374	-	537	-
	13	2319	-	554	-
4.5 mg/kg bw methamidophos	4	1867	21.4	459	14.6
	13	2138	7.8	496	10.4
15 mg/kg bw TOCP	4	811	65.8	266	50.5
	13	560	75.9	186	66.5

Necropsy: There were no test substance-related findings in those hens which died intercurrently, were terminated in moribund condition, at 1st interim necropsy (1st NTE assay) or 2nd interim necropsy (2nd NTE assay) or at the end of the treatment or recovery period. The necropsy findings of the TOCP group were not reported.

Histopathology: There was no histological evidence of treatment-related effects in the nervous system. The histological findings in the TOCP group were not reported.

Cholinesterase: The activities and inhibition of plasma ChE at various intervals are tabulated below. Biologically significant inhibition (≥ 20%) of plasma ChE occurred at 4.5 mg/kg bw. The inhibition had fully reversed by the the end of the recovery period.

Activity and inhibition of plasma ChE

Interval (wk)	**Activity (kU/L) and % inhibition of plasma ChE at each dose (mg/kg bw) of methamidophos**				
	0	**0.5**	**1.5**	**4.5**	**15 (TOCP)**
Initiation (-1)	0.90	0.94	1.00	0.99	0.94
3	0.95 -	0.99 -	0.83 13.0	0.57* 40.0	0.87 8.0
6	0.84 -	0.85 -	0.75 11.0	0.49* 42	0.75 11.0

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13[a]	0.89 -	0.83 7.0	0.78 12.0	0.51* 43.0	0.82 8.0
17[b]	0.82	-	-	0.88	-

* Statistically significantly less ($p < 0.05$) than controls (Dunnett test)
[a] End of the treatment period; [b] End of the recovery period

Conclusions

(1) There was no evidence of delayed neurotoxicity following the repeated (1/d 5 times/wk for 13 wk) dermal application of 0.5, 1.5 or 4.5 mg methamidophos/kg bw.

(2) On the basis of biologically significant inhibition (≥ 20%) of plasma ChE, a NOEL was established at 1.5 mg/kg bw/d.

Lotti M, Moretto A, Bertolazzi M, Peraica M & Fioroni F (1995b): Organophosphate polyneuropathy and neuropathy target esterase: studies with methamidophos and its resolved optical isomers. Archives of Toxicology, 69(5):330-336.

Test Substances:	R-(±) methamidophos analytical grade. Purity: 98.3%. D-(+) and L-(-) methamidophos isomers. Purity: 100%. Source: Bayer AG, Leverkusen, Germany. Dibutyl dichlorovinylphosphate (DBDCVP) Purity: >98% Source: Chemsyn Science Laboratories, Lenexa, KS, USA Phenylmethanesulfonyl (PMSF) Purity: > 99% Source: Fluka AG, Buchs, Switzerland.
Test Species:	Hen: Warren strain, 1.7-2.5 kg bw of an unspecified age obtained from an unspecified local breeder.
Dose:	R-(±) methamidophos 50, 120 mg/kg bw po; D-(+) methamidophos 25, 60 mg/kg bw po; L-(-) methamidophos 60, 120 mg/kg bw.

Prelude

This study reported comparative biochemical and clinical studies in hens treated with single doses of R-(±), D-(+) and L-(-) methamidophos to elucidate which of them initiates, protects from or promotes OPIDP. The initiation of OPIDP by the 3 forms of methamidophos, the promotion of methamidophos initiated-OPIDP by phenylmethanesulfonyl and the promotion of dibutyl dichlorovinylphosphate initiated-OPIDP and diisopropyl fluorophosphinate-initiated-OPIDP by methamidophos will be considered but protection against dibutyl dichlorovinylphosphate initiated-OPIDP by pre-treatment with methamidophos will not be included in this evaluation.

Method

An unspecified number of hens were caged in groups of 5-10 and allowed food and water *ad libitum*. Racemic methamidophos and the two isomers were each dissolved in water before single dose administration by gavage at 1 mL dosing solution/kg bw. Phenylmethanesulfonyl (PMSF), dibutyl dichlorovinylphosphate (DBDCVP) and diisopropyl fluorophosphonate

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(DFP) (positive control substance > 95% purity) were dissolved in glycerol formal immediately before single dose sc administration at 0.1-1 mL/kg bw at 1-4 skin sites. The numbers of hens used in each treatment group were variable and are shown in the results with the doses used.

Pretreatment with atropine sulfate dissolved in saline (20 mg/kg bw ip) was given to all hens 10-20 min before treatment with organophosphorous compounds. The same dose of atropine was given every 2-5 h when needed, usually for 48 h after dosing but when high doses of R-(±) methamidophos (≥ 50 mg/kg bw po), D-(+) methamidophos (≥ 25 mg/kg bw po) and L-(-) methamidophos (≥ 25 mg/kg bw po) were used, this treatment was prolonged up to 4 d. Signs of ataxia were blindly evaluated on a 0 to 8-point scale according to (Lotti *et al.*, 1991) as follows:

0 = no defects in posture, standing ability and walking performance
1 = minor changes in walking performance
2 = clear changes in walking performance with some falling of the hind end
3 = walking limited to short steps with frequent falling of the hind end
4 = still able to walk but often hens remain sitting on metatarsus
5 = unable to walk, sitting on metatarsus but able to stand when lifted
6 = unable to stand when lifted
7 = unable to stand, wings drop
8 = unable to stand, extended legs and wings

Hens were killed by decapitation. Brain, lumbosacral spinal cord and sciatic nerve were excised immediately and either placed in an ice-cold 50 mL mM TRIS-HCl buffer (pH 8.0 at 23^{o}C) containing 0.2 mM EDTA and assayed for NTE or stored at -80^{o}C prior to assay. NTE was measured in brain and spinal cord (Johnson, 1977) and in peripheral nerve (Moretto *et al.*, 1989). AChE was measured in brain, spinal cord and peripheral nerve homogenates (Ellman *et al.*, 1961).

Statistical analyses (Kruskal-Wallis, Mann-Whitney *U*-test) were performed.

Results

Preliminary note: NTE and AChE activities of controls were always within the historical range of the study authors' laboratory (NTE: 2000-2700, 400-600 and 70-130 nmol/min/g tissue and AChE: 15-25, 2-5 and 0.7-1.5 µmol/min/g tissue in brain, spinal cord and peripheral nerve respectively)

Time course of AChE and NTE inhibition: The data for D-(+) and L-(-) methamidophos were presented in the form of curves and the following tables have been constructed as accurately as possible. The time-course of NTE inhibition over 1-13 d was determined. The doses were selected to obtain comparable inhibition. AChE inhibition was measured as a control. With D-(+) methamidophos, AChE activity was fully restored by d 4 whereas with L-(-) methamidophos, at 60% of the dose of D-(+) methamidophos, AChE activity had restored to 70-77% of control activity by d 13.

Time course of AChE inhibition

Nerve tissue	**AChE activity* (% controls) over time (d) (n = 3)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**11**	**13**

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D-(+) methamidophos 25 mg/kg bw										
Brain	22	28	78	95	90	117	90	82	95	102
SpC	25	20	58	100	111	118	110	105	-	115
PN	20	30	70	92	100	102	90	92	123	98
L-(-) methamidophos 15 mg/kg bw										
Brain	8	10	38	42	52	52	54	58	62	72
SpC	12	12	38	58	52	52	60	78	98	77
PN	15	12	40	40	35	40	42	50	72	70

SpC = Spinal cord; PN = Peripheral nerve

*Control values were brain, 16.8, spinal cord, 2.5 and peripheral nerve, 0.9 μmol/min/g tissue (n = 10)

NTE inhibition by D-(+) methamidophos remained steady from d 1-4. NTE activity was fully restored between d 7 and 9. Maximum NTE inhibition by L-(-) methamidophos, at 4.8 times the dose of D-(+) methamidophos, was observed on d 4. At d 8, NTE activity was restored to 48-60% control activity.

Time course of NTE inhibition

Nerve	**NTE activity* (% controls) over time (d) (n = 3)**										
tissue	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**11**	**13**
D-(+) methamidophos 25 mg/kg bw											
Brain	25	32	35	35	39	40	65	-	89	90	100
SpC	37	40	42	50	50	62	60	-	90	75	100
PN	28	29	30	30	37	-	45	-	58	72	70
L-(-) methamidophos 120 mg/kg bw											
Brain	72	50	-	14	-	60	-	59	-	-	-
SpC	76	60	-	20	-	62	-	60	-	-	-
PN	39	40	-	7	-	50	-	48	-	-	-

SpC = Spinal cord; PN = Peripheral nerve; - = no result reported

*Control values were brain, 2370, spinal cord, 444 and peripheral nerve, 109 nmol/min/g tissue (n = 8)

A limited experiment with R-(±) methamidophos at 50 mg/kg bw indicated that NTE activity in nervous tissue on d 1 was brain 49, spinal cord 54 and peripheral nerve 47% of control activity. On d 8 NTE activity was 56, 51 and 62% of control activity respectively.

Preliminary note on the initiation or promotion of neuropathy: Promotion (or potentiation) was described by the study authors as an elicitation or intensification of a neuropathic response by NTE antagonists (e.g. PMSF) following its initiation by NTE agonists (e.g. methamidophos) (Pope & Padilla, 1990; Lotti *et al.*, 1991; Johnson & Read, 1993). Where the initiating dose(s) of the agonist were subthreshold, neuropathy initiated by agonists became clinically expressed by antagonists. In the work that follows, the induction of clinical signs in hens by PMSF following methamidophos treatment, meant that mild sub-clinical neuropathy had been initiated by methamidophos. PMSF itself has been shown to cause only mild neuropathy in hens at extremes of exposure i.e. 6 repeated doses of 120 mg/kg bw sc (Lotti *et al.*, 1993a). Histopathological evidence in nervous tissue was not reported but clinical outcomes have been shown to correlate with biochemical and morphological criteria in spinal and peripheral nerve (Lotti *et al.*, 1987; Moretto *et al.*, 1989). The induction or increased severity of clinical signs caused by methamidophos following treatment with DBDCVP or DFP, meant that methamidophos had promoted the neuropathy induced by DBDCVP or DFP. Methamidophos itself has been shown to cause only mild neuropathy in hens at 200 mg/kg bw po (Flucke *et al.*, 1990b). An account of the use of the terms agonist or antagonist in relation to organophosphorus inhibition and induction of OPIDP can be found in (Lotti *et al.*, 1993a).

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Initiation of neuropathy by methamidophos and its promotion by PMSF: The degree of NTE inhibition and the severity of clinical effects following treatment with methamidophos, DFP and PMSF are tabulated below. PMSF at 120 mg/kg bw, was given 24 h after the initial treatment except in the L-(-) methamidophos group (120 mg/kg bw) where PMSF was given 120 h afterwards. On the basis of clinical signs assessed 15-20 d after dosing, R-(±) methamidophos did not cause OPIDP at 120 mg/kg bw despite high NTE inhibition (72-76% at 24 h). The minimal neuropathic dose (if defined as the dose causing unpromoted observable mild OPIDP in some hens only) was 60 mg/kg bw for D-(+) methamidophos and 120 mg/kg bw for L-(-) methamidophos. In both cases, OPIDP correlated with high NTE inhibition (≥ 79% at 24 and 96 h respectively). Positive control hens (DFP treated) developed mild OPIDP at 67-77% NTE inhibition at 24 h. The poor promotion by PMSF of neuropathy caused by D-(+) and L-(-) methamidophos was attributed by the study authors to occupation by the isomers of the promotion site which would then be unavailable to PMSF.

Methamidophos as initiator of OPIDP

Initial treatment (single dose mg/kg bw)	Biochemical and clinical effects of methamidophos, DFP and PMSF							
	Mean % NTE inhibition[a]			T (h)	Median clinical scores (range)[b]			
	Brain (n)	Spinal cord (n)	Periph. nerve (n)		No PMSF	n	Plus PMSF	n
R-(±) meth (50)	51 (4) 57 (4)	46 (4) 48 (4)	63 (4) 51 (4)	24 48	0 (0-0)	3	0 (0-0)	6
R-(±) meth (120)	76 (3)	72 (3)	76 (3)	24	0 (0-0)	3	1 (1-2)*	3
D-(+) meth (25)	77 (4) 60 (3)	60 (4) 48 (3)	76 (4) 52 (3)	24 48	0 (0-0)	5	0 (0-0)	6
D-(+) meth (60)	87 (5) 83 (3)	79 (5) 81 (3)	84 (5) 83 (3)	24 48	0 (0-1)	5	1 (1-2)*	5
L-(-) meth (60)	31 (6) 29 (3)	25 (5) 35 (3)	45 (5) 38 (3)	24 48	0 (0-0)	6	1 (0-1)*	3
L-(-) meth (120)	85 (3)	79 (3)	91 (3)	96	0.5 (0-2)	4	1 (0-2)	9
DFP (0.3)	75 (3)	77 (3)	67 (3)	24	1 (1-1)	3	8 (4-8)*	3

T = Time of assay after dosing; n = group size; meth = methamidophos; Periph = Peripheral

a: NTE inhibition was calculated from the mean activity of 12 concurrent control hens (brain, 2447, spinal cord, 516 and peripheral nerve, 103 nmol/g tissue

b: Clinical scores assessed at 15-20 d after dosing

* Statistically different from the corresponding group not treated with PMSF (Mann-Whitney *U* test, $p < 0.05$)

The promotion by PMSF of neuropathy initiated by methamidophos suggested that doses of either R-(±) or D-(+) methamidophos causing more than 70% NTE inhibition at 24 h were associated with very mild neuropathy. Lower levels of NTE inhibition (25-45% at 24 h) obtained with L-(-) methamidophos at 60 mg/kg bw were also associated with mild OPIDP. The neuropathy caused by L-(-) methamidophos at 120 mg/kg bw (NTE inhibition 79-91% at 96 h) was not further promoted by PMSF. In contrast, the severity of the neuropathy initiated by DFP (67-77% NTE inhibition at 24 h) was greatly enhanced by PMSF.

Methamidophos as a promoter of OPIDP: The clinical effects of D-(+) and L-(-) methamidophos and PMSF given after OPIDP initiation by DBDCVP or DFP are tabulated below. The % NTE inhibitions at 24 h after dosing with DBDCVP alone were for brain, spinal cord and peripheral nerve 90, 80 and 83% respectively; and for DFP alone were 75, 77 and 67%. PMSF was used as a positive control. Neuropathy initiated by DBDCVP was promoted by D-(+) and L-(-) methamidophos. On the basis of clinical signs assessed at 15-20 d after dosing, the promoting effects of 25 mg/kg bw of either isomer of methamidophos on

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neuropathy initiated by DBDCVP were similar. Neuropathy initiated by DFP was promoted by D-(+) methamidophos at 24 mg/kg bw. D-(+) and L-(-) methamidophos promoted OPIDP at doses lower than needed either to cause or initiate OPIDP.

Methamidophos as a promoter of OPIDP

Initiator (a)	Promoter (b)	Dose[c] mg/kg bw	Clinical score (d)	n
DBDCVP	Vehicle		3 (1-6)	16
DBDCVP	D-(+)	25	6 (4-8)*	6
DBDCVP	L-(-)	15	4 (2-5)	5
DBDCVP	L-(-)	25	5 (4-8)*	5
DBDCVP	PMSF	120	8 (8-8)*	3
DFP	Vehicle		1 (0-2)	7
DFP	D-(+)	1.5	0 (0-1)	6
DFP	D-(+)	6	0 (0-1)	3
DFP	D-(+)	12	0 (0-2)	3
DFP	D-(+)	24	2 (2-7)**	5
DFP	PMSF	120	4 (2-8)*	5

D-(+) = D-(+) methamidophos; L-(-) = L-(-) methamidophos; SpC = spinal cord; Periph. n = peripheral nerve
a: DBDCVP dose: 0.4 mg/kg; DFP dose: 0.3 mg/kg bw
b: administered 24 h after the initiator
c: dose of promoter
d: median (range) - the time of assessment was not specified but is assumed to be 15-20 d after dosing as previously
* Statistically different from corresponding controls treated with initiator and vehicle (Kruskal-Wallis, $p < 0.05$)
** Statistically different from hens treated with DFP alone (Kruskal-Wallis $p \approx 0.08$)

Conclusions

(1) D-(+) methamidophos is a less potent inhibitor of AChE than L-(-) methamidophos but is a more potent inhibitor of NTE.

(2) A single dose of 120 mg/kg po of R-(±) methamidophos induced 72-76% NTE inhibition at 24 h after dosing. However no clinical signs of unpromoted OPIDP were observed at 15-20 d after dosing. On the basis of the absence of signs at 120 mg/kg po, the minimum neuropathic dose of R-(±) methamidophos was > 120 mg/kg bw po.

(*Comment*: The study authors did not specify the minimum neuropathic dose of R-(±) methamidophos. However, on the basis that PMSF at 120 mg/kg sc promoted mild OPIDP when administered 24 h after 120 mg/kg po R-(±) methamidophos, mild sub-clinical neuropathy was initiated by 120 mg/kg po R-(±) methamidophos. The minimum neuropathic dose of R-(±) methamidophos was then 120 mg/kg bw po. This determination can be compared with the observation by (Flucke *et al.*, 1990b) evaluated in Section 10.1), that the minimum neuropathic dose was between 200-400 mg/kg bw po.

(3) A single dose of 60 mg/kg bw po D-(+) methamidophos induced 81-83% NTE inhibition at 24 h after dosing. Mild unpromoted clinical signs of OPIDP were observed at 15-20 d after dosing. On the basis of these clinical signs, the minimum neuropathic dose for D-(+) methamidophos was considered to be 60 mg/kg bw po.

(4) A single dose of 120 mg/kg bw po L-(-) methamidophos induced 79-91% NTE inhibition at 96 h after dosing. Mild unpromoted clinical signs of OPIDP were observed 15-20 d after

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dosing. On the basis of these signs the minimum neuropathic dose for L-(-) methamidophos was considered to be 120 mg/kg bw po.

(*Comment*: The study authors specified the minimum neuropathic dose of L-(-) methamidophos as 120 mg/kg bw po. However, on the basis that PMSF at 120 mg/kg sc produced clinical signs of OPIDP when administered 24 h after 60 mg/kg po L-(-) methamidophos, mild sub-clinical neuropathy was initiated by 60 mg/kg po L-(-) methamidophos. The minimum neuropathic dose of L-(-) methamidophos was then 60 mg/kg bw po. The study authors did not comment on this possibility).

(5) Because D-(+) and L-(-) methamidophos promoted OPIDP at doses lower than needed either to cause or initiate OPIDP, neuropathy induced by either isomer is self-promoted.

Kellner T, Sanborn J & Wilson B (2000): In vitro and in vivo assessment of the effect of impurities and chirality on methamidophos-induced neuropathy target esterase aging. Toxicological Sciences, 54: 408-415.

Test Substance:	Racemic methamidophos analytical grade Purity: 99.4% Racemic methamidophos technical grade Purity: 73.1% Source: Chevron Chemical Company, Richmond, California, USA Methamidophos enantiomers D-(+) and L-(-) Purity: D-(+); chemical 93.1%, optical >99% Purity: L-(-); chemical 95.1%, optical 97.5% Source: Synthesised.
Test Species:	Hen: domestic hen of unspecified strain, 3 and 48-64 wks of age, of unspecifed bw or source
Dose:	35 mg/kg bw technical methamidophos; 30 mg/kg bw analytical methamidophos

Method

Hens were housed in individual cages. The room conditions were optimal for maintaining hens in a laying condition. A light/dark cycle of 16/9 h was established and an unspecified ration was fed *ad libitum*. Before *in vivo* or *in vitro* experiments, analytical and technical grade methamidophos were analysed using TLC and high-resolution GC-MS. The optical isomers (and organic synthesis intermediates) were analysed by NMR after being dissolved in deuterated chloroform. NTE aging assays were conducted using brain homogenates from adult hens and 3 wk old chicks treated *in vivo* and from 18 d old chick embryos treated *in vitro*. In both experimental systems, potassium fluoride (KF) was used as the nucleophilic reactivator. The *in vitro* assay incorporated metabolic activation, NTE inhibition and measurement of aging. It was used to determine the role of metabolic activation in NTE inhibition and aging by various forms of methamidophos and to validate an *in vitro* alternative to whole-hen testing.

Aging measurements in vivo: Solutions containing 70 mg analytical or technical methamidophos/mL were prepared in distilled water. Seven hens were dosed at 35 mg technical methamidophos/kg bw im (at 73.1% purity = 25.6 mg/kg bw). Five hens were dosed with 30 mg analytical methamidophos/kg bw im (at 99.4% purity = 29.8 mg/kg bw). An

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unspecified number of negative control hens received distilled water and 4 positive control hens received DFP (diisopropyl fluorophosphonate) at 0.2 mg/kg bw im. The positive control material DFP rapidly forms aged-inhibited NTE and was used to verify if the assay could detect the loss of NTE reactivation capacity. The ability of the KF system to reactivate a reversible inhibitor was also tested with 2 control birds receiving 10mg/kg bw phenyl di-n-pentylphosphinate (PNPP), a compound that can inhibit NTE without aging. Six 3 wk old chicks received 25 mg/kg bw D-(+) methamidophos and single chicks received either 25 mg/kg bw L-(-) methamidophos, 25 mg/kg bw methamidophos analytical or 0.2 mg/kg bw DFP respectively. All birds were dosed with 20 mg/kg bw atropine and 50 mg/kg bw 2-PAM im before being dosed with methamidophos to reduce the occurrence and/or severity of acute cholinergic signs.

Twenty four h after dosing, the hens and chicks were terminated by exsanguination and whole brain was homogenised using teflon glass and sonication in ice-cold 50 mM Tris-HCl, 0.2 mM EDTA and pH 8 buffer (NTE assay buffer). A solution of KF reactivation buffer and KCl was prepared and reactivation was initiated by adding 1 mL brain homogenate to 10 mL KCl-containing buffer in plastic test tubes. After 30 min incubation the tubes were cooled and the reaction slowed with 25 mL ice-cold distilled water. The cooled tubes were centifuged and the pellet was resuspended in 2 mL NTE assay buffer. NTE activity was determined (Johnson & Read, 1987) and reported as nmoles/min/mg protein.

Aging measurements in vitro: Microsomal protein from phenobarbital-induced livers was isolated from chick embryos and NTE inhibition assays using chick embryo brain (with and without metabolic enzymes) were performed according to the method of (Chow *et al.*, 1986). Incubation time of test compound (1 mM technical and analytical grade methamidophos or 5 mM D-(+) and L-(-)) with embryo brain was 1 h or 30 min for positive controls. After incubation the tubes were cooled and centrifuged and the pellet resuspended in 1 mL 10 mM Tris-HCl buffer by sonication while in an ice bath. Inhibited NTE was reactivated with fresh solutions of KF and enzyme activity measured as before. Leptophos (*O*-[4-bromo-2,5-di-chlorophenyl] *O*-methyl phenylphosphonothioate) with metabolic activation and leptophos oxon without activation were incubated at 37^{o}C for 30 min at 0.1 mM concentrations and served as positive metabolic activation and positive aging controls, respectively. Leptophos requires metabolic activation to become a potent NTE inhibitor and was used to verify the effectiveness of the *in vitro* metabolic activation system. Leptophos oxon forms aged-inhibited NTE.

One undescribed *in vitro* assay (1 nM concentration for 1 h incubation) was conducted to compare NTE inhibitions by the optical isomers with and without metabolic activiation. The outcome but not the data were provided with the results.

Statistical analyses used are described as necessary in the results.

Results and discussion

Chemical analysis: GC-MS analysis of technical grade methamidophos showed a principal peak of *O,S*-dimethyl phosphoramidothioate (methamidophos) accounting for 80% of the total based on total ion current values. The other major components were *O,O*-dimethyl phosphoroamidothioate, 10%, *O,O,N*-trimethyl phosphoroamidothioate, 2%, and *O,O,S*-trimethyl phosphorothioate, < 1%.

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Clinical signs and mortality: Signs in adult hens were not reported. Two/6 chicks receiving D-(+) methamidophos died less than 1 h after dosing. The brain of 1 of them was immediately removed and frozen and was assayed at 24 h with the other samples. The remaining chicks dosed with D-(+) methamidophos required additional doses of atropine to relieve cholinergic signs. The chick that received L-(-) methamidophos showed no clinical signs.

NTE inhibition/aging in vivo: Values for unaged and aged inhibited NTE are tabulated below. The negative control NTE activity values were not provided. The ratio of the unaged-inhibited NTE for technical and analytical methamidophos (43/52 = 0.83) reflected the difference in actual concentration of pure methamidophos administered (25.6/29.8 mg/kg bw = 0.86). The amount of aged-inhibited NTE (6%) produced with analytical methamidophos was significantly less ($p \leq 0.05$) than that produced with technical methamidophos (18%). With L-(-) methamidophos there was a low total inhibition and all inhibited NTE was of the aged type. The positive controls showed inhibited NTE of the aged type (48 and 63%) and where PNPP was used, the opposite effect was observed as expected.

NTE inhibition in hen and chick brain after methamidophos treatment *in vivo*

Compound	**Mean activities (% control) and % NTE 24 h after dosing**				
	A	B	C - Unaged NTE (%) (B - A)	D - Aged NTE (%) (100-B)	Total NTE inhib (%) (C + D)
Methamidophos technical and analytical (hen)					
Methamidophos technical	39	82	43	18*	61
Methamidophos analytical	42	94	52	6	58
Diisopropyl fluorophosphonate (DFP)	54	52	<1	48	48
Phenyl di-n-pentylphosphinate (PNPP)	44	100	56	0	56
Methamidophos resolved optical isomers (chick)					
Methamidophos D-(+)	61	90	29	10	39
Methamidophos D-(+)[#]	80	96	16	4	20
Methamidophos L-(-)	100	86	0	14	14
Methamidophos analytical	58	86	28	14	42
Diisopropyl fluorophosphonate (DFP)	45	37	0	63	63

A = activity after treatment with KCl (% control); B = activity after treatment with KF (% control)

* Statistically significantly different from analytical methamidophos value by Wilcoxon Rank-Sum Test ($p \leq 0.05$)

Found dead < 1 h after dosing; brain frozen and assayed at 24 h

NTE inhibition/aging in vitro: Values for unaged and aged inhibited NTE are tabulated below. The negative control values were not provided. The total NTE inhibition by L-(-) isomer (33%) was less than half that of D-(+) (73%). The data were not shown for 1 assay (1 mM concentration, 1 h incubation) which demonstrated that the absence of metabolic activation did not influence the results observed for the isomers.

NTE inhibition in chick embryo brain after methamidophos treatment *in vitro*

C'pound	**Mean activities (% control) and % NTE following 1 h incubation**						
	Metab. activation	**Conc (mM)**	**A**	**B**	**C - Unaged NTE (%) (B - A)**	**D - Aged NTE (%) (100 - B)**	**Total NTE inhib (%) C + D**
Methamidophos technical and analytical							
M-tech.	+	1	40	89	50	11	60

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M-tech	-	1	37	95	58	5	64
M-analyt	+	1	41	95	54	5	59
M-analyt	-	1	43	95	52	5	57
Leptophos	+	0.1	16	88	72	12	84
Leptophos	-	0.1	63	80	17	20	37
L & oxon	-	0.1	2	75	74	25	99
Methamidophos resolved optical isomers							
D-(+)	+	5	28	97	69	4	73
L-(-)	+	5	67	94	27	6	33
Leptophos	+	0.1	23	83	60	17	77
L & oxon	-	0.1	1.4	77	76	23	99

M-tech or M-analyt = methamidophos technical or analytical; L = Leptophos;
A = activity after treatment with KCl (% control); B = activity after treatment with KF (% control)

Conclusions

(1) *In vivo*, methamidophos technical and methamidophos analytical showed a similar capacity to inhibit NTE (61 and 58% total inhibition respectively) within 24 h of dosing. Methamidophos technical produced a significantly higher proportion of aged-inhibited NTE (18%) than methamidophos analytical (6%).

(2) The study authors attributed the enhancement of the NTE aging potential of methamidophos technical to the presence of impurities but considered that the effect demonstrated in this study was not sufficient to fully explain the development of OPIDN in humans after exposure to methamidophos.

(3) *In vitro*, methamidophos technical and methamidophos analytical also showed a similar capacity to inhibit NTE in 1 h of incubation as they did *in vivo*. The total inhibition of NTE by methamidophos technical was not affected by metabolic activation (60% with metabolic activation, 64% without) but the proportion of aged-inhibited NTE formed with metabolic activation (11%) was twice as high as that formed without activation (5%). The proportion of aged-inhibited NTE formed with methamidophos analytical was not affected by metabolic activation (5% with or without).

(4) These *in vitro* studies suggested that bioactivation was required for the enhanced aging potential of methamidophos technical. The study authors attributed the enhancement to the presence of impurities (probably via reactions with impurities).

(5) *In vivo*, the capacity of D-(+) methamidophos to inhibit NTE was 2.8 times greater than that of L-(-) methamidophos within 24 h of dosing (39 and 14% total inhibition respectively). For D-(+) and L-(-) the proportions of aged-inhibited NTE were 10% and 14% respectively.

(6) *In vitro*, the capacity of D-(+) methamidophos to inhibit NTE was 2.2 times greater than that of L-(-) methamidophos within 1 h of incubation (73 and 33% total inhibition respectively). For D-(+) and L-(-) the proportions of aged-inhibited NTE were 4 and 6% respectively. Metabolic activation did not influence these proportions.

(7) Metabolic activation had no discernible effect on the NTE inhibition/aging potential of analytical racemic methamidophos or the resolved isomers suggesting that, under the conditions of this study, they were poor substrates for microsomal mixed-function oxidase systems and for glutathione-dependent transferases.

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Comment

The principal interest of the study authors was the possible contribution made by impurities to the capacity of methamidophos technical to cause NTE inhibition. The methamidophos precursor, *O,O*-dimethyl phosphoroamidothioate, and an *N*-methyl analog, *O,O,N*-trimethyl phosphoroamidothioate, were identified as impurities in the methamidophos technical used in this study. They were also reported to be present in samples obtained from the methamidophos poisoning incidents in Sri Lanka (Senanayake & Johnson, 1982) evaluated in Section 11.2). Many technical products of organophosphorus insecticides contain appreciable amounts of reactive phosphorylating impurities e.g. isomalathion and *O,O,S*-trimethyl phosphorothioate (also found as an impurity here) in malathion (Aldridge *et al.*, 1979). *O,O,S*-trimethyl phosphorothioate has an oral LD_{50} in rats of 60 mg/kg bw cf malathion 10700 mg/kg bw. Rats dosed with 3 x LD_{50} *O,O,S*-trimethyl phosphorothioate, gradually deteriorated in condition and were found dead 3-4 d later. After removing these compounds from technical malathion, the oral toxicity to rats of malathion was reduced from an LD_{50} of 1580 mg/kg bw to 8000 mg/kg bw (Pellegrini & Santi, 1972). Trimethyl phosphorothioates have also been found in technical acephate and are believed to contribute to its toxicity (Umetsu *et al.*, 1977).

10.2 Hens and Humans

Bertolazzi M, Caroldi S, Moretto A & Lotti M (1991): Interaction of methamidophos with hen and human acetylcholinesterase and neuropathy target esterase. Archives of Toxicology, 65(7):580-585.

Test Substance:	Methamidophos resolved optical isomers. Purity: >99.9%. Source: Bayer AG, Leverkusen, BRD.
Test Species:	Hen: 1.5-2.3 kg bw, no further details provided.

Method

Eight hens were decapitated, brains and sciatic nerves were excised immediately, washed in ice-cold 50 mM TRIS/HCl buffer (pH 8.0) containing 0.2 mM EDTA and stored at -80^{o}C. Human nucleus caudatus and cerebral cortex were obtained during necropsy examinations performed within 36 h of death, from the bodies of two patients who died of extranervous causes. Samples were subdivided into fragments of about 300 mg, washed in TRIS buffer and stored at -80^{o}C. Hen tissues and a 1:1 (by weight) mixture of human cortex and nucleus caudatus were then homogenised (10% w/v, and 40% w/v for sciatic nerve) in ice-cold TRIS. In most experiments, unless otherwise indicated, tissue concentrations were 5-7 mg of wet tissue per mL TRIS pH 8.0 buffer for NTE and of 100 mM phosphate buffer, pH 7.4 for AChE experiments. All experiments were performed at 37^{o}C.

Human and hen brain NTE activity was assayed according to (Johnson, 1977). For peripheral nerve NTE assay a modification of that method was used (Caroldi & Lotti, 1982; Moretto *et al.*, 1989). AChE activity was measured according to (Ellman *et al.*, 1961) with slight modifications.

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In determining rate constants for NTE inhibition, hen and human homogenates were incubated in TRIS with D-(+) methamidophos (200 μM) or L-(-) methamidophos (2 mM) and NTE activity was measured at regular intervals up to 360 min. Activity of uninhibited control NTE did not change within this period. D-(+) and L-(-) methamidophos did not undergo significant non-enzymatic hydrolysis when incubated in the same conditions for 360 min. The k_as of methamidophos isomers were then calculated from fixed time (20 min) incubation of enzymes (8 concentrations, 0.06-2 mM). Hen sciatic nerve k_as were similarly calculated..

For AChE, hen and human brain homogenates were incubated in phosphate buffer with D-(+) methamidophos (20 μM) or L-(-) methamidophos (2 μM) and AChE activity was measured at regular intervals up to 500 min. Activity of uninhibited control AChE did not change within this period. D-(+) and L-(-) methamidophos did not undergo significant non-enzymatic hydrolysis when incubated in the same conditions for 500 min. The k_as were calculated from fixed-time incubations of enzymes in phosphate buffer (6-8 concentrations; for D-(+) methamidophos: 50-300 μM and for L-(-) methamidophos: 1-30 μM). Incubation time for L-(-) methamidophos was 20 min while for D-(+) methamidophos it was reduced to 10 min, corresponding to about 1/10th of reactivation half time as suggested by (Aldridge & Reiner, 1972).

Apparent reactivation rate constants, k_{+3}s, were calculated in two separate experiments after removal of the inhibitor. Hen brain homogenates (TRIS pH 8.0) were incubated with either D-(+) (800 μM and 1 mM) or L-(-) (50 and 100 μM) methamidophos for 20 min at 37°C. The 30000 g pellets (20 min at 4°C) were washed and resuspended in TRIS. Nucleus caudatus homogenates (TRIS pH 8.0) were incubated with either D-(+) methamidophos (400 μM or 1 mM) or L-(-) methamidophos (25 or 100 μM) and similarly processed. The % of residual AChE after incubation at 37°C for different times (t_x up to 16 h) was calculated from similarly processed homogenates that did not contain inhibitors. Percentage of reactivation was then calculated as follows:

$$\frac{\text{activity } t_x - \text{activity } t_0}{100 - \text{activity } t_0} \times 100$$

Apparent k_{+3} was calculated from the initial linear portion of a semilog plot of % reactivation against time. Physostigmine was used as positive control and $10^3.k_{+3}$ (min^{-1}) for hen and human AChE were 9 and 15 respectively, when enzymes were 100% inhibited with 1μM physostigmine for 20 min. Paraoxon was used as negative control (no reactivation after 100% AChE inhibition obtained with paraoxon 50 nM for 20 min).

Rate constants were also calculated from the plotting of time-points vs log % remaining activity by solving the following equations (Aldridge & Reiner, 1972):-

$$\ln \frac{\%\text{ inhibition}_{st.st}}{\%\text{ inhibition}_{st.st} - \%\text{ inhibition}_t} = (k_a.[C] + k_{+3})t$$

$$\frac{100 - \%\text{ inhibition}_{st.st}}{\%\text{ inhibition}_{st.st}} . [C] = \frac{k_{+3}}{k_a}$$

Results and discussion

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NTE: The time-course of both hen and human NTE inhibition by D-(+) or L-(-) isomers of methamidophos was observed to follow first order kinetics extrapolating through the origin. The data for each isomer are tabulated below.

Linear regression data of time-courses of hen and human brain NTE inhibition

Isomer/conc of methamidophos	**Linear regression parameters**			
	Species	**Slope**	**Intercept**	**r**
L-(-)	Hen	-0.004	1.981	-0.99
(2 mM)	Human	-0.006	2.014	-0.99
D-(+)	Hen	-0.003	2.043	-0.98
(200 μM)	Human	-0.002	1.975	-0.95

Fixed-incubation time inhibitions were reported as linear regression plots of log % activity NTE against concentration (mM) of isomer. With L-(-), the respective r values for hen and human homogenates were -0.999 and -0.994; with D-(+), the respective r values for hen and human homogenates were - 0.999 and -1.000.

In fixed-incubation time inhibition (20 min), the D-(+) isomer was a more potent an inhibitor of NTE than the L-(-) isomer. At a concentration of 1 mM, the D-(+) isomer inhibited hen and human NTE activity by 25 and 40% respectively. By comparison, a 10 mM concentration of the L-(-) isomer inhibited hen and human NTE activity by 10 and 15% respectively.

The study authors indicated without elaboration that the inhibition of NTE was progressive and irreversible.

AChE: The time course of AChE inhibition by the two isomers did not follow first order kinetics because of spontaneous reactivation of AChE. The data were presented as curves and will not be reproduced here. Regression lines of hen and human AChE inhibition by different concentrations of L-(-) or D-(+) isomers intercepted the ordinate below log % activity suggesting that non-progressive inhibition also occurred (Aldridge & Reiner, 1972). With L-(-), the respective r values for hen and human brain homogenates were -0.981 and -0.999; with D-(+), the respective r values for hen and human brain homogenates were - 0.907 and -1.004.

In fixed-time incubation inhibitions (L-(-) 20 min, D-(+) 10 min), the L-(-) isomer was a more potent an inhibitor of AChE than the D-(+) isomer. At a concentration of 30 μM the L-(-) isomer inhibited hen and human AChE activity by 20 and 40% respectively. By comparison, a 300 μM concentration of the D-(+) isomer inhibited hen and human AChE by 20 and 30% respectively.

Spontaneous reactivation of AChE: The time courses of spontaneous reactivation for each isomer in each species were presented as curves of % reactivation against time up to 16 h. These curves will not be reproduced here. Complete reactivation was not observed. The study authors suggested that it was possible that aging had occurred. Approximately 60% of human and 30% of hen AChE inhibited by L-(-) isomer had spontaneously reactivated by 6 h. No further reactivation was observed. Approximately 20% of human and 12% of hen AChE inhibited by D-(+) had spontaneously reactivated at 6 h. No further reactivation was observed.

Rate constants were calculated for AChE in brain and NTE in brain and peripheral nerve. They are tabulated below. The study authors regarded the rate constants for NTE inhibition in hen brain and sciatic nerve to be similar. For each isomer, the k_a(AChE) in humans was twice

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that in hens. The ratios between apparent k_{+3}s for human and hen AChE were 4.3 and 1.2 for L-(-) and D-(+) isomers respectively.

Rate constants of hen and human brain AChE and NTE

Species	Isomer	Rate constants for AChE and NTE			
		AChE		NTE	k_a(AChE)/ k_a(NTE)
		k_a(M^{-1} min^{-1})[a]	k_{+3}(min^{-1})[b]	k_a(M^{-1} min^{-1})[a]	
Hen	D-(+)	0.13×10^3	0.69×10^{-3}	59 (51)[c]	2.2
	L-(-)	2.8×10^3	1.52×10^{-3}	3.0 (5.0)[d]	933
Human	D-(+)	0.24×10^3	0.83×10^{-3}	88	2.7
	L-(-)	5.70×10^3	6.50×10^{-3}	3.2	1781

a: rate constants calculated from experiments performed with fixed incubation times
b: rate constants calculated from apparent first order kinetics for time-points up to 2 h after removal of the inhibitor
c and d: corresponding value for sciatic nerve was 51 and 5.0

Conclusions

(1) The respective rate constants for the inhibition of AChE by D-(+) methamidophos in hen and human brain homogenates were 0.13×10^3 and 0.24×10^3 M^{-1} min^{-1}; for the inhibition of NTE by D-(+) methamidophos they were 59 (51 sciatic nerve) and 88 M^{-1} min^{-1} respectively.

(2) The respective rate constants for the inhibition of AChE by L-(-) methamidophos in hen and human brain homogenates were 2.8×10^3 and 5.7×10^3 M^{-1} min^{-1}; for the inhibition of NTE by L-(-) methamidophos they were 3.0 (5.0 sciatic nerve) and 3.2 M^{-1} min^{-1} respectively.

(3) On the basis of the rate constants, the L-(-) isomer was 22 to 24 times more potent inhibitor of AChE than the D-(+) isomer in hen and human brain homogenates respectively, but the D-(+) isomer was a more potent inhibitor of NTE than the L-(-) isomer; 20 and 28 times more potent in hen and human brain homogenates respectively.

(4) Each isomer was a more potent inhibitor of AChE than NTE in both hen (D-(+) 2.2; L-(-) 930 times as potent) and human (D-(+) 2.7; L-(-) 1780 times as potent) brain homogenates.

(5) For each isomer, the rate constants for inhibition of AChE in human brain homogenates were twice those for hen brain homogenates.

(6) The respective rate constants for the spontaneous reactivation of AChE inhibited by D-(+) methamidophos in hen and human brain homogenates were 0.69×10^{-3} and 0.83×10^{-3} min^{-1}.

(7) The respective rate constants for the spontaneous reactivation of AChE inhibited by L-(-) methamidophos in hen and human brain homogenates were 1.52×10^{-3} and 6.50×10^{-3} min^{-1}.

(8) The rate of spontaneous reactivation of AChE inhibited by L-(-) methamidophos is 2 and 8 times greater than that following inhibition by the D-(+) isomer in hen and human brain homogenates respectively.

Comment

The study authors considered the neuropathic potential of an organophosphorus compound can be assessed by measuring its relative capacity to inhibit AChE and NTE. It had been

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shown (Lotti & Johnson, 1978) that the ratio AChE I_{50}/NTE I_{50} for a given compound correlates with its *in vivo* response. This study indicated that the optical isomers of methamidophos are more potent inhibitors of AChE than NTE both in hen and human brain homogenates. Another study (Vilanova *et al.*, 1987) showed also that racemic methamidophos was a more potent inhibitor of AChE than NTE in hen brain homogenates. These results suggest that *in vivo*, the development of OPIDP would be possible in hens and humans only after potentially fatal acute intoxications. This was the clinical picture observed in humans after methamidophos poisoning (Senanayake & Johnson, 1982). In that instance OPIDP developed after severe cholinergic signs. Other work (Flucke *et al.*, 1990b) showed that OPIDP could be induced in antidote protected hens using D-(+) methamidophos at 5 x LD_{50} for that isomer. The occurrence of OPIDP in unprotected hens has not been demonstrated.

10.3 Humans

Robinson CP & Beiergrohslein D (1982): Inhibition of human erythrocyte and plasma cholinesterases by methamidophos. Journal of Applied Toxicology, 2(4):217-218

Test Substance:	Methamidophos. Source: Chevron Chemical Company, Richmond, California, USA. No further details provided.
Dose:	*In vitro* study. Concentrations (M x 10^{-5}) of methamidophos used: with plasma 4.01, 7.22, 8.67, 10.80; with erythrocytes 1.91, 4.78, 6.69, 9.56

Method

Twenty mL blood was collected from an adult human male in a heparinised syringe. RBC and plasma were separated and the RBC washed. Samples of lysed RBC and plasma were diluted with 100 or 10 volumes of normal saline respectively. Ten mL diluted sample was placed in a chamber maintained at 37^{o}C and a concentration of methamidophos was added. Four concentrations of methamidophos were used for plasma and RBC ChE activity measurements. Aliquots of each sample were removed at approximately 1 min intervals for several minutes and their ChE activity determined with an automated colorimetric method (Fowler & McKenzie, 1967) with acetylthiocholine as substrate. This was repeated with 3 other concentrations of methamidophos for both plasma and RBC dilutions. The logarithm of the ChE activity was plotted against time. The slope and correlation coefficient were calculated for each concentration of methamidophos from the linear regression lines by the method of least squares. The inhibitory rate constant (k_i) was calculated for each concentration and the mean k_i was determined.

Results and discussion

The slopes, correlation coefficients and rate constants for each concentration of methamidophos are tabulated below. ChE activities in RBC and plasma decreased during the 7-12 min observation period. The rate of inhibition of RBC ChE was greater than that of plasma ChE ($p < 0.05$). The rate constant of inhibition for human plasma ChE was 1.85 x 10^3 /M/min compared with 1.57 x 10^3 /M/min for rats (Robinson & Beiergrohslein, 1980). The rate of inhibition for human RBC ChE was 3.14 x 10^3 /M/min compared with 8.86 x 10^3 /M/min for rats. Although the inhibition rate constant for methamidophos in plasma ChE is similar in rats and humans, the inhibition rate constant for RBC ChE in rats is 2.8 times as

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high as it is in humans. The study authors commented that the rates of inhibition for both types of ChE in humans were slow compared to that of other ChE inhibitors.

Rate constants of inhibition of human male cholinesterases by methamidophos

Material	Rate constants for each concentration of methamidophos over 7-12 min				
	Methamidophos concentration (M x 10^{-5})	**Slope* (x 10^{-4})**	**Correlation coefficient**	**k_i (x 10^3 /M/min)**	**Mean k_i**
Plasma	4.01	5.30	-0.994	1.82	
	7.22	9.82	-0.996	1.88	1.85
	8.67	11.50	-0.999	1.83	
	10.80	11.64	-0.998	1.87	
RBC	1.91	5.17	-0.997	3.73	
	4.78	9.88	-0.998	2.85	3.14
	6.69	13.45	-0.997	2.75	
	9.56	22.20	-0.997	3.21	

* Slope = logarithm of the change in ChE activity/min

Conclusion

Methamidophos was a comparatively weak inhibitor of human ChEs. However this finding does not preclude high toxicity to humans as methamidophos is highly toxic to rats despite a weak plasma ChE inhibition in that species.

10.4 Rats

Robinson CP & Beiergrohslein D (1980): Cholinesterase inhibition by methamidophos and its subsequent reactivation. Pestic Biochem Physiol, 13:267.

Test Substance: Methamidophos laboratory grade
Purity: 98%
Source: Chevron Chemical Company, Richmond, California, USA.

Test Species: Rat: adult, male, obtained from Holtzman Co, Madison, Wisc., USA: no further details provided.

Dose: *In vivo study*: 0, 12.5, 15.0, 17.5, 20, 30, 40, 45, 60 mg/kg ip.
In vitro study: unspecified concentrations.

Method

Determination of first order rate constants for in vitro inhibition of plasma, RBC and brain ChE: An unspecified number of adult male rats were anaesthetised with ether and their abdominal cavities surgically opened. Twelve mL of blood was collected in a heparinised syringe from the abdominal aorta. Their brains were then removed, washed with normal saline, blotted dry and frozen for later assay. The blood was centifuged for 15 min to separate cells from plasma. A plasma sample was diluted with 3 parts of normal saline and maintained at 37^{o}C. An "appropriate" concentration of methamidophos in water was added to the diluted plasma in the chamber. The plasma ChE activity was determined at 1.5 min intervals for 15 min using the Technicon autoanalyzer method of (Fowler & McKenzie, 1967). This was repeated with different unspecified concentrations of methamidophos. The sedimented RBC were re-suspended in normal saline, centrifuged and the supernatant discarded. This washing was repeated once. The RBC were then lysed and diluted. Various concentrations of methamidophos were added to the haemolysate and RBC ChE activity was determined using

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the same method. Brains were homogenised in cold normal saline, diluted and their ChE activity determined.

Spontaneous and pralidoxime-induced reactivation of inhibited ChE in vitro: Four mL aliquots of plasma was divided into 2 parts. To one 2 mL sample excess methamidophos (20 mg) was added; nothing was added to the 2nd 2 mL sample which served as control. Both samples were incubated at 37^{o}C for 15 min. Excess unreacted methamidophos was then removed by desalting column chromatography. The control sample was treated in the same way. Plasma ChE activity was determined spectrophotometrically (Ellman *et al.*, 1961). The substrate used was acetylthiocholine iodide. To determine spontaneous reactivation, 100 µL of eluate containing inhibited enzyme was added to the sample cuvette at intervals of 0.5, 2.5, 4.5, 6.5 and 24.5 h after the removal of excess methamidophos and ChE activity was recorded. In determining spontaneous and pralidoxime-induced reactivation of brain ChE, rat brains were homogenised, diluted and processed in the same way. The effectiveness of pralidoxime in reactivating the inhibited enzyme was determined by adding 20 µL of 2 x 10^{-3} M pralidoxime solution to sample and reference cuvettes immediately after the rate of spontaneous reactivation had been determined. Identical fractions of uninhibited and inhibited enzymes from the desalting column were collected. To correct for any differences in amount of ChE collected, protein determinations of the samples were made using the method described by (Quistad *et al.*, 1970). Rates of hydrolysis were corrected for differences in protein content. Uninhibited brain and plasma enzyme preparations incubated at 37^{o}C lost approximately 25% of their initial ChE activity during the 24.5 h incubation.

Determination of in vivo protection by atropine and pralidoxime: One hundred and thirty male rats were divided into 3 groups each containing 40, 40 and 50 rats respectively. These groups were further subdivided into subgroups of 10. All rats were fasted for 18 h prior to dosing. Forty rats were given unspecified graded doses of methamidophos ip to determine the acute LD_{50}. The 40 rats of the 2nd group were given unspecified graded doses of methamidophos ip and 5 s later were given 10 mg/kg bw atropine im. The 50 rats of the 3rd group were given unspecified graded doses of methamidophos ip and 5 s later was given 60 mg/kg bw pralidoxime im. Deaths were observed and recorded for up to 24 h. Methamidophos, atropine and pralidoxime were prepared in water immediately before use. The LD_{50} at 24 h were determined for each group using the method of (Miller & Tainter, 1944).

[Note: The dosing regimes used were not specified in the methodology section of this study, but the tables and curves presented in the results section reported doses].

The brains of all rats that died were quickly removed and ChE activity was determined. The survivors of the LD_{50} determinations were terminated by decapitation 24 h after dosing. The brain and stomach of each was quickly removed and frozen for later ChE determination. Blood samples collected from the cervical area following decapitation were heparinised and centrifuged to separate cells and plasma. The plasma was refrigerated and assayed for ChE activity 24 h later.

[Note: A perusal of result tables and curves suggests that survivors of the protective dosing studies were also processed in a similar way]

Recovery of plasma, stomach and brain ChE activity at various times following administration of methamidophos or methamidophos and pralidoxime. One hundred and

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twenty male rats were divided into 2 groups of 60. One group was given 12.5 mg/kg of methamidophos ip (an LD_{20}) and the other was given the same dose of methamidophos plus 60 mg/kg pralidoxime im 5 s later. Ten additional rats served as controls. The brains of all rats that died were removed and assayed for ChE activity. The surviving rats were subdivided into subgroups which were terminated by decapitation at intervals of 1, 3, 6, 24 and 48 h after methamidophos was given. The plasma, brains and stomachs of treated and control rats were collected, homogenised and ChE was determined (Ellman *et al.*, 1961).

Statistical analyses used were not described.

Results and discussion

First order rate constants: Plasma, RBC and brain ChE activities decreased in the presence of methamidophos at an exponential rate for the duration of the incubation period. The inhibition of plasma ChE ($k_i = 1.57 \times 10^3\ M^{-1}\ min^{-1}$) proceeded at a slower rate than did the inhibition of RBC ChE ($k_i = 8.86 \times 10^3\ M^{-1}\ min^{-1}$) or brain ChE ($6.58 \times 10^3\ M^{-1}\ min^{-1}$). Inhibition rate constants for RBC and brain ChE were not significantly different ($p < 0.05$). Both rate constants were significantly different ($p < 0.05$) from that for inhibition of plasma ChE.

In vitro reactivation of inhibited ChE: The data obtained was presented as curves which will not be reproduced here. They indicated imprecisely that at 0.5 h, 3 and 8% of control activity remained for brain ChE activity and plasma ChE activity respectively. Brain and plasma ChE spontaneously reactivated. Approximately 50% of brain ChE control activity was restored in 4 h compared with 14 h for plasma ChE. Inhibited brain and plasma ChE continued to reactivate for at least 25 h, achieving 88% (3.5%/h) and 65% (2.6%/h) of control activity respectively.

The addition of pralidoxime to inhibited enzyme preparations within a few minutes of removal of excess inhibitor, resulted in a partial restoration of enzyme activity (e.g. when pralidoxime was added at 0.5 h, brain ChE activity was restored from 3 to 60% of control activity). Complete reactivation of inhibited enzyme was not observed. The ability to restore ChE activity progressively declined at each test interval over 0.5-25 h The data were presented as curves which will not be reproduced here. It was apparent that at 2.5 h and 6.5 h, brain ChE activity was approximately 30% and 8% restored respectively. At the same intervals plasma ChE activity was approximately 10% and 0% restored respectively. At 25 h, pralidoxime reactivation of inhibited enzyme was no longer observed. The addition of a 2nd dose of pralidome at the various time intervals did not further increase the rate of return of the ChE activity but usually slightly decreased it.

In vivo protection: All rats dosed with methamidophos exhibited typical signs of peripheral and central cholinergic stimulation including salivation, lacrimation, fasiculations, urination, piloerection, respiratory depression and convulsions. All rats that died after dosing with methamidophos did so within 15 min after dosing. After 24 h survivors showed marked improvement. The ip LD_{50} for methamidophos was 15 mg/kg bw.

Atropine and pralidoxime protected against the lethality of methamidophos, increasing the LD_{50} to 60 and 52 mg/kg respectively. The rats that died following treatment with atropine generally showed less frequent tremors and convulsions. All of those treated with atropine that died did so within 15 min of dosing. Most of the deaths observed in rats given methamidophos plus pralidoxime occurred between 1-2 h after dosing. The signs of

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cholinergic stimulation in rats after treatment with pralidoxime were as severe as those in rats given only methamidophos.

Brain ChE activity of the rats that died was markedly depressed with activities ranging from 5.3-12.4% of control. The relationship between treatment and brain ChE activity at the time of death is tabulated below. The number of control rats was not specified.

Brain ChE activity in male rats that died following administration of methamidophos

Dose (mg/kg ip) methamidophos	Mean ChE activity (µmol Acetylthiocholine hydrolysed/g) at death			
	Protectant*	No. of rats	ChE activity	% control
Control	-	?	7.03	100
12.5	Nil	19	0.81	11.5
15	Nil	5	0.87	12.4
17.5	Nil	8	0.52	7.4
30	Atropine	1	0.69	9.8
40	Pralidoxime	1	0.37	5.3
45	Atropine	3	0.62	8.8
45	Pralidoxime	3	0.57	8.1
60	Atropine	5	0.64	9.1
60	Pralidoxome	7	0.45	6.4

* Atropine: 10 mg/kg im 5 s after dosing; Pralidoxime: 60 mg/kg im 5 s after dosing

Plasma, stomach and brain ChE activity in rats alive at 24 h after various dosing regimes were presented only as curves of activity against dose of methamidophos which will not be reproduced here. The following table is constructed using data interpreted as precisely as the curves permit.

Plasma, stomach and brain ChE activity 24 h after treatment with methamidophos

Dose (mg/kg ip) methamidophos	Mean ChE activity at 24 h (% of control)			
	Protectant*	Plasma	Stomach	Brain
12.5	Nil	54	20	67
15	Nil	60	25	49
17.5	Nil	49	19	49
20	Nil	52	23	39
20	Atropine	52	23	64
20	Pralidoxime	-	15	52
30	Atropine	58	20	59
40	Pralidoxime	42	12	50
45	Atropine	21	16	50
45	Pralidoxime	32	8	44
60	Atropine	36	9	50
60	Pralidoxome	23	7	43

* Atropine: 10 mg/kg im 5 s after dosing; Pralidoxime: 60 mg/kg im 5 s after dosing

In vivo recovery of plasma, stomach and brain ChE activity at various times following administration of methamidophos or methamidophos and pralidoxime: Plasma, stomach and brain ChE activities at 1, 3, 6, 24 and 48 h after dosing with 12.5 mg methamidophos/kg bw ip or 12.5 mg methamidophos/kg bw ip plus 60 mg pralidoxime/kg bw im 5 s later, were presented as curves of activity (% control) against time which will not be reproduced here. Data was interpreted from the curves and text to produce the following table. In the methamidophos treated group the rate of recovery of ChE activity up to 24 h was 2.1, 1.9 and 0.8%/h for brain, plasma and stomach respectively. At 48 h plasma, stomach and brain ChE

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had returned to 60, 62 and 80% of control values. The study authors regarded the differences in ChE recovery rates between the methamidophos treated group and the methamidophos plus pralidoxime treated group as slight and did not attribute to pralidoxime any effect on ChE activity restoration rate.

Recovery of ChE activity in male rats

ChE/group	Recovery of plasma, stomach and brain ChE activity (% control)* at various intervals (h) after treatment				
	1	**3**	**6**	**24**	**48**
Plasma A	5	8	20	47	60
B	10	10	20	45	70
Stomach A	8	9	12	25	62
B	4	7	9	25	70
Brain A	22	27	50	74	80
B	25	26	52	82	83

A-12.5 mg methamidophos/kg bw; B-2.5 mg methamidophos/kg bw plus 60 mg pralidoxime/kg bw
* At time 0 h the plasma, stomach and brain ChE activity for each group was shown as 100% of that of untreated controls

Discussion: The study authors considered that there was a poor correlation between the acute lethality of methamidophos and its ability to inhibit ChEs *in vitro* using data obtained in this study and elsewhere. Methamidophos is 1/40th as lethal to the rat as paraoxon (Robinson *et al.*, 1976; Robinson & Beiergrohslein, 1980) but is only 1/30,000th as rapid an inhibitor of ChE *in vitro* (paraoxon: $k_i = 2.7 \times 10^7$ M^{-1} min^{-1}; methamidophos: $k_i = 9.2 \times 10^2$ M^{-1} min^{-1} for house-fly head ChE; (Quistad *et al.*, 1970)). *In vitro* ChE inhibition data for paraoxon in rats were not given for comparison. The phenomenon of aging of the phosphorylated enzyme was considered as possibly enhancing the *in vivo* lethality of methamidophos by interfering with the spontaneous reactivation of inhibited ChE, as happens with house fly ChE which does not spontaneously reactivate following methamidophos inhibition and for which pralidoxime produced only a partial reactivation (Quistad *et al.*, 1970).

In this study spontaneous reactivation of inhibited ChEs was observed *in vitro* and *in vivo* but pralidoxime produced only partial or no recovery of ChE activity. The loss of pralidoxime-induced capacity to reactivate decreased with time, suggesting that aging was occurring. The *in vitro* work suggested that aging and spontaneous reactivation were occurring simultaneously. The rate of spontaneous regeneration of inhibited ChE decreased after the 1st 3 h. The study authors believed that this reduction may be due to the formation of an aged enzyme that is not completely refractive to nucleophilic displacement, allowing some reactivation.

Conclusions

(1) The rate constants (k_is) for the *in vitro* inhibition of plasma, RBC and brain ChE by methamidophos were 1.6×10^3, 8.9×10^3 and 6.6×10^3 M^{-1} min^{-1} respectively. The rate constants for RBC and brain ChE inhibition were not significantly different ($p < 0.05$). Both RBC and brain ChE inhibition rate constants were significantly different ($p < 0.05$) from that for inhibition of plasma ChE.

(2a) *In vitro* inhibited brain and plasma ChE spontaneously reactivated. Inhibited brain ChE took 4 h to reactivate to 50% of control activity compared to 14 h for inhibited plasma ChE.

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After 25 h the inhibited brain and plasma enzyme activities had regained 88 and 65% of control activity, respectively

(2b) The addition of pralidoxime to *in vitro* inhibited enzyme preparations partially restored enzyme activity (maximum 37 and 27% restoration of brain ChE and plasma ChE respectively at 0.5 h). Complete reactivation of inhibited enzyme was not observed. The ability to restore ChE activity progressively declined with time over 0.5-25 h. The addition of a 2nd dose of pralidoxime at the various test intervals did not further increase the rate of return of the ChE activity.

(3) The ip LD_{50} for methamidophos was 15 mg/kg bw. All rats that died after dosing with methamidophos did so within 15 min after dosing. In these rats mean brain ChE activity after death was reduced to 12.4% of control activity. In those that survived the LD_{50} dose, brain, plasma and stomach ChE was observed to be 49, 49 and 19% of control activity respectively 24 h after dosing.

(4) Atropine and pralidoxime protected against the lethality of methamidophos, increasing the ip LD_{50}s to 60 and 52 mg/kg respectively. All rats treated with methamidophos and atropine that died, did so within 15 min of dosing. Most of the deaths observed in rats given methamidophos plus pralidoxime occurred between 1-2 h after dosing.

(5) *In vivo* inhibited plasma, stomach and brain ChE activity spontaneously reactivated. During the 1st 24 h after a 12.5 mg/kg bw dose of methamidophos, the rates of restoration of activity were 2.1%/h, 1.9%/h and 0.8%/h for brain, plasma and stomach ChE respectively. At 48 h after dosing, plasma, stomach and brain ChE had returned to at least 60% of control values. Pralidoxime did not improve reactivation rates.

Guo L, Chen Y, Lu R, Liu Y, Xia L, Chen J, Zhang X & Mao J (1986b): Acute, cumulative and sub-chronic toxicity, delayed neurotoxicity and experimental treatment of intoxication. Part 1: Acute Toxicity. Section (2): Threshold dose for inhibition of whole blood ChE activity and dynamic change in ChE activity. Zhonghua Laodong-Weisheng Zhiyebing Zazhi, 4(5):257-260. [BAL: ECRP Submission, Dossier file No. 5.2.1.1/15, OCS Ref.12153, Vol 4/31, Tab 14 (Copy of the published article)].

[Preliminary note: *The LD_{50} determinations reported in Section 1 of Part 1 (Guo et al., 1986a) are listed in the table in Section 3.1.1 of this review]*

Test Substance:	Methamidophos "direct isomerisation crystal". Purity: 90.4% Source: Zhejiang Research Institute.
Test Species:	Rat: Wistar, male and female, approximately 215 g bw of an unspecified age and obtained from an unspecified source.
Dose:	1.0, 1.5, 2.0 mg/kg bw ig; 20, 30, 50 mg/kg bw percutaneously; 5 mg/kg bw ig

Prelude

The techniques for whole blood ChE activity measurement were not described and the measurements of activity were not provided. Activity was presented as percentages of pre-dosing whole blood ChE activity which are presumed to be percentage declines in group mean activity.

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Method

The conditions under which the rats were maintained and the nature of their diet were not specified. Groups of rats (8/sex) were dosed ig with an aqueous solution of crystalline methamidophos. Blood samples were collected 0.5 h after dosing and whole blood ChE activity was determined by photoelectric colorimetry. Percutaneous exposure was also carried out using 20 male rats in 3 groups of unspecified sizes. Blood samples were collected before and at 4 h after dermal administration of an aqueous solution of methamidophos and whole blood ChE activity was determined. The solution was applied in a volume of 0.1 mL/100 g bw to an area of shaved skin of approximately 4 x 5 cm^2 on the back of rats. To measure the dynamic change in whole blood ChE activity, the pre-exposure ChE activity was determined twice in 7 male rats. The mean result was accepted as normal ChE activity. The rats were then given 5.0 mg/kg bw of an aqueous solution of methamidophos ig. Blood samples were collected at 0.5, 2, 6, 12, 24, 48, 96, 144 h and ChE activity was determined.

Results and discussion

Clinical signs and mortality: After oral administration of 2 mg/kg bw, some of the rats developed acute undescribed intoxication signs. After percutaneous exposure at 30 mg/kg crawling, prostration, general muscular tremor and salivation were observed. At 50 mg/kg percutaneously, well-defined toxic signs appeared. In the determination of the dynamic change in whole blood ChE, the decline and restoration of ChE activity with time was consistent with the appearance and alleviation of toxic signs and the time of death observed in earlier work (Guo *et al.*, 1986a). No further details were provided.

Whole blood ChE activity at 0.5 h after oral dosing: The ChE activity measurements were not reported. At 1.0 mg/kg there was a decrease in ChE activity to 72-79% of the pre-dosing activity in 1 male and 3 female rats. At 1.5 mg/kg there was a decrease to 50-77% in 6 male and 7 female rats and at 2.0 mg/kg there was a decrease to 39-77% in 6 males and 7 females.

Whole blood ChE activity at 4 h after cutaneous application: The ChE activity measurements were not reported. At 20 mg/kg, ChE activity rose to 120% of the pre-dosing activity in a small number of rats. At 30 mg/kg the ChE activity decreased to 55-58% in half of the rats. At 50 mg/kg ChE activity decreased to 18-50% in all rats. The number of rats affected at each dose level was not specified.

Dynamic change in whole blood ChE activity following 5 mg/kg ig methamidophos: The ChE activity measurements were not reported. ChE activity declined to 40.1% at 0.5 h after dosing, 21.6% at 2 h and had recovered substantially at 24 h. ChE activity was restored to pre-dosing levels within 48-72 h.

Conclusion

(1) Inhibition of whole blood ChE (21-28%) was first observed at 0.5 h following intragastric administration of 1 mg/kg bw of methamidophos. On this basis the ig threshold dose eliciting inhibition was 1 mg/kg bw.

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(2) Inhibition of whole blood ChE (42-45%) was first observed at 4 h following cutaneous application of 30 mg/kg. It was not demonstrated at 20 mg/kg possibly due to biological or methodological variation.

(3) Methamidophos appears to be relatively poorly absorbed percutaneously compared to absorption through the gastro-intestinal mucosa. This may be related to its hydrophilic nature. At 0.5 h after ig dosing at 1.5 mg/kg bw, 23-50% whole blood ChE inhibition was observed. At 4 h after percutaneous dosing at 30 mg/kg/bw, 42-45% inhibition was observed. Twenty times the dose and up to 8 times the exposure time were required for percutaneous administration to produce ChE inhibition within the range of that produced by intragastic administration of 1.5 mg/kg bw.

[In Section 1 Part 1 (Guo *et al.*, 1986a), the study authors determined the oral LD_{50} for methamidophos as 23 mg/kg bw in male rats and 13 mg/kg bw in females; the percutaneous LD_{50} as 355 mg/kg bw in male rats and 380 mg/kg bw in females. Fifteen times the ig dose was required in males to produce median lethality percutaneously. The equivalent factor for females was 29.]

(4) Sixty per cent inhibition of whole blood ChE activity was observed at 0.5 h following the intragastric administration of 5.0 mg/kg bw methamidophos. At 2 h the degree of inhibition was 78%. The degree of inhibition had recovered "substantially" at 24 h and "within 48-72 h" it had fully reversed.

Easter MD & Rosenburg DW (1986): The cholinesterase inhibition potential of analytical grade methamidophos (SX-1672) and methamidophos technical (SX-1490) following topical application of a single dose to male and female rats. Study No. SOCAL 2211 Report No. USASRA 51301287. Lab: Environmental Health Centre, Chevron Chemical Company, Richmond, California, USA. Sponsor: Corporate Toxicology Department, Mobay Chemical Corporation, Stilwell, Kansas, USA. Study Duration: 5 December 1985 - 27 June 1986. Report Date: 27 June 1986. Unpublished. (QA: Yes: GLP: USEPA/FIFRA; 40 CFR 160 & 40 CFR 792; Test Guidelines: not specified).

Test Substances:	Methamidophos analytical grade & Methamidophos technical Identification Numbers: SX-1672 & SX-1490 respectively. Purity: 99.1% & 74.7% respectively. Source: Chevron Chemical Company, California, USA
Test Species:	Rats: Sprague-Dawley, Crl:CD (SD)BR, males, females, aged 43-70 d, of bw 173-263 g, obtained from Charles River Laboratories, Michigan USA.
Dose:	0, 1.0, 2.5, 6.25, 15.6 mg/rat, applied dermally.

Method

The methods used in this study were a modification of those previously published (Knaak *et al.*, 1980). The rats were housed individually in ventilated wire-bottom cages and were maintained under standard environmental conditions except that the humidity ranged from 21-73%. Cage conditioning periods ranged from 17-23 d. The rats had free access to Purina Laboratory Rodent Chow #5002 and water. The rats were randomly allocated as 5

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rats/sex/treatment group. There were 20 treatment groups (2 test substances, 5 dose levels and 2 exposure intervals).

All dosing mixtures were prepared the d prior to dosing and stored in the dark at 5^{o}C until used. The test substance was dissolved in distilled water and 0.1% Tween 80 was added to reduce the surface tension of the dosing mixture. The test material concentration was adjusted so that dosing volume for each animal was 0.250 mL. All dosing mixtures were sampled after dosing and analysed for test substance concentration.

On the d before dosing, the application site was clipped free of hair. On the d of dosing the site was washed with mild soap and water and dried. A neoprene rubber template that defined a 6.25 x 4.0 cm application site was glued to the back of each rat with cyanoacrylate glue. A 6.0 x 4.25 cm site was used for males treated with SX-1672 for 72 h. A Queen Anne collar was placed on each rat to prevent access to the template and test substance. The rats were weighed immediately prior to dosing. This initial bw was used to calculate the % of total body surface area using the equation: total body surface area = 9.64 x bw$^{0.86}$ (Knaak *et al.*, 1984).

A single aliquot of dosing mixture was then applied dermally with an SMI adjustable volume micro/pettor™ and spread evenly over the application site with a glass capillary tube. Each rat was observed 4 times on the d of dosing and 1/d thereafter for signs of toxicity. Viability checks were made 2/d at 6 h intervals. The rats were weighed and killed with sodium pentobarbital at 24 and 72 h after treatment. A blood sample of at least 2 mL was obtained from the descending aorta in a syringe coated with 0.01% w/v sodium heparin and containing physiological saline and placed into a tube with anticoagulant. The spinal cord was cut at the level of the foramen magnum and the whole brain including cerebral hemispheres, cerebellum and brain stem was removed. The brain was cut in half along the midline and each half was weighed separately. The left half of the brain was used for ChE determination and the right half of the brain was retained frozen.

ChE activities were determined using a spectrophotometric method (Ellman *et al.*, 1961). ChE activities in brain and RBC samples were determined with acetylthiocholine as the substrate and were defined as acetylcholinesterase (AChE) activities. The ChE activity in plasma was determined using butyrylthiocholine as a substrate and was defined as non-specific cholinesterase (BChE) activity. The Hct of each blood sample was determined in order to express the RBC activity as mL of whole blood. RBC protein concentration was determined (Wong & Kodama, 1979). The ID_{50}, slope, y-intercept and correlation coefficient for ChE inhibition was determined by linear regression analysis (Sokal & Rohlf, 1969) of log-dose [µg test substance/total body surface area (cm^2)] vs individual ChE activity (per mL or g of tissue). The ID_{50} value was derived as the dose corresponding to 1/2 the mean control activity.

All rats found dead and those terminated were subjected to a gross necropsy. The following organs and tissues were examined histologically: skin, spleen, pancreas, stomach, small and large intestine, liver, adrenals, kidneys, gonads, uterus or seminal vesicles, bladder, heart, thymus, salivery glands, lungs, trachea, thyroid and fat.

The mean ChE activities, protein concentrations, Hct values and mean bw of the treated rats were compared to those of controls using one-way analysis of variance (anova) (Sokal & Rohlf, 1969). Significant differences were evaluated using the two-sided Least Significant Difference test.

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Results and discussion

Dosing mixture and achieved dose: Throughout the study, the mean concentrations of methamidophos SX-1672 and methamidophos SX-1490 in the dosing mixtures ranged from 84.4-102% and 78.8-91.7% of the target nominal concentrations respectively. These were within acceptable limits. The components of SX-1490 other than the active ingredient methamidophos were not specified. The corrected applied dose based on analysis was calculated as follows: (µg/cm^2 x application area (cm^2))/mean initial bw (g). The doses are tabulated below.

The actual applied dose

Test substance	**Applied dose mg/kg - males**									
	Target dose mg/rat and exposure period (h)									
	0		**1.0**		**2.5**		**6.25**		**15.6**	
	24	**72**	**24**	**72**	**24**	**72**	**24**	**72**	**24**	**72**
SX-1672	0	0	3.9	4.27	9.47	9.86	25.0	26.9	61.5	70.2
SX-1490	0	0	3.9	2.07	9.71	8.02	24.2	20.6	63.7	53.3
Test substance	**Applied dose mg/kg - females**									
	Target dose mg/rat and exposure period (h)									
	0		**1.0**		**2.5**		**6.25**		**15.6**	
	24	**72**	**24**	**72**	**24**	**72**	**24**	**72**	**24**	**72**
SX-1672	0	0	4.59	4.11	12.1	10.0	31.7	27.3	81.8	64.2
SX-1490	0	0	4.12	3.65	10.6	8.8	26.6	22.2	74.1	58.3

Clinical signs, mortality and body wt: No mortalities were observed in males. At 15.6 mg/rat 3/5 SX-1672 24 h females died at 6 h-1 d and 2/5 SX-1490 24 h females died at 1.5 h post dosing. One SX-1490 72 h female at 15.6 mg/rat also died. Pre-mortality signs were not observed in the 2 SX-1490 24 h females. The signs observed in the others that died were not different from those in survivors. Cholinergic signs (tremors, fasciculations, salivation, dyspnoea, ataxia, lacrimation and collapse) were observed in most or all rats treated at 15.6 mg/rat and in females treated at 6.25 mg/rat but were absent at 2.5 or 1.0 mg/rat. The time to earliest onset of treatment-related signs varied from 1.5 h to 1 d. No substantial differences in this respect were observed between the two highest dose groups, the periods of exposure or the grades of methamidophos. No significant differences were observed between groups for the initial or terminal bw.

ChE activity: Since the protein-specific ChE activities were almost identical to the non-protein-specific ChE activity they are not reproduced here. The RBC AChE activity in male rats is tabulated below. With one exception (SX-1672 at 72 h at 1.0 mg/rat, $p \leq 0.05$), statistically significant inhibition was first observed at 2.5 mg/rat and above. Inhibition was dose-dependent over 2.5-15.6 mg/rat. Generally, inhibition had partially reversed at 72 h.

Mean RBC AChE activity values in male rats

Group	**Mean RBC activity values and % of controls - males**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
RBC AChE activity[#]					
SX-1672 24 h	0.54 100.0	0.43 79.6	0.29* 53.7	0.14* 25.9	0.04* 7.9
SX-1672 72 h	0.56 100.0	0.42** 75.0	0.29* 51.8	0.24* 42.9	0.02* 3.6
SX-1490 24 h	0.50 100.0	0.37 74.0	0.36 72.0	0.16* 32.0	0.06* 12.0

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SX-1490 72 h	0.50 100.0	0.49 98.0	0.47 98.0	0.25* 50.0	0.12* 24.0

#µmoles/min/mL whole blood
* Statistically significantly different ($p \leq 0.01$)
** Statistically significantly different ($p \leq 0.05$)

The RBC AChE activity in female rats is tabulated below. With one exception (SX-1672 at 24 h at 1.0 mg/rat, $p \leq 0.01$), statistically significant inhibition was first observed at 2.5 mg/rat and above. One hundred % inhibition occurred at 24 h at 15.6 mg/rat with both SX-1672 and SX-1490. Inhibition was dose-dependent. Generally, inhibition had partially reversed at 72 h.

Mean RBC AChE activity values in female rats

Group	**Mean RBC activity values and % of controls - females**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
RBC AChE activity#					
SX-1672 24 h	0.51 100.0	0.33* 64.7	0.20* 39.2	0.02* 3.9	0[a] 0
SX-1672 72 h	0.46 100.0	0.39 82.6	0.44 95.7	0.14* 30.4	0.05* 10.9
SX-1490 24 h	0.44 100.0	0.35 79.5	0.16 * 36.4	0.01* 2.3	0[b] 0
SX-1490 72 h	0.44 100.0	0.34 77.3	0.29** 65.9	0.15* 34.1	0.02[c]* 4.5

#µmoles/min/mL whole blood
* Statistically significantly different ($p \leq 0.01$)
** Statistically significantly different ($p \leq 0.05$)
a: n = 2; b: n = 3; (anova was not performed for n = 2 or 3); c: n = 4

The plasma BChE activity in male rats is tabulated below. With one exception (SX-1490 at 24 h at 1.0 mg/rat, $p \leq 0.05$), statistically significant inhibition occurred at 2.5 mg/rat and above. Inhibition was dose-dependent. Generally, inhibition had partially reversed at 72 h.

Mean plasma BChE activity values in male rats

Group	**Mean plasma activity values and % of controls - males**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
Plasma BChE activity#					
SX-1672 24 h	63.8 100.0	67.9 106.4	30.8* 48.3	19.0* 29.8	7.5* 11.8
SX-1672 72 h	63.8 100.0	66.2 86.5	54.5 85.4	37.4* 58.6	22.5* 35.3
SX-1490 24 h	85.0 100.0	60.7** 71.4	40.2* 47.3	24.4* 28.7	8.7* 10.2
SX-1490 72 h	58.3 100.0	50.4 86.4	41.8* 71.7	25.6* 43.9	14.9* 25.6

nmoles/min/mL plasma
* Statistically significantly different ($p \leq 0.01$)
** Statistically significantly different ($p \leq 0.05$)

The plasma BChE activity in female rats is tabulated below. Statistically significant inhibition occurred with SX-1672 and SX-1490 at 24 h at 1.0 mg/rat and above. Inhibition was dose-dependent. Greater than 90% inhibition occurred with SX-1672 and SX-1490 at 24 and 72 h at 15.6 mg/rat. Generally, inhibition had partially reversed at 72 h.

Mean plasma BChE activity values in female rats

Group	**Mean plasma activity values and % of controls - females**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
Plasma BChE activity#					
SX-1672 24 h	262.5 100.0	110.1* 41.9	69.7* 26.6	17.8* 6.8	15.6[a] 5.9
SX-1672 72 h	315.9 100.0	254.4 80.5	184.0* 58.2	73.0* 23.1	14.6* 4.6

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SX-1490 24 h	318.8 100.0	94.8* 29.7	93.0* 29.2	15.2* 4.8	7.1[b]* 2.2
SX-1490 72 h	336.5 100.0	186.9 55.5	240.9 71.6	78.0* 23.2	16.5[c]* 4.9

nmoles/min/mL plasma
* Statistically significantly different ($p \leq 0.01$)
** Statistically significantly different ($p \leq 0.05$)
a: n = 1 (anova not performed for n = 1); b: n = 3; c: n = 4

Brain AChE activity in male rats is tabulated below. With one exception (SX-1672 at 72 h at 2.5 mg/rat, $p \leq 0.05$) statistically significant inhibition occurred at 6.25 and above. There was no effect at 1.0 mg/rat but there were no indications of AChE inhibition at 2.5 mg/rat albeit without statistical significance. Inhibition was dose-dependent over 2.5-15.6 mg/rat. Inhibition had partially reversed at 72 h at 6.25 and 15.6 mg/rat

Mean brain AChE activity values in male rats

Group	**Mean brain activity values and % of controls - males**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
Brain AChE activity[#]					
SX-1672 24 h	10.66 100.0	11.2 103.4	9.38 88.0	5.60* 52.5	4.10* 38.5
SX-1672 72 h	10.96 100.0	11.04 100.7	10.05 91.7	7.25* 66.2	4.57* 41.7
SX-1490 24 h	9.75 100.0	10.11 103.7	8.18 83.9	5.51* 56.5	2.41* 24.7
SX-1490 72 h	10.38 100.0	9.69 93.4	9.57 92.2	7.65** 73.7	4.67* 45.0

µmoles/min/g brain
* Statistically significantly different ($p \leq 0.01$)
** Statistically significantly different ($p \leq 0.05$)

Brain AChE activity in female rats is tabulated below. Statistically significant inhibition occurred at 2.5 mg/rat and above. Inhibition was dose-dependent. Inhibition had partially reversed at 72 h.

Mean brain AChE activity values in female rats

Group	**Mean brain activity values and % of controls - females**				
	Target dose (mg/rat)				
	0	**1.0**	**2.5**	**6.25**	**15.6**
Brain AChE activity[#]					
SX-1672 24 h	8.24 100.0	7.95 96.5	6.49[a] 78.8	2.87* 34.8	0.93[b] 10.1
SX-1672 72 h	9.64 100.0	9.66 100.2	7.99* 82.9	5.99* 62.1	3.21* 33.3
SX-1490 24 h	6.30 100.0	4.58 72.7	4.56 72.4	1.39* 22.1	0.46[c*] 7.3
SX-1490 72 h	10.24 100.0	9.87 96.4	8.38* 81.8	6.17* 60.3	3.5[a*] 34.2

µmoles/min/g brain; * Statistically significant ($p \leq 0.01$); ** Statistically significant ($p \leq 0.05$)
a: n = 4; b: n = 2; (anova was not performed); c: n = 3

ID_{50}: The doses of test substances (µg/total body surface area (cm^2)) required to produce 50% ChE inhibition are tabulated below. They were calculated by linear regression analysis of RBC AChE (µmoles/min/mL whole blood), plasma BChE (µmoles/min/mL plasma) and brain AChE (µmoles/min/g brain). The regression line coefficients (y-intercepts, slopes and r values) were provided by the study authors. The lowest ID_{50} dose was observed for plasma BChE in 24 h females treated with SX-1490. For all types of ChE except RBC AChE, the ID_{50} was least in 24 h females treated with SX-1490.

ID_{50} doses for each test substance

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Type of ChE	ID_{50} doses (µg/total body surface area (cm^2)) for each test substance, sex and exposure period (h)							
	SX-1672				SX-1490			
	Males		Females		Males		Females	
	24	72	24	72	24	72	24	72
Plasma BChE	9.51	26.83	1.61	7.20	5.85	10.93	0.57	5.82
RBC AChE	6.98	8.00	4.94	12.33	10.00	15.09	5.41	7.81
Brain AChE	23.40	34.33	14.04	24.37	18.55	47.24	8.33	21.33

Gross pathology and histopathology: No treatment-related gross pathological changes were observed. Histological examination of the treated areas of the skin showed no differences between treatment groups and controls. No other histological examination results were reported.

Note: The study authors recommended that since there was no known physiological function of plasma BChE in neural transmission, RBC AChE data in females be considered as conservative estimates of exposure limits for methamidophos. They did not elaborate further.

Conclusion

(1) The treatment of male and female rats with a single dose of dermally applied analytical grade methamidophos or methamidophos technical resulted in dose-dependent inhibition (up to 15.6 mg/rat) of RBC and brain AChE and plasma BChE at 24 and 72 h after exposure. For each grade of methamidophos the degree of inhibition at 24 h was greater than at 72 h.

(2) Females were more sensitive to the effects of methamidophos. For both grades of methamidophos and for all types of ChE, the degree of inhibition at 24 h was up to 85% greater in females than males. This observation parallels the more extensive appearance of clinical signs and mortality in females at 6.25 and 15.6 mg/rat after 24 h exposure to both grades of methamidophos. The ID_{50} for all types of ChE and both grades of methamidophos was least in females at 24 and 72 h except for RBC AChE at 72 h in those treated with SX-1672.

(3) The capacity of methamidophos technical to produce ChE inhibition was greater than that of analytical grade methamidophos. The ID_{50} values for methamidophos technical were less than those for analytical grade methamidophos except for RBC AChE in rats treated with SX-1490.

(4) NOELs in respect of each type of ChE inhibition occurring with each grade of methamidophos in each sex established by this study are tabulated below. A NOEL was established for males treated with analytical grade methamidophos as 3.9 mg/kg bw/d on the basis of statistically significant ChE inhibition. A NOEL for analytical or technical grade methamidophos on the basis of statistically or biologically significant ChE inhibition in rats was not established.

NOEL for analytical and technical grade methamidophos using two types of significance

Type of ChE	NOEL (mg/kg bw/d) established by this study							
	Analytical grade				Technical grade			
	Males		Females		Males		Females	
	SSI	BSI	SSI	BSI	SSI	BSI	SSI	BSI
RBC AChE	3.9	0	0	0	3.9	0	4.1	0

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Plasma BChE	3.9	3.9	0	0	0	0	0	0
Brain ChE	9.5	9.5	4.6	4.6	9.7	9.7	0	0

SSI = statistically significant (cf controls) ChE inhibition at 24 h post-dosing ($p \leq 0.05$)
BSI = biologically significant ChE inhibition ($\geq$ 20% ChE inhibition) at 24 h post-dosing

Sheets LP, Schoeling GL, Hamilton BF, Moore KD & Sangha GK (1993): An acute oral neurotoxicity screening study with technical grade methamidophos (MONITOR^R) in rats. Bayer AG File No. 7133. Study No. 92-412-QL. Report No. 105053. Lab: Toxicology, Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Study Duration: 21 September 1992 - 7 October 1993. Report date: 5 November 1993. Unpublished. (QA: Yes; GLP: FIFRA 40 CFR Part 160; OECD C(81)30 (1981); Test Guidelines: FIFRA EPA 540/09-91-123, PB 91-154617).

[This evaluation has considered information supplied to the California Environmental Protection Agency in response to questions put by that agency. These questions were contained in a separate submission as follows: Sheets LP, Schoeling GL, Moore KD & Hamilton BF (1995): An acute oral neurotoxicity screening study with technical grade methamidophos (MONITOR^R) in rats. Bayer AG File No. 7133. Study No. not specified; attachment to original study 92-412-QL & its supplement 94-412-YW. Report No. not specified. Lab: Toxicology, Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Study Duration: not applicable. Report Date: 14 October 1995. Unpublished. (QA: Yes; GLP: FIFRA 40 CFR Part 160; OECD C(81)30 (1981); Test Guidelines: FIFRA EPA 540/09-91-123, PB 91-154617).

Test Substance:	Methamidophos technical. Batch Number: 0-06-7009 Purity: 75.6% Source: Miles Inc, Stilwell, Kansas, USA.
Test Species:	Rat: Sprague-Dawley (Sas:CD(SD)BR), males, females, 9 wk of age of unspecified bw, obtained from Sasco Inc, St Louis.
Dose:	0, 1, 3, 8 mg/kg bw nominal po

Method

Rats of normal appearance and behaviour were placed into individual cages and acclimatised to standard laboratory environmental conditions over 6 d. They were permitted Purina Mills Rodent Lab Chow 5001-4 and tap water *ad libitum*. The composition of the food and water were determined. The rats were weighed and those with bw more or less than 20% of the mean bw for each sex were rejected. Those accepted were randomly assigned to a control group or one of 3 dose level groups.

The identity of the active ingredient of the test substance was confirmed as methamidophos using NMR and MS. The concentration of methamidophos was measured within 6 mo of administration and at termination of the rats to verify stability under freezer conditions. The stability, concentration and homogeneity of the test substance in the vehicle following room temperature exposure, were established by GC.

The results of a preliminary dose range-finding study were used to select dose levels of 0, 1, 3 and 8 mg/kg bw for each sex. The high dose was selected to produce clear evidence of toxicity without causing mortality. The middle dose was selected to produce an intermediate level of cholinergic toxicity and 1 mg/kg bw was chosen to produce no evidence of toxicity.

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The treated groups received the test substance by gavage as a single dose in 0.5% w/v methylcellulose with 0.4% w/v Tween 80 in deionised water in a dosing volume of 10 mL/kg bw. The doses as specified in this study have taken into account the 75% purity of the test substance.

Twelve rats/sex/dose were subjected to a functional observation battery (FOB) of behavioural testing and motor activity testing at 1 wk prior to treatment, on the d of treatment (d 0) and 7 and 14 d after treatment. On d 0, FOB testing began between 90 min to 2 h after treatment and measurement of motor activity occurred between 2.5 - 4.5 h after treatment in each individual. These times were selected to coincide with an expected peak in behavioural and motor effects. One half of these rats (6 rats/sex/dose) were randomly allocated for use in neuropathology studies. The rats were terminated 15 d following exposure. At a later date satellite groups of 6 rats/sex/dose were used for ChE determinations and 6 rats/sex/dose for clinical chemistry and haematology determinations.

The functional observation battery (FOB) used was based on the battery of procedures described by (Moser, 1989) and is summarised in Appendix V of this review. Scoring criteria and explicitly-defined scales were used to rank the severity of observations. All data were computer analysed (SAS FOB Computer Program).

Motor and locomotor activity was measured in individual rats over 10 min intervals during a 90 min session in a figure-eight maze with infrared emitter/detector pairs. Motor activity was measured as the number of infrared beam interruptions that occurred during the test session. Locomotor activity was measured by ignoring the second and subsequent counts for a given beam. Only one interruption of a given beam was thus counted until the rat relocated in the maze and interrupted another beam. Habituation was evaluated as a decrement in activity during the test session. The order of testing and assignment of rats to mazes was semi-random. All data were computer analysed (SAS).

Except for the satellite rats, all rats in the study were subjected to a complete gross necropsy. They were killed by CO_2 asphyxiation except for the rats allocated for neuropathology studies. These were deeply anaesthetised using 50 mg/kg ip pentobarbital and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by *in situ* fixation using universal fixative (4% w/v glutaraldehyde and 4% w/v EM-grade formaldehyde) in phosphate buffer. The entire brain and spinal cord, both eyes (and optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle and the tail were dissected from each rat and post-fixed in 10% buffered formalin. The brain was weighed upon removal prior to placement in formalin and the brain:body wt ratio was calculated.

In one set of satellite rats (6/sex/dose), RBC, plasma and brain ChE activities were measured at 2 h after administration of the dose in order to have ChE determinations coincide with the time of neurobehavioural evaluation in the rats of the main study groups. The results tables indicate that blood and brain were collected when the rats were terminated at 2 h after treatment. The methods of termination were not specified. ChE determinations were made using a modification of the method of (Ellman *et al.*, 1961). Other clinical biochemical and haematological parameters were measured in a second set of satellite rats (6/sex/dose). The biochemical and haematological parameters examined were those listed in Appendix II of this review. Urinalysis was not performed. Blood samples were collected from the orbital plexus of these rats. They were not fasted beforehand. All rats of this set were blood sampled 20-24 h

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following treatment. The females of this second set were also blood sampled 2 wk before treatment.

Coronal sections from 6 levels of the brain (olfactory region, forebrain, midbrain, pons, medulla oblongata and cerebellum) and cross and longitudinal sections from 3 levels of the spinal cord (cervical, thoracic and lumbar) were collected from the perfused control and high-dose rats. They were embedded in paraffin and examined using hematoxylin and eosin stain. Dorsal root ganglia (including dorsal and ventral root fibres), cada equina, gasserian ganglion, eyes, optic nerves, gastrocnemius muscle and additional tissue from the hippocampus and cerebellar cortex were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned and stained using Modified Lee's stain. Peripheral nerve tissues were embedded in Epon, sectioned and stained with Toluidine Blue. Additional sections from each region of the brain and spinal cord were stained using Luxol Fast Blue/Cresyl Violet and Sevier-Munger stains.

The tissues from rats that received the high dose were examined against those from control groups. Since no treatment-related lesions were evident further analysis was not performed. Previous studies with trimethyltin and acrylamide established the sensitivity and reliability of these histological procedures for detecting lesions in peripheral nerves and the central nervous system.

The rats were observed twice daily for mortality or clinical signs and weighed weekly. Perfused rats were also weighed on the d of termination prior to perfusion for terminal bw measurement.

In general, continuous data and interval data were analysed using a repeated-measures Analysis of Variance (ANOVA) and/or a one-way ANOVA followed by Dunnett's test if a significant F-value was determined in the ANOVA. Categorical data were analysed using General Linear Modeling and Categorical Modeling procedures with Dunnett's test or Analysis of Contrast respectively.

Results and discussion

Test substance analysis: The analytically confirmed doses were 0, 0.9, 3.3 and 9.0 mg/kg for main study group rats and 0, 0.9, 3.1 and 8.9 mg/kg for the satellite groups.

Clinical signs and mortality: There were no treatment-related deaths. Cholinergic signs appeared after treatment on d 0 in rats administered 3 or 8 mg/kg bw. They had reversed within 5 d. At 3 mg/kg bw males showed muscle fasciculations (7/12) and ataxia (1/12). At 8 mg/kg males showed muscle fasciculations (12/12), tremors (2/12), ataxia (6/12) and salivation (1/12). These signs were not seen in controls or at the lowest dose. Their incidence was dose-related. Also dose-related was the incidence of urine, oral and lacrimal staining. In females at 8 mg/kg bw, 7/12 showed muscle fasiculations, 2/12 ataxia, and 1/12 salivation. These signs were not seen in controls or at the two lowest doses. Urine and nasal staining was observed in females at 3 mg/kg bw but had its highest incidence at 8 mg/kg bw.

Body wt: There were no treatment-related effects on mean bw at 7 and 14 d after treatment.

FOB: Abnormalities of behaviour or response were not observed pretreatment. Treatment-related effects were observed after treatment on d 0 at all dose levels in males and at 3 and 8

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mg/kg bw in females. They were not observed on d 7 or d 14. At 1 mg/kg bw 1/12 males was sitting or lying in a normal position (rather than standing) during open field observations. This effect was not statistically significant but it was considered by the study authors to be a treatment-related effect because it was not observed in any control animal and it occurred in males at the two higher doses.

Males and females of the 3 or 8 mg/kg groups showed muscle fasiculations, tremors, incoordinated gait, repetitive chewing, a lower incidence of vocalisation upon removal from the home cage, evidence of decreased activity during open field observation (sitting or lying in a normal position with either minimal head or body movement or only some exploratory movement and a decreased incidence of rearing), decreased response to touch, impaired righting response, decreased body temperature and decreased grip strength.

Grip strength of the male forelimb was 0, 20 or 69% less than that of controls at 1, 3 or 8 mg/kg respectively. For the female forelimb it was 2, 17 or 61% less than that of controls. At 8 mg/kg grip strength of the male and female hindlimb was 26 and 29% less than that of controls respectively. There was no loss of hindlimb grip strength at 1 or 3 mg/kg.

The incidence and/or severity of these effects were generally greater at the higher dose. The response to other stimuli (approach, auditory and tail pinch) was reduced in high-dose males and females. At 3 mg/kg, response to approach was reduced in 2 males and response to tail pinch was reduced in 1 female. Urine stain was present in females that received a dose of 3 or 8 mg/kg and in males at 8 mg/kg. Additional treatment-related effects in males and females at 8 mg/kg consisted of flattened posture, lacrimation and salivation. One male at 8 mg/kg also had red oral stain on d 0 which may have resulted from treatment.

Motor and locomotor activity: After treatment on d 0, the 90 min test sessions showed that the respective % loss of motor activity in males compared to controls at 1, 3 and 8 mg/kg was 23, 84* and 73*. In females the respective % losses were 14, 85* and 81* (*significantly different from controls, $p \leq 0.05$). After treatment on d 0, the respective % loss of locomotor activity in males compared to controls at 1, 3 and 8 mg/kg was 25, 95* and 92*. In females the respective % losses were 14, 89* and 98* (*significantly different from controls, $p \leq 0.05$). The study authors ascertained from their data that differences of < 20% were within the range of normal variability in these parameters in their laboratory for groups of 12 rats/sex/dose level. Accordingly they did not regard the 14% decrease in motor or locomotor activity measured in females at 1 mg/kg as treatment-related. Decreases of 15-16% in motor and locomotor activity in high-dose males were observed on d 7 and were considered to reflect incomplete recovery. No decreases in motor or locomotor activity were observed on d 14. The examination of results at 10 min intervals in each 90 min session verified that where decreased motor or locomotor activity compared to controls was observed, it was observed in the 1st few intervals while control rats were still active before settling into a slower habituated activity period during which differences between groups were difficult to discern.

Cholinesterase: Plasma, RBC and brain ChE activities in satellite rats at 2 h after treatment are tabulated below. Inhibition of plasma, RBC and brain ChE was biologically ($\geq$ 20%) and statistically significant at 1 mg/kg and above in both sexes. Inhibition of plasma, RBC and brain ChE was dose-dependent.

Plasma, RBC and brain ChE determinations

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ChE	Plasma, RBC and brain ChE activities (IU/mL or IU/g) and % inhibition 2 h after treatment at each nominal dose (mg/kg bw)							
	Male				Female			
	0	1	3	8	0	1	3	8
Plasma	0.57 -	0.35* 40.0	0.11* 80.0	0.05* 92.0	1.08 -	0.82* 24.0	0.36* 67.0	0.12* 89.0
RBC	1.21 -	0.82* 33.0	0.33* 72.0	0.10* 92.0	1.27 -	0.86* 32.0	0.41* 68.0	0.18* 85.0
Brain	15.1 -	10.1* 33.0	4.5* 70.0	2.7* 82.0	16.1 -	11.5* 29.0	4.5* 72.0	2.5* 84.0

* Statistically significant compared to controls, $p < 0.05$

Clinical chemistry: The serum enzyme, lipid or electrolyte values which were statistically significantly different $p \leq 0.05$ from controls are tabulated below. Elevations in AST and ALT activity were considered by the study authors to be treatment-related and caused by liver and/or muscle injury sufficient to trigger enzyme release. At 3 mg/kg mean female AST was not elevated statistically significantly but the increase was also considered treatment-related since it was similar to the increase in mean male AST at 3 mg/kg. The increased mean male cholesterol at 8 mg/kg and decreased mean female triglycerides at 3 and 8 mg/kg, were considered treatment-related through unknown mechanisms.

Statistically different serum enzyme lipid and electrolyte values 24 h after treatment

Enzyme/ lipid/ electrolyte	Serum enzyme, fat and electrolyte values statistically different from controls – those marked * (AST and ALT U/L; Chol and Trig mg/dL; Cl mmol/L)							
	Male				Female			
	0	1	3	8	0	1	3	8
AST	86	95	123*	515*	76	74	106	509*
ALT	60	67	59	102*	52	45	50	94*
Cholesterol	43	46	49	56*	49	50	47	46
Triglycerides	108	143	146	94	118	82	66*	51*
Chloride	101	103	104*	104*	102	103	102	103

* $p \leq 0.05$

Haematology: There were no treatment-related haematological findings.
Gross and histopathology: There were no treatment-related gross lesions at any dose and no treatment-related microscopic lesions at 8 mg/kg. As a result, tissue from the lower dose groups was not examined microscopically.
Terminal bw and brain wts: There were no treatment-related effects on terminal bw, absolute or relative brain wt.

Conclusion

A NOEL was not established in this study due to statistically and biologically significant inhibition of plasma, RBC and brain ChE activity, and decreased motor and locomotor activity (in males) at the lowest dose of 0.9 mg/kg bw and above. There was no histological evidence of injury to the central or peripheral nervous system at the highest dose of 9 mg/kg bw

Sheets LP, Gilmore RG, Moore KD & Stuart BP (1994a): An acute oral neurotoxicity screening study with technical grade methamidophos (MONITOR^{R}) in rats. Bayer AG File No. 7133. Study No. 94-412-YW (Supplement to Original Study 92-412-QL). Report

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No.105053-1. Lab: Toxicology, Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Study Duration: 11 April 1994 - 12 August 1994. Report date: 12 August 1994. Unpublished. (QA: Yes; GLP: FIFRA 40 CFR Part 160; OECD C(81)30 (1981); Test Guidelines: FIFRA EPA 540/09-91-123, PB 91-154617).

[The original screening study (Sheets et al., 1993) evaluated above in this Section, established that a low dose of 0.9 mg/kg bw resulted in minimal evidence of cholinergic toxicity in males and females. The purpose of this supplemental study was to test lower doses of methamidophos, in order to establish an overall NOEL]

Test Substance:	Methamidophos technical. Batch Number: 0-06-7009 Purity: 75.6% Source: Miles Inc, Stilwell, Kansas, USA.
Test Species:	Rat: Sprague-Dawley (Sas:CD(SD)BR), males, females, 9 wk of age of unspecified bw, obtained from Sasco Inc, St Louis.
Dose:	0, 0.3, 0.6 mg/kg bw nominal po

Method

The methods employed in this supplemental study were those employed in the original study (Sheets *et al.*, 1993). In this study 3 dose groups (1 control and 2 treatment groups) received the vehicle or test substance in vehicle as a single dose by gavage as previously described. Eighteen rats/sex/dose level were used. Twelve rats/sex/dose level were subjected to neurobehavioural testing. The remaining rats (6/sex/dose level) were used as satellite groups for ChE determinations. The rats used in this study were not subjected to gross pathology or histopathology examinations since there were no treatment-related lesions seen at higher doses in the original study. Clinical chemistry or haematological examinations were not performed.

Results and discussion

Test substance analysis: The analytically confirmed doses were 0.3 and 0.7 mg/kg bw.

Clinical signs and mortality: There were no treatment-related clinical signs or deaths prior to termination 14-15 d after treatment.

Body wt: There were no treatment-related effects on mean bw at 7 and 14 d after treatment.

FOB: There were no treatment-related effects at any interval or dose.

Motor and locomotor activity: There were no treatment-related effects at any interval or dose.

Cholinesterase: Plasma, RBC and brain ChE activities in satellite rats 2 h after treatment are tabulated below. At 0.3 mg/kg bw there was no statistically significant inhibition (cf controls) in males or females although plasma ChE in females was 24% lower than their respective controls. The study authors did not consider this biologically significant because of the lack of statistical significance and the absence of a proportionately dose-dependent reduction of effect relative to the 0.9 mg/kg bw dose (Sheets *et al.*, 1993). Further, the presence of a control

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female whose plasma ChE activity (2.27 IU/mL) after treatment was believed to have made the mean value of 1.48 artificially high. The pretreatment plasma ChE activity for this individual was 1.51 IU/mL and the mean pretreatment value for this group was 1.06 IU/mL.

Plasma, RBC and brain ChE determinations

ChE	Plasma, RBC and brain ChE activities (IU/mL or IU/g) and % inhibition 2 h after treatment at each nominal dose (mg/kg bw)					
	Male			Female		
	0	0.3	0.6	0	0.3	0.6
Plasma	0.67 -	0.63 6.0	0.49* 27.0	1.48 -	1.13 24.0	1.11 25.0
RBC	1.43 -	1.36 5.0	1.13* 21.0	1.44 -	1.33 8.0	1.07* 26.0
Brain	14.6 -	14.7 -	12.4* 15.0	15.5 -	14.5 6.0	11.4* 26.0

* Statistically significantly different compared to controls, $p \leq 0.05$

Conclusion

This study showed that on the basis of plasma, RBC and brain ChE inhibition a single oral dose of 0.3 mg/kg bw was a NOEL for methamidophos.

Sheets LP, Schoeling GL, Moore KD & Hamilton BF (1994b): A subchronic dietary neurotoxicity screening study with SRA 5172 (methamidophos) in Fischer 344 rats. Bayer AG File No. 7252. Study No. 92-472-RE. Report No. not specified. Lab: Toxicology, Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Project Duration: 11 January 1993 - 15 April 1993. Report date: 13 April 1994. Unpublished. (QA: Yes; GLP: FIFRA 40 CFR Part 160; OECD C(81)30 (1981); Test Guidelines: FIFRA EPA 540/09-91-123, PB 91-154617).

This evaluation has considered information supplied to the California Environmental Protection Agency in response to questions put by that agency. These questions were contained in a separate submission as follows:

Sheets LP, Schoeling GL, Moore KD & Hamilton BF (1996): A subchronic dietary neurotoxicity screening study with SRA 5172 (methamidophos) in Fischer 344 rats. Supplemental submission. Bayer AG File No. 7252. Study No. not specified; supplement to original study 92-472-RE. Report No. California EPA DPR Document No. 315-135; Record No. 129816. Lab: Toxicology, Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Sponsor: Agriculture Division, Miles Inc, Stilwell, Kansas, USA. Project Duration: not applicable. Report date: 3 May 1996. Unpublished. (QA: Yes; GLP: FIFRA 40 CFR Part 160; OECD C(81)30 (1981); Test Guidelines: FIFRA EPA 540/09-91-123, PB 91-154617).

Test Substance:	Methamidophos technical. Batch Number: 0-06-7009 Purity: 75.6% Source: Miles Inc, Stilwell, Kansas, USA.
Test Species:	Rat: Fischer 344 CDF(F-344)/BR, males, females, 8 wk of age of unspecified bw, obtained from Sasco Inc, St Louis.
Dose:	0, 1, 12, 60 ppm in diet

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Method

Rats used were selected on the basis of general appearance and behaviour. They were caged individually and acclimatised to standard environmental conditions for 1 wk. They were fed Purina Mills Rodent Lab Chow 5001-4 and tap water *ad libitum*. Following acclimatisation the rats were weighed. Those with bw more or less than 20% of the mean bw for each sex were rejected. The rats were randomly assigned to a control group or 1 of 3 dietary levels. The dietary test substance concentrations used were selected on the basis of a previous subchronic study (Loser & Lorke, 1970a) evaluated in Section 5.1.2, and a chronic study (Hayes *et al.*, 1984b). Sixty ppm produces clear evidence of toxicity without causing mortality. Twelve ppm was selected to produce an intermediate level of cholinergic toxicity and 1 ppm was chosen to produce no evidence of toxicity.

The composition of the food, corn oil vehicle and water were determined. The chemical identity and structure of methamidophos were confirmed by NMR and MS prior to the start of the study. The concentration of active ingredient in the test substance was measured within 6 mo of the start of dietary exposure and after termination in order to verify stability under freezer conditions. The stability of the test substance at room temperature and in the freezer and the homogeneity of the test substance in the feed were established by analysis of samples at nominal concentrations of 1 ppm and 60 ppm. The concentration of the test article in the ration was measured for the ration used in wk 1, 5, 9 and 14 of the study.

Four dose groups (18 rats/sex/dietary level) were administered the test substance in the diet for 14 wk. Twelve of the rats/sex/dietary level were subjected to behavioural testing with half of these used for neuropathology. The remaining 6 rats/sex/dietary level were included as satellite groups for ChE determinations. Corn oil was used as the vehicle for the test substance at 1% by wt of diet. A small amount of acetone served as a solvent in the diet preparation process but evaporated before the diets were administered. The amount of test substance used to prepare dietary levels was corrected for the test substance purity based on % active ingredient. A given batch of food was available for consumption for 1 wk prior to changing to the next batch.

The rats were observed twice daily for mortality or clinical signs. Individual bw and food consumption measurements were made weekly. Pre-exposure and pre-terminal (wk 12) ophthalmic examinations were conducted on all rats in a semi-darkened room. The pupillary reflex, eyelids, conjunctive, cornea, aqueous humor and lens were examined. Also examined were the vitreous humor, retina, choroid and optic disc. Rats with optical defects likely to interfere with interpretation of results were excluded from the study.

The neurobehavioural studies using a Functional Obervational Battery (Appendix V), motor and locomotor activity testing, ChE determinations, gross and histological pathology and neuropathology studies and statistical analyses were carried out as described in an acute neurotoxicity study evaluated in this Section (Sheets *et al.*, 1993). Clinical chemistry and haematologic examinations were not performed.

Results and discussion

Analytical chemistry: The concentration of methamidophos was not affected by freezer storage. Batches of methamidophos mixed with ration at 1 and 60 ppm were found to have a

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homogeneous distribution. Methamidophos mixed with ration at 1 and 60 ppm was stable for 2 wk at room temperature and 4 wk at freezer temperature.

Concentration/dose: The calculated mean daily food consumption and intake of methamidophos is tabulated below.

Intake of methamidophos for each nominal dose

Determined/calculated parameter	**Daily food consumption and intake of methamidophos**							
	Males				**Females**			
Nominal dose[a]	**0**	**1**	**12**	**60**	**0**	**1**	**12**	**60**
Analytical dose[a]	0	0.99	11.6	58.6	0	0.99	11.6	58.6
Mean food consumption[b]	67.8	68.0	67.8	72.6	77.1	75.4	77.5	84.4
Mean intake of active ingredient[c]	-	0.07	0.79	4.3	-	0.07	0.90	4.9

a: ppm in food; b: g/kg bw/d; c: mg active ingredient/kg bw/d

Clinical signs and mortalities: There were no deaths before termination. There were no treatment-related signs in controls, at 1 ppm or at 12 ppm in males. At 60 ppm, males showed muscle fasciculations (12/12), increased reactivity (11/12), perianal stain (11/12), urine stain (8/12) and lacrimation (6/12). At 12 ppm in females, urine stain (10/12 cf controls 4/12) was observed. At 60 ppm, females showed showed muscle fasciculations (12/12), increased reactivity (11/12), perianal stain (2/12), urine stain (12/12) and lacrimation (9/12). The 1st treatment-related signs were seen during the 1st 2 wk of exposure and signs of toxicity generally persisted throughout the rest of the exposure period.

Ophthalmological examination: There were no treatment-related ophthalmological findings.

Food consumption: There were no treatment-related effects on food consumption in males. In females at 60 ppm, the mean weekly food consumption was statistically significantly higher ($p \leq 0.05$) than that of controls at wk 4, 5, 7, 8 and 9 (wk 4: 12.6 g/rat/d cf controls 11.6; wk 9: 13.3 g/rat/d cf controls 11.5).

Body wt: There were no statistically significant differences from controls in bw in males or females at any interval. In males at 60 ppm there was a sustained difference in bw (6-7% less than controls) which began with the initiation of treatment (wk 1: 155.6 g/rat/d cf controls 168.5; wk 13 268.7 g/rat/d cf controls 286.3). This was regarded by the study authors as treatment-related because it was consistent with findings of other studies at this dietary level.

FOB: Treatment-related effects were observed in males and females at 12 and 60 ppm. The findings for grip strength (a measure of ChE inhibition) are tabulated below. Significant decreases ($p \leq 0.05$) in forelimb grip strength were observed at 60 ppm in males at wk 4 - 13 and in females at wk 8 and 13. The differences between males and females in hindlimb grip strength at 60 ppm were considered by the study authors to be incidental and unrelated to treatment with methamidophos.

Grip strength in males and females

Interval	**Grip strength - % controls**					
	Male			**Female**		
	1	**12**	**60**	**1**	**12**	**60**
Pre-treatment: Forelimb Hindlimb	94 100	96 104	98 100	100 105	98 105	100 95
Wk 4: Forelimb	90	99	84*	99	103	86

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Hindlimb	92	95	95	108	100	131
Wk 8: Forelimb	104	102	78*	94	98	75*
Hindlimb	107	102	88	104	107	122
Wk 13: Forelimb	96	94	69*	103	102	72*
Hindlimb	112	112	83	107	117	121

* Statistically significantly different ($p \leq 0.05$) from controls

Other treatment-related FOB findings were seen at 12 and 60 ppm. They included perianal and urine stains, muscle fasciculations and tremors in both sexes. Treatment-related effects persisted during the treatment period. All treatment-related effects in males and females were consistent with cholinergic toxicity.

Motor and locomotor activity: The measurements of motor and locomotor activity are tabulated below. A 20% difference from controls was regarded by the study authors as being within the range of normal variability for their laboratory for groups sizes of 12 sex/dose level. At 60 ppm statistically significant decreases ($p \leq 0.05$) in motor activity were seen in males over the whole treatment period. This effect was also seen in females at wk 4 at 12 and 60 ppm. At 60 ppm motor and locomotor activity was decreased compared to controls in males and females at each test interval. There were no biologically significant decreases in motor or locomotor activity at 1 ppm.

Motor and locomotor activity

Interval	**Motor (MA) and Locomotor (LA) activity - % difference from controls**					
	Male			**Female**		
	1	**12**	**60**	**1**	**12**	**60**
Pre-treatment: MA	+12	-3	-10	+28	-2	+14
LA	+16	-4	-10	+26	+1	+16
Wk 4: MA	+17	-4	-44*	-6	-32*	-34*
LA	+9	-3	-48	-2	-24	-32*
Wk 8: MA	-2	-22	-57*	-3	-2	-27
LA	0	-17	-56	0	+3	-26
Wk 13: MA	-5	0	-39*	+8	+5	-27
LA	-7	-3	-35	+13	+11	-26

* Statistically significantly different ($p \leq 0.05$) from controls

Cholinesterase: The degree of inhibition of plasma, RBC and brain ChE at each nominal concentration is tabulated below. Plasma and brain ChE activities were statistically significantly decreased ($p \leq 0.05$) in males and females at 12 and 60 ppm at 4 wk after treatment and at termination. The statistically significant differences in plasma and brain ChE at 1 ppm in females and males respectively were not considered biologically significant by the study authors. No biologically significant inhibition ($\geq$ 20% inhibition) was observed at 1 ppm.

Plasma, RBC and Brain ChE inhibition

Interval	**Plasma, RBC and brain ChE activities (IU/mL or IU/g) and % inhibition (cf controls) at each nominal concentration dose (ppm)**							
	Male				**Female**			
	0	**1**	**12**	**60**	**0**	**1**	**12**	**60**
Pretreatment Plasma	0.64	0.64	0.64	0.65	1.1	1.16	1.18	1.19
RBC	1.57	1.53	1.64	1.74	1.56	1.65	1.49	1.52

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Wk 4 Plasma	0.57	0.53	0.32*	0.12*	1.7	1.41*	0.61*	0.16*
	-	6.0	44.0	78.0	-	17.0	64.0	90.0
RBC	1.46	1.45	0.44*	0.31*	1.24	1.26	0.28*	0.03*
	-	1.0	70.0	79.0	-	-	77.0	97.0
Wk 14 Plasma	0.58	0.57	0.34*	0.15*	2.35	2.2	0.94*	0.25*
(Termination)	-	1.0	41.0	74*	-	6.0	60.0	90.0
RBC	1.5	1.39	0.36*	0.05*	1.34	1.22	0.41*	0.06*
	-	7.0	76.0	97.0	-	9.0	70.0	95.0
Brain	15.6	14.7*	6.5*	2.4*	16.4	15.5	6.5*	2.4*
	-	6.0	58.0	84.0	-	6.0	60.0	86.0

* Statistically significantly different ($p \leq 0.05$) from controls

Gross, histological pathology and terminal body and organ wt: There were no treatment-related gross lesions observed. There were no treatment-related histological lesions at 60 ppm and as a consequence, tissues from the groups at 1 and 12 ppm were not examined histologically. There were no effects on terminal bw or brain wt in males or females at any dietary concentration.

Conclusion

This study showed that on the basis of plasma, RBC and brain ChE inhibition and neurobehavioural signs 1 ppm in diet (0.07 mg/kg bw/d) was a NOEL for methamidophos.

Camara AL, Braga MFM, Rocha ES, Santos MD, Cortes WS, Cintra WM, Aracava Y, Maelicke A & Albuquerque EX (1997): Methamidophos: an anticholinesterase without significant effects on postsynaptic receptors or transmitter release. Neurotoxicology, 18(2)589-602.

Test Substance: Methamidophos analytical. Purity: 99.8%. Source: Bayer AG, Leverkusen, Germany

Test Species: Rat: Wistar, adult, female, male, bw 200 g, no further details provided

Prelude

Aspects of this study involving sciatic nerve-sartorius muscle from frog and the organphosphorous compound paraoxon are considered here only where outcomes were available for comparison with methamidophos. Paraoxon was used in this study as a representative of the organophosphorous class of compounds that exert a range of potentially toxic effects other than the inhibition of ChE.

Method

The pre-experimental management of the rats was not described. Aliquots of 1 M methamidophos were prepared and kept frozen until ready for use. They were diluted with double-distilled water daily just before experiments.

AChE activity assay: ChE activity in homogenates of rat hemidiaphragm, brain and hippocampus was measured using Ellman's colorimetric method (Johnson, 1975a) with acetylthiocholine as the substrate. The homogenates were incubated with methamidophos (0.1-300 μM) for 30 min. ChE activity was also measured in homogenates of rat

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hemidiaphragm muscles exposed for 2 h to methamidophos (100 μM) and washed for another 2 h after the treatment with methamidophos.

Nerve-elicited twitch and tetanic contraction in rat hemidiaphragm muscle: Left hemidiaphragm muscles with phrenic nerve were removed from rats in an unspecified manner. The nerve-muscle preparation was mounted in a tissue bath and was continuously bathed at room temperature in Tyrode's physiological solution which was bubbled with 95% O_2 and 5% CO_2. The nerve was stimulated continuously at 0.1 Hz with 0.1 ms pulses of supramaximal strength except during tetanic stimulation. Tetanic stimuli of 4 s duration were delivered at 4 min intervals to the nerve at 20 and 50 Hz. The muscle response was observed every 15 min. When a control steady-state recording was obtained, the muscle was exposed to 100 μM methamidophos for 2 h. Then the muscle was washed with physiological solution for 4 h to remove methamidophos. An initial and final tension during a tetanic stimulation was defined as the amplitude measured from the base line to the peak at the beginning and at the end, respectively, of the tetanic response. The tetanus sustaining ability (TSA) was defined as the ratio between the final tension and initial tension.

Also, indirectly elicited twitches were obtained in rat hemidiaphragm muscle by stimulating the phrenic nerve with supramaximal pulses of 0.1 ms duration at a frequency of 0.1 Hz. After the initial 30 min for muscle stabilisation, the neuromuscular transmission was partially blocked with 0.5-0.7 μM d-Tubocurarine (d-TC). After a stable blockade of about 70% was reached, 1-300 μM methamidophos was added and twitch contractions of the muscle were recorded continuously for 2 h.

Intracellular recording of Nerve-evoked End-plate Potentials (EPPs) and Miniature End-plate Potentials (MEPPs): These experiments were conducted on rat phrenic nerve-hemidiaphragm muscle preparation bathed as described above. The EPPs and MEPPs were recorded by lowering external Ca^{2+} concentration to 0.5 mM and by adding 1-2 mM Mg^{2+} to the bathing solutions. The nerve was stimulated with 0.1 ms pulses of supramaximal strength at a frequency of 0.5 Hz. The potentials recorded using an intracellular KCl (3M) filled electrode were amplified and stored on video cassette for later analysis of the peak amplitude, rise time, and decay-time constant (τ) of both EPPs and MEPPs. The quantal content was determined from the ratio between the mean EPP and the mean MEPP.

Single channel currents: The cell-attached configuration of the patch-clamp technique (Hammill *et al.*, 1981) was used to record single channel-currents from rat myoballs in culture. Bathing solutions were made from HEPES-buffered solution. Tetrodotoxin (TTX) (0.3 μM) was present in all solutions. Agonist (ACh) and/or methamidophos were added to the microelectrode solution. All recordings were performed at 10^{o}C and stored on video for off-line analysis.

Whole cell currents experiments: Pregnant female rats at 17-18 GD gestation were terminated by cervical dislocation after CO_2 narcosis. The hippocampi obtained from 10-15 foetuses were dissected out, minced and treated with trypsin. Dispersed neurons were plated at a density of 250,000 to 350,000/mL on collagen-coated dishes and were incubated at 36^{o}C in water-saturated 10% CO_2-90% air atmosphere. Seven d after plating, proliferation of non-neuronal cells was inhibited by further treatment of the medium. Neurons cultured for 1-5 wk were used. Upon removal of culture dishes from the incubator, the nutrient medium was replaced and the culture dish was continuously perfused with an external solution containing

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sodium, potassium and calcium chloride, glucose and HEPES 10. One μM atropine and 150 nM were added to the external solution in all experiments.

Whole cell currents were recorded from hippocampal neurons in culture according to standard patch-clamp technique (Hammill *et al.*, 1981). Whole-cell currents activated by different agonists were obtained using a "U"-shaped glass tube of 400 μm inner diameter. This allowed the intermittent and fast (20 ms onset) application of agonist solution to the perineuronal region, without continuous perfusion of agonist solution in the neuronal culture. When continuous superfusion of the neurons was desired, as in the case for continuous recording of the miniature postsynaptic currents (MPSCs), a set of 6 coplanar glass tubes was used. MPSCs were identified as mediated by GABA (gamma amino butyric acid) or glutamate or AMPA-type (L-alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate) glutamate receptors based on their reversal potential and their specific blockade by inhibitors, 100 μM picrotoxin for GABA or glutamate and 10 μM CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione) for AMPA receptors. Analysis of the frequency, peak amplitude, rise time and decay time constant were performed off-line as previously described.

Statistical analysis: The peak amplitude, 10-90% rise time and decay-time constants of whole-cell currents were analysed using the pCLAMP program. The nature of this program was not described. The EPPs and the MPSCs recorded were analysed with programs designed manage electrophysiological signal data (Dempster, 1988). The significance of differences was determined with the student's t test at level of $p < 0.05$ and two-way ANOVA.

Results and discussion

ACh hydrolysis rates: ACh hydrolysis rates are tabulated below. The study authors suggested that the difference in ACh hydrolysis rates among tissues supported previous findings about different levels and/or isoforms of ChE within the rat and between species (Ellman *et al.*, 1961; Massoulie & Bon, 1982; Atack *et al.*, 1986).

The effects of methamidophos on ACh hydrolysis rates in various rat tissues in vitro

Concentration of methamidophos (μM)	ACh hydrolysis rates (μmoles substrate/min/g tissue		
	Diaphragm	Brain	Hippocampus
0	0.29	4.4	2.7
0.1	-	4.5	2.8
1	0.3	4.1	2.5
10	0.24	3.3	1.9
30	0.14	2.1	1.0
50	0.11	1.4	-
100	0.06	0.56	0.19
300	0.04	-	-

Methamidophos as an anti-ChE: Inhibition of ChE by methamidophos is shown in the table below. The data was derived as accurately as possible from curves presented by the study authors. The methamidophos I_{50} values derived by the study authors from their curves were 30, 29 and 20 μM for diaphragm, brain and hippocampal homogenate respectively. On the basis of this data, methamidophos is a much less potent inhibitor of ChE than paraoxon (I_{50} 0.01 μM) (Lotti & Johnson, 1978; Chambers & Carr, 1993). ChE in frog sartorius muscle was 50% inhibited at 300 μM methamidophos.

Inhibition of ChE by methamidophos in various rat tissues in vitro

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Concentration of methamidophos (µM)	Mean ChE activity (% activity)		
	Diaphragm	Brain	Hippocampus
0.1	100	100	100
1	102	95	95
10	83	76	70
30	-	50	38
50	38	32	-
100	20	10	5
300	16	-	-

Further elucidation of the time course and reversibility of ChE inhibition by methamidophos: In control conditions, when the diaphragm muscles were bathed in physiological solution for up to 6 h, there was a 15-20% reduction in single twitch tension. The control tetanic contractions, evoked at rates of 20 and 50 Hz for a 4 s period, consistently produced a 2-3-fold increase in muscle tension as compared to single twitch, and the TSA of the muscles was 100%. The effect of methamidophos on the tetanic tension of the hemidiaphragm muscle of the rat is tabulated below. There was no significant effect on single twitches recorded at 0.1 Hz following exposure of the hemidiaphragm muscle to 100 µM muscle. The peak tetanic tension and TSA for either 20 or 50 Hz stimulation were severely depressed with exposure to methamidophos for 2 h at 100 µM. The study authors attributed the depression to accumulation of ACh in the synaptic cleft. These effects occurred when ChE activity was 20% of control activity. After washing the nerve-muscle preparation for 4 h muscle contraction parameters had returned to normal and ChE activity had returned to 90% of control activity. Statistical significance determinations were not provided with this data.

Effects of methamidophos on the tetanic tension of hemidiaphragm muscle of rat

Incubation time/methamidophos concentration (µM)	ChE activity (% of control)	Muscle contraction parameters			
		Peak tetanic tension (% of control)		TSA (x 100)	
		20 Hz	50 Hz	20 Hz	50 Hz
1 h/methamidophos (100)	-	96	56	100	53
2 h/methamidophos (100)	21	69	43	63	0
Methamidophos washout 4 h	88	100	100	100	100

Confirmation that methamidophos is an anti-ChE: *reversal of d-tubocurarine-induced inhibition of neuromuscular transmission in rat hemidiaphragm muscle*: The effect of methamidophos in reversing d-TC induced-inhibition of neuromuscular transmission is tabulated below. Seventy % inhibition of transmission was established with 0.5-0.7 µM d-TC. Twitches were elicited via phrenic nerve stimulation at 0.1 Hz. From 10-100 µM methamidophos the reversal of the 70% inhibition induced by d-TC was concentration-dependent but at 300 µM methamidophos no further enhancement of the twitches was observed. Statistical significance determinations were not provided with this data.

Methamidophos and d-tubocurarine-induced inhibition of neuromuscular transmission

Twitch tension (% control) of d-tubocurarine-induced inhibition at various concentrations of methamidophos (µM)					
0	**1**	**10**	**30**	**100**	**300**
30 (base)	32	42	83	98	90

An alternative mechanism for the capacity of methamidophos to reverse d-tubocurarine-induced inhibition of neuromuscular transmission?: *methamidophos and presynaptic*

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transmitter release in rat hemidiaphragm muscle: At 1-10 μM methamidophos did not significantly change either the amplitude or the decay phase of MEPPs or EPPs. At 100 μM methamidophos had limited but significant effect ($p \leq 0.05$) on the amplitude and the decay phase of both EPPs and MEPPs. Under control conditions, the amplitude of spontaneous MEPPs range between 0.2-2.1 mV and the time constant of decay varied from 1.0-4.3 ms. After exposure to 1 μM methamidophos the amplitude and time constants of decay of MEPPs were between 0.3-3.1 mV and 1.5-5.5 ms respectively. The EPPs in control preparations had amplitudes that varied from 0.2-2.5 mV and had time constants of decay of EPPs of 1.0-4.6 ms. After exposure to methamidophos (100 μM), the amplitudes and time constants of decay of EPPs ranged from 0.2-3.3 mV and 2.0-6.0 ms respectively. At 300 μM no further increases in amplitude and decay phase of EPPs and spontaneous MEPPs were observed. Under these experimental conditions, the quantal content of the EPPs was low (m = 1 and 2) and the frequency of the MEPPs ranged between 0.2-0.5/s. Methamidophos at 1-300 μM had no significant effect on the frequency of the MEPPs or quantal content of the EPPs.

The observed limited changes in amplitude and decay time were attributable to the antiChE activity of methamidophos and not to direct action on rat muscle neurotransmitter release.

Methamidophos and interaction with postsynaptic receptors in rat myoballs: Using 0.2 μM ACh activated single channel currents, the effects of 100 and 300 μM methamidophos on nicotine ACH receptors (nAChR) of rat myoballs (or isolated frog muscle fibres) were analysed. Methamidophos produced no significant changes in the τ values of the open, closed or burst durations at all concentrations and voltages tested. The frequency of openings and the single channel conductance were not affected by methamidophos. In some patches 1 mM methamidophos was tested and no alterations in the single channel current parameters were seen. The data showed that methamidophos did not interact with foetal or adult nAChRs in rat or frog muscle preparations.

Methamidophos and presynaptic transmitter release in CNS of the rat: GABA and glutamate mediated MPSCs reversed polarity at -20 mV and 0 mV respectively. The frequency of MPSCs in these studies ranged from approximately 20 to 100 events/min. Methamidophos (0.1 to 100 μM) did not change significantly the frequency, peak amplitude or decay-time constant of MPSCs recorded from hippocampal neurons. When 100 μM picrotoxin, a noncompetitive antagonist of $GABA_A$ receptors was added to the external solution, the spontaneous MPSCs recorded were mediated only by AMPA-type glutamate receptors and were completely blocked by addition of 10 μM CNQX. In the presence of picrotoxin, the application of up to 100 μM methamidophos did not affect the frequency or amplitude of glutamate mediated MPSCs.

In contrast, paraoxon (300 nM) caused a presynaptic effect in the cultured hippocampal neurons characterised by a significant increase in the frequency of GABA- and glutamate-mediated MPSCs. Paraoxon at 300 μM decreased the peak amplitude of the nicotinic currents evoked by 10 μM (+)anatoxin-*a*.

Methamidophos and interaction with postsynaptic receptors in rat CNS neurons: Whole cell currents were recorded from hippocampal neurons in culture. At a holding potential of -60 mV, 1 mM ACh activated currents with a mean peak amplitude of 364 pA. These currents were blocked by 1 n M MLA (methyllycaconitine citrate) which indicated that they were type IA currents. Application of 1 mM methamidophos did not affect the peak amplitude or decay time constant of the currents evoked by ACh. Application of 50 μM NMDA (N-methyl-D-

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aspartate) and 10 μM glycine to neurons elicited currents that had a mean peak amplitude of 2494 pA. Methamidophos at 1 mM did not affect the NMDA-induced currents when applied as an admixture with the agonist.

[Note: The hippocampal receptors involved in the postsynaptic whole cell current experiment were α-bungarotoxin-sensitive neuronal nicotinic ACh receptors (the most prevalent in hippocampal neurons). Whether or not methamidophos effects other types of nAChRs present on hippocampal neurons was not determined. Because the preparation was studied in the presence of atropine, possible interaction of methamidophos with muscarinic receptors was also not determined].

Conclusions

(1) Methamidophos exhibited species selectivity in its anticholinesterase activity, being a more potent inhibitor of ChE from rat skeletal muscle than ChE from frog skeletal muscle.

(2) ChE I_{50} values for methamidophos for rat diaphragm muscle, brain and hippocampal homogenate were 30, 29 and 20 μM respectively suggesting that methamidophos is a weak inhibitor of ChE.

(3) At 100 μM methamidophos caused 80% inhibition of rat skeletal muscle ChE in 2 h. Upon removal of methamidophos from the system, spontaneous reactivation of ChE from 20% to 90% control activity occurred in 4 h. On the basis of this data, the inhibition half life for ChE inhibition was 1.2 h and the spontaneous reactivation half life was 2.2 h. Reversal of inhibition took twice as long as inhibition.

(4) The effect of methamidophos on neuromuscular transmission is most likely to be solely an inhibition of ChE because it:-

a. reversed a partial blockade of twitch tension in rat skeletal muscle induced by d-tubocurarine,
b. decreased the ability of rat skeletal muscle to maintain tension during tetanus,
c. failed to induce any change in spontaneous or evoked presynaptic release of ACh from motor nerve terminals in skeletal muscle of rat and,
d. showed no signs of direct postsynaptic effects on muscle nicotinic ACh receptors in skeletal muscle of rat.

[a. and b. indicated an accumulation of ACh in the synaptic cleft].

(4) Methamidophos failed to induce any pre- or postsynaptic effects unrelated to ChE inhibition in rat hippocampal neurons because neither the miniature postsynaptic currents mediated by GABA and AMPA-type glutamate response nor NMDA-induced macroscopic currents were affected by it. It did not have any significant effect on NMDA-induced whole cell current and did not affect the α-bungarotoxin-sensitive neuronal nicotinic ACh receptor.

In contrast, paraoxon induced a significant increase in spontaneous transmitter release as evidenced by an augmentation in frequency of GABA and glutamate-mediated miniature postsynsaptic currents. Also, paraoxon is able to block the ion channels of glycine, $GABA_A$, NMDA and neuronal nicotinic ACh receptors (Rocha *et al.*, 1996).

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10.5 Rats and Monkeys

Wong ZA & Kodama JK (1979): Characterisation of the in vitro inhibition of rat and monkey red blood cell and brain acetylcholinesterase by acephate, technical acephate and methamidophos and their mixtures. Study No. SOCAL 1628. Report No. S-1747. Lab: Environmental Health & Toxicology, Standard Oil Company, San Francisco, California, USA. Sponsor: Chevron Chemical Company, Richmond, California, USA. Study Duration: not specified. Report Date: 17 September 1979. Unpublished. (QA: No; GLP and Test Guidelines: not specified).

[Note: *The evaluation below is concerned only with methamidophos except insofar as to compare its AChE inhibition characteristics with those of acephate laboratory grade (99.3%)]*

Test Substance:	Methamidophos laboratory grade. Purity: 99.6%. Source: Chevron Chemical Company, California, USA.
Test Species:	Rats: Sprague-Dawley, bw 201-295 g, no further details provided. Monkey: Rhesus and African Green, obtained from California Primate Research Centre and the University of California Naval Biosciences Laboratory, Oakland, California, no further details provided.

Method

In the preparation of enzymes, rats were anaesthetised with diethyl ether and blood was drawn from the abdominal aorta using a heparinised syringe. Blood was drawn from the saphena vein of monkeys which were under Ketamine^{TM} anaesthesia. In the recovery of brain, rats were killed by a blow to the head and the monkeys with Ketamine^{TM}. The whole brain was immediately excised and dissected longitudinally at the midline. The right half was homogenised 1:10 (w/v) in 0.1% cold saponin, using a Potter Elvehjem homogeniser. Approximately 2 mL of the blood was centrifuged for 5 min at 2000x g to separate plasma and RBC. The RBC were then washed twice in cold saline. Each washing was followed by centrifugation for 5 min at 2000x g. Finally 50µL of the washed RBC lysates were haemolysed by diluting to 1 mL with cold 0.1% saponin. Brain homogenates and RBC was incubated with various concentrations of the test materials for 60 min at 37^{o}C with mixing. An untreated sample for each enzyme source was also incubated concurrently and served as the control. Each sample was then assayed for AChE activity as described. The AChE inhibition measured in the test samples was expressed as a % of control activity. I_{50} values for methamidophos in rat brain and RBC AChE, and in monkey (rhesus) brain and RBC AChE were calculated from the data obtained.

Results and discussion

Rat: The *in vitro* inhibition of rat brain and RBC AChE by methamidophos is tabulated below. The *in vitro* I_{50} for methamidophos was determined from this data to be 5.0×10^{-6} M and 9.0×10^{-7} M for brain and RBC AChE respectively. At 10^{-6} M methamidophos caused substantial *in vitro* inhibition of both brain (by 25%) and RBC AChE (by 55%) compared to acephate which was inactive. When I_{50} values were compared, methamidophos was 5.5 times more potent in inhibiting RBC AChE than brain AChE.

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Inhibition of rat brain and RBC AChE by methamidophos

Methamidophos Conc. (M)	Inhibition of RBC and brain AChE	
	Enzyme Activity (% of control)	
	Brain	RBC
10^{-8}	92.5	102.9
10^{-7}	87.9	77.5
10^{-6}	75.0*	45.1*
10^{-5}	28.3	33.3
10^{-4}	6.3	13.7
10^{-3}	6.9#	9.8#

* cf Acephate: no inhibition of brain or RBC AChE;
# cf Acephate: 70% AChE activity in brain and 100% in RBC

Monkey: The *in vitro* inhibition of monkey brain and RBC AChE by methamidophos is tabulated below. The *in vitro* I_{50} for methamidophos in Rhesus was determined from this data to be 3.5 x 10^{-6} M and 9.0 x 10^{-6} M for brain and RBC AChE respectively. At 10^{-6} M methamidophos caused substantial *in vitro* inhibition in brain (by 29% in rhesus; by 39% in African Green) and no inhibition of RBC AChE. By comparison acephate was inactive on brain AChE at 10^{-6}M. When I_{50} values in Rhesus were compared, methamidophos was 2.6 times more potent in inhibiting brain AChE than RBC AChE.

Inhibition of monkey brain and RBC AChE by methamidophos

Methamidophos Conc. (M)	Inhibition of RBC and brain AChE			
	Enzyme Activity (% of control)			
	Brain		RBC	
	Rhesus	African Green	Rhesus	African Green
10^{-8}	99	-	100	-
10^{-7}	88.6	92.8	100	100
10^{-6}	71.1*	60.6	100	100
10^{-5}	24.0	25.7	29.4	-
10^{-4}	0	5.8	0	-
10^{-3}	0#	1.2	0	-

* cf Acephate: no inhibition; # cf Acephate: 51% AChE activity
Note: Acephate tested only for brain inhibition in Rhesus over 10^{-3}-10^{-6} M concentrations

Conclusion

(1) The *in vitro* I_{50} values for methamidophos in rats were determined to be 5.0 x 10^{-6} M and 9.0 x 10^{-7} M for brain and RBC AChE respectively.

(2) The *in vitro* I_{50} values for methamidophos in Rhesus monkeys were determined to be 3.5 x 10^{-6} M and 9.0 x 10^{-6} M for brain and RBC AChE respectively.

(3) On the basis of the determined I_{50} values, methamidophos was 1.4 times more potent in inhibiting brain AChE in monkey as in rat and 10 times less potent in inhibiting RBC AChE in monkey than in rat.

[*Note*: In comparing AChE inhibition for methamidophos laboratory grade, acephate laboratory grade, acephate technical grade and various mixtures of the 3 chemicals, the study authors concluded that the AChE inhibitory capacity of acephate technical was due to the presence of methamidophos as an impurity.]

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10.6 Developmental neurotoxicity studies

Wang S & Huang XS (1987): Behavioral toxicity in the offspring of mice following maternal exposure to methamidophos. Chin J Pharmacol Toxicol, 1:371-375.

Test Substance:	Methamidophos: no further details provided Source: not specified
Test Species:	Mouse: Kunming; adult female, no further details provided
Dose:	0, 0.4, 2.2 and 4 mg/kg bw/d

[Prelude: The only parts of this study available in English were an abstract and tables of results]

Method

This study was designed to examine the effects of perinatal methamidophos exposure in female mice at lower levels (1/6-1/60 LD_{50}) on the development of cerebral cortex and behaviour in their offspring. Adult pregnant mice of an unspecified number were administered methamidophos daily with "feeding needles" from GD 16 to LD 21 (weaning of offspring). A positive control group was administered an antithyroid methimazole at 100 mg/kg bw/d over the same interval. The offspring were assessed on the basis of a series of tests known as the "Cincinnati pyschoteragenicity screening test battery" (Vorhees *et al.*, 1983, 1984) .

The testing schedule for developmental psychotoxicity in offspring was as follows:

1. Maternal observations: Body wt (GD 15, LD 1, 5, 10, 15); gestation length (LD 1) and number of offspring delivered (LD 1).
2. Physical development: Body wt (LD 3, PND 35, 120); pinna detachment (LD 3 to event) and eye opening (LD to event).
3. Reflex development: Surface righting (LD 7 to event); auditory startle (LD 10 to event) and reflex suspension (LD 15).
4. Locomotor activity: Pivoting activity (LD 5); swimming ability (LD 8, 12) and open field (PND 30).
5. Learning ability: T-water maze (PND 60, 90) and avoidance conditioning study (PND 90).
6. Histology and biochemistry in cerebral cortex: Brain wt (LD 3, PND 35, 120); neuron density (Nissl) (PND 30); thickness (Nissl) (PND 30) and protein (Folin-phenol) (PND 120).

Results and discussion

The time for all members of a litter to open their eyes, achieve a capacity to right their bodies, respond to an auditory signal or to reflex suspend their bodies is tabulated below. For the first 3 parameters a statistically significant ($p < 0.05$ or <0.01; cf controls) increase in the time taken to reach the criterion and a statistically significant ($p< 0.01$ cf controls) decrease in capacity for reflex suspension were observed. These differences were treatment-related. The effect plateaued at 2.2 mg/kg bw/d.

Physical and reflex development of offspring

Dose (mg/kg	Litters	Time (d) to criterion or reduction in capacity (s)

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bw/d)		Eye opening (d)	Surface righting (d)	Auditory startle (d)	Reflex suspension (s)
Control	34	14.8	9.2	11.9	97.7
Me 0.4	19	14.8	9.4	12.5*	75.2**
2.2	21	15.4**	10.7**	13.3**	62.2**
4.0	19	15.3*	10.6**	13.0**	63.6**
Mz 100	19	15.3**	11.4**	13.6**	52.1**

Me: methamidophos; Mz: methimazole (positive control)
* Statistically different from controls $p < 0.05$; ** $p < 0.01$

The scores for swimming ability and the number of T-water maze trials needed to meet the criterion are tabulated below. The swimming scores and the number of T-water maze trials were statistically significantly ($p < 0.05$ or 0.01) less than that of controls at each dose of methamidophos. These difference were treatment-related.

Swimming ability and T-water maze study of offspring

Dose (mg/kg bw/d)	**Litters**	**Swimming ability score and maze trials to meet the criterion**			
		Swimming ability		**T-water maze**	
		LD 8	**LD 12**	**PND 60**	**PND 90**
Control	34	6.0	6.7	3.9	3.9
Me 0.4	19	5.6*	6.4*	6.6*	6.5*
2.2	21	5.5*	6.3*	7.6**	6.8*
4.0	19	5.5*	6.3**	8.2**	8.5**
Mz 100	19	5.2**	6.1**	8.7**	11.6**

Me = methamidophos; Mz = methimazole (positive control); LD = lactation d; PND = postnatal d
* Statistically different from controls $p < 0.05$; ** $p < 0.01$

The neuron density, thickness and protein content in cerebral cortex of offspring are tabulated below. At 2.2 and 4.0 mg/kg bw/d methamidophos at PND 30, neuron density was statistically significantly ($p < 0.01$) higher than controls. It was also dose dependent.

Neuron density, thickness and protein content in cerebral cortex of offspring

Dose (mg/kg bw/d)	**Neuron density No neurons/VE unit PND 30**	**Thickness (mm) PND 30**	**Protein mg/g brain PND 120**
Control	90	0.334	132
Me 0.4	94	0.783	120
2.2	105**	0.785	120
4.0	120**	0.737*	119
Mz 100	114**	0.776	118

Me = methamidophos; Mz = methimazole (positive control); VE = Von Economo unit = 10^6 μm^3; PND = postnatal d
* Statistically different from controls $p < 0.05$; ** $p < 0.01$

Conclusion

Methamidophos produced evidence of cortical injury and behavioural toxicity. At the doses used a NOEL could not be established.

11. OTHER HUMAN STUDIES

11.1 Occupational Exposure

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Kollert W (1981): BBA-requirement: effect on humans. Medical observations on production workers making SRA 5172 / Tamaron at the Bayer AG plant at Elberfeld. Bayer Medical Department. Report Reference: Ko/Me. Report Date: 5 June 1981. Unpublished. (QA: No; GLP & Test Guidelines: not relevant).

A Bayer internal letter indicating that occasional temporary inhibition of ChE to a minor degree was observed in production workers making SRA 5172/Tamaron™. No further details were provided.

Miksche L (1981): Effect on humans: internal experiences. Medical observations on production workers making Tamaron at the Bayer AG plant at Elberfeld. Bayer Medical Department. Report Reference: PF - A/Beratung. Report Date: 12 June 1981. Unpublished. (QA: No; GLP & Test Guidelines: not relevant).

A Bayer internal letter indicating that staff members engaged in the formulation of Tamaron™ were medically examined regularly and that no damaging effects on their well-being had been observed.

Faul J (1990): Methamidophos (SRA 5172) - Occupational medical experience. Medical observations on production workers making formulations containing methamidophos at the Bayer AG plant at Elberfeld. Bayer Medical Department. Report Reference: sde/wl. Report Date: 26 February 1990. Unpublished. (QA: No; GLP & Test Guidelines: not relevant).

A Bayer internal letter indicating that methamidophos produced at the Elberfeld plant had been processed into liquid formulations on a scale of 600 - 800 metric tons annually at the FVL II Plant in Dormagen for about 15 yr. Under the protection of the occupational hygeine measures practiced, no cases of dermal or mucous membrane irritation had been observed in employees.

Sitt R (1990): Methamidophos (SRA 5172). Medical observations on production workers making Tamaron at the Bayer AG plant at Elberfeld. Bayer Medical Department. Report Reference: Si/Me. Report Date: 3 June 1990. Unpublished. (QA: No; GLP & Test Guidelines: not relevant).

A Bayer internal letter indicating that slight transient inhibition of ChE activity had been observed occasionally in employees engaged in the production of Tamaron™. No further details were provided.

Ospenson JN, Magee PS, Thompson CM & Others (1968): Monitor human exposure report. Sixty seven reports. Chevron Chemical Company, 12 June 1968 - 1 September 1970. (QA: not applicable).

This submission contained 67 reports to the Chevron Chemical Company from 12 June 1968 to 1 September 1970. They were reports of human exposure to Monitor. Fifty four of the reports concerned individuals who were exposed in some particular way following their activities in the field. These field activities involved mixing formulations for spraying and/or applying spray to crops either by knapsack or by air. The other 13 reports involved exposure in research laboratories or in manufacturing plants. There were no adverse effects reported in any of these exposure incidents.

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De Haro L, Arditti J, David JM & Jouglard J (1999): Methamidophos intoxication: immediate and late neurological toxicity; two case reports. [French]. Acta Clinica Belgica - Supplementum, 1:64-67. [Sourced from NCBI, NLM, NIH, PubMed, PMID: 10216986].

This clinical report concerns two women (aged 45 and 55 y, respectively) engaged in lettuce seed extraction in a French agricultural research station. The women were hospitalised following exposure to methamidophos (in a product named Orthotox) that had been applied by thermal fogging to lettuce grown within hoop-houses.

The first patient was working in an untreated greenhouse downwind of the application site and noticed a foul odour shortly after fogging commenced. She left the area, returned home and washed herself. At 7:30 the next morning, she re-entered the greenhouse and resumed work. The second patient was exposed to the pesticide after she commenced work within a treated hoop-house at 6:30 the following morning. At least 8 h had elapsed between treatment and re-entry, during which time the hoop-house had been ventilated mechanically. Neither worker was wearing gloves, and both had bare arms and legs.

At 8:00 am the first signs of toxicity appeared. Both patients presented at hospital bearing the odour of the pesticide, and were decontaminated. They showed typical signs of acute anticholinesterase poisoning (pallor, salivation, sweating, nausea or vomiting, headache and abdominal pain and/or swelling and arterial hypotension). However, there was no evidence of depressed plasma or RBC ChE activity in either patient, one of whose blood was sampled 4 h after the onset of symptoms. The second patient was not assayed until at least 24 h after admission.

Following resolution of the acute cholinergic crisis, each patient developed proximal neurological signs that were diagnosed as intermediate syndrome (parasthesia and bursts of pain in the thighs of the first patient, while the second patient lost visual acuity and muscular strength and had hyper-reflexivity of the upper limbs). On d 30, retrobulbar optical neuritis developed in the second patient, recovering by 2 mo post-exposure.

Subsequently, both patients developed sensory and peripheral neuropathy, which the study authors attributed to OPIDN. The time course of onset was different in each case. The first patient developed numbness of the distal legs and calves from d 9, while parasthesia, diminished sensation, cramps in the hands and legs and hyporeflexivity became evident in the second patient from mo 2 onwards. By 7 mo post-exposure, intense sensory impairment and motor dysfunction had developed in the legs and hands of both patients, accompanied by behavioural and mood disorders. The condition persisted for 16 mo in each patient, after which there was improvement in the clinical picture, neuromuscular function, psychometric tests and sensory threshold measurements. At 18 mo, the first patient was well but symptoms had not resolved totally in the second.

Comment:

This paper is unique in describing the onset of OPIDN in persons exposed to methamidophos by the dermal route, although at least one patient may have had additional inhalation exposure. However, the value of this study is reduced by the unexplained absence of plasma or RBC ChE inhibition in both cases.

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McConnell R, Keifer M & Rosenstock L (1994): Elevated quantitative vibrotactile threshold among workers previously poisoned with methamidophos and other organophosphate pesticides. American Journal of Industrial Medicine, 25(3):325-334.

[Preliminary note:

1. The study authors in this work examined workers from Nicaragua's Northern Pacific coastal plain who were previously hospitalised for acute organophosphate poisoning. The authors had previously reported evidence of persistent central nervous system effects among this population (Rosenstock et al., 1991).

2. They also reported that methamidophos was a common cause of organophosphate poisoning in this area and in Central America (Conroy et al., 19xx) and accounted for 25% of the 766 poisonings reported to the Nicaraguan Ministry of Health, in 1986 (Nicaraguan Ministry of Health, 1986).

Hospital inpatient discharge diagnoses were received for all admissions between 1 July 1986 and 31 July 1988. Fifty two male patients, age 15-44 yr at the time of admission, were identified as having been treated for occupational organophosphate poisoning. Thirty six agreed to participate in this study. The 36 treated patients all had medical record documentation of symptoms consistent with severe organophosphate insecticide poisoning and all received atropine during treatment. Each previously poisoned person was matched by sex and age (within 5 yr) to a sibling or friend in the same community who had never been medically treated for a pesticide poisoning. During 1989, the matched pairs were brought to the regional capital for examination.

Of the 36 previously poisoned participants, 21 were classified as previously poisoned with methamidophos (group M). The hospital record had identified methamidophos as the cause of poisoning in 14 cases but in a questionnaire, an additional 7 men reported having been treated for methamidophos poisoning on that or another occasion. The questionnaire enquired into previous poisonings, demographic characteristics and a variety of potentially confounding characteristics or exposures. The remaining 15 persons reported never having been treated for methamidophos poisoning and were poisoned with other organophosphates or mixtures of organophosphates and other pesticides (group NM). The non-poisoned controls, were well matched with respect to age, height, presence of callus, previous work with pesticides, recent pesticide exposure, alcohol consumption in previous month, history of solvent exposure and history of work with vibrating machinery.

Vibrotactile threshold was measured using a Vibraton II (Gerr & Letz, 1988; Gerr *et al.*, 1990) for right and left index finger and right and left great toe. Univariate and multivariate analyses were conducted. Trends for means for ordered categories were evaluated using a Jonckheere test.

The mean vibration thresholds are tabulated below. The previously poisoned subjects had an increased vibrotactile threshold in all digits tested. The group poisoned with methamidophos had higher mean thresholds than the group poisoned with other material. Compared with the control group, vibration thresholds were significantly elevated in persons who had experienced organophosphate poisoning with mean vibration sensitivity being lowest in the group poisoned with methamidophos. The finding was consistent across all 4 digits examined. The proportion of the 2 organophosphate poisoned groups having "abnormal thresholds"

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(criteria referenced but not specified) was greater than in the control group, especially on the toes. However "abnormal thresholds" incidence appears not to have been influenced by the type of organophosphate identified as the cause of poisoning.

Mean vibration thresholds

Test digit	Mean vibration thresholds[#] (SD); number and (%) abnormal thresholds among the 3 categories of persons studied			
	Controls	M	NM	Trend*
	Threshold	Threshold	Threshold	p value
Right index finger	-0.2 (0.9); 0 (0)	0.5 (0.9); 2 (9)	0.1 (1); 1 (7)	0.007
Left index finger	-0.17 (0.9); 0 (0)	0.34 (0.8); 1 (5)	0.09 (1); 1 (7)	0.002
Right first toe	0.07 (1); 2 (6)	1 (0.9); 5 (24)	0.7 (1); 2 (13)	0.001
Left first toe	0.19 (1); 2 (6)	1 (0.9); 6 (29)	0.6 (1); 4 (27)	0.003

M = poisoned with methamidophos; NM = poisoned with other organophosphates (OPs) or mixtures of other OPs and other substances

Log microns of peak vibration amplitude normalised for age and height - North American reference formula (Gerr *et al.*, 1990)

* Jonckheere test

Data on the effect of confounding factors is not reproduced here. The time elapsed between poisoning and examination was not statistically significantly associated with vibrotactile threshold in a multivariate model adjusting for age, height, exposure and callus formation.

The study authors suggested that a chronic sensory impairment, manifest as a decreased vibrotactile sensitivity, may be a consequence of methamidophos poisoning and that the frank OPIDP reported in the literature may represent only the worst cases of a spectrum of neuropathy.

11.2 Poisoning Incidents

Senanayake N & Johnson MK (1982): Acute polyneuropathy after poisoning by a new organophosphate insecticide. The New England Journal of Medicine, 306(3):155-157. Lab: Department of Medicine, University of Peradeniva, Peradeniya, Sri Lanka & MRC Toxicology Unit, Medical Research Council Laboratories, Carshalton, Surrey, England. Sponsor: not specified. (QA, GLP or Test Guidelines: not specified).

Preliminary note: These cases were of special interest at the time of their occurrence, because polyneuropathy in humans had not been attributed to methamidophos. It had not produced neurologic damage in animals. It had been assumed that methamidophos was free of delayed neurotoxicity (FAO, 1977). Following the publication of these findings a special study of neurotoxicity in chickens was conducted at the Bayer AG Institute of Toxicology (Thyssen *et al.*, 1982). The evaluation of this study is presented in section 10.1 of this review.

The material: The Tamaron™ sold in Sri Lanka at the time of the poisonings was formulated locally and was believed to have contained a 60% w/v methamidophos in ethylene glycol monomethyl ether with an unidentified dispersing agent (5%). In one case the trace of material remaining in the container was identified as a typical Sri Lankan formulation of Tamaron™. According to the study authors, such formulations also contain as impurities small amounts of several compounds related to methamidophos, principally an unidentified isomer and the *N*-methyl analogue of methamidophos. No further details were provided.

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Occurrence: The study authors report 10 isolated cases of acute polyneuropathy in humans seen over 3 years in Sri Lanka. All persons involved developed the illness after poisoning by formulations of Tamaron™. In all cases severe cholinergic signs occurred and medical attention was sought. All patients were unconscious on admission to hospital. Seven of the cases were attempted suicides by ingestion. Of the other 3, one person had sucked the insecticide through a tube from a spraying machine, another had spilled the formulation on his body while opening the bottle and another had become intoxicated while spraying the insecticide. All patients survived. A summary of the cases is tabulated below.

A summary of 10 cases of human neuropathy in Sri Lanka attributed to methamidophos

Age (yr)/Sex	Mode of poisoning	Amount ingested (mL)	Time to neuropathy (wk)	Duration of follow up (wk)
22/M	Ingestion	80	3	8*
14/F	Ingestion	?	2	11*
18/M	Inhalation/skin	-	?4	36*
20/F	Ingestion	?	?2	10*
28/M	Ingestion	45	3	80*
21/M	Ingestion	30	3	32*
16/M	Ingestion	?	3	5
19/F	Ingestion	45	3	7
16/F	Ingestion	30	3	7
22/M	Skin	-	2	7

* Signs of pyramidal (corticospinal) tract involvement were seen in this period

Clinical signs: The main signs observed in the 22 y old male who had ingested 80 mL of Tamaron™ were pupillary constriction, muscular fasciculations, profuse sweating. He was treated with atropine (270 mg wthin 24 h) and remained unconscious for 24 h with gradual recovery over 3 d. He was discharged 5 d after admission with no symptoms except blurring of vision which lasted for several d. Ten d after discharge he had calf pain with numbness in the feet lasting for 3 d. This was followed by weakness of the feet and a day later by weakness of the hands. One mo after the onset of weakness, he was unable to move his fingers against resistance and had bilateral footdrop with marked weakness of the dorsiflexor muscles and the evertors of the feet. The muscle power of the knee flexors and the hip flexors was slightly impaired but the tone of the proximal muscles was increased and of a spastic type. The tendon reflexes were exaggerated, except that the ankle jerks were absent. The plantar responses were flexor. Sensory testing, including two-point discrimination, revealed no abnormality. Electromyography of the distal muscles of the limbs showed changes due to denervation but the motor-conducting velocity in the fastest conducting fibres of the peripheral nerves supplying those muscles was relatively normal.

All of the patients had shown an acute cholinergic crisis soon after exposure to the insecticide with a polyneuropathy developing 2-3 wk later. Six patients followed for 8 wk or more also had evidence of pyrimidal tract involvement.

[*Note*: The principal findings (Schaumburg, 2000) in pyramidal tract (corticospinal tract) disease are weakness, hyperactive tendon reflexes and Babinski responses (dorsiflexion of the great toe following scratch of the sole of the foot). Weakness involves extensor groups of muscles in the upper limb and flexors in the lower. It may be accompanied by increased tone and resistance to stretch (spasticity) and ankle/patellar clonus. Muscle atrophy is mild and due to disuse. Gait is stiff-legged and may feature scissoring if adductor muscles are hypotonic.]

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Clinical pathology: No ChE assays could be performed on these patients. In the 22 yr old male referred to above, clinical pathology findings within normal limits were ESR, Hb, RBC, WBC, WBC-DC, fasting blood sugar, liver function, urine albumin, porphobilinogen and sediment, CSF sugar, protein and cells.

Diagnosis: The diagnosis was based on the early clinical signs and their control by treatment with aggressive atropine therapy or pralidoxime. The signs suggestive of delayed neuropathy were claimed to be typical of those caused by organophosphates.

Discussion: The volume ingested by the 22 y old male was equivalent to an oral dose of 800 mg/kg assuming 60 kg bw. An oral LD_{50} for methamidophos in glycol monoethyl ether had been previously established in rats as 30 mg/kg bw (Kimmerle & Lorke, 1967a). It is possible that the atropine therapy prevented death in this patient enabling the development of delayed neurotoxicicity. Predominant motor paralysis affecting the distal muscles of the limbs and calf pain preceding the onset of weakness are typical of polyneuropathy caused by organophosphate compounds, as are the electrophysiologic findings of partial denervation with the surviving fibres conducting at normal rates and the pyramidal tract signs noted during late stages of the illness (Hopkins, 1975).

Comment: The study authors later compared Tamaron™ formulation purchased in Sri Lanka with methamidophos (>95%). The Sri Lanken Tamaron™ was more potent in acute effect and NTE inhibition than an equivalent wt of methamidophos in water. Sixty five mg formulation/kg administered to hens po produced a severity of acute effects (the highest of 3 ratings) and a % NTE inhibition (31% brain) similar to that produced by the po administration of 100 mg 95% methamidophos/kg. The unpublished data of the comparison studies conducted in the UK ("the Carshalton experiments") were not provided but were reported in a review by (Johnson & Lotti, 1989).

The Tamaron™ formulation sold in Sri Lanka was thought to have been a solution of Tamaron™ (methamidophos technical) (60% w/v) in ethylene glycol monomethyl ether with the addition of dispersing agent (5%). Methamidophos technical in the formulation contained impurities similar to those found in methamidophos technical from other sources. They were *O,O*-dimethyl phosphoroamidothioate (isomer of methamidophos) at < 5%, *O,O,O*-trimethyl phosphorothioate at 5%, *O,O,S*-trimethyl phosphorothioate at 2% and *N,O,S*-trimethyl phosphoramidothioate (*N*-methyl analog of methamidophos) at 1%.

The study authors stated that the enhanced potency appeared not to have been due to any of the identified impurities in methamidophos technical but that it was probably due to alterations to the pharmacokinetics of methamidophos induced by components of the formulation. The solvent may have increased its absorption or another component had prolonged its circulating life. The results of further work were reported by (Thyssen *et al.*, 1982).

Pauluhn J & Machemer L (1984a): Toxicological assessment of the neurotoxic potential of methamidophos (the active ingredient of Monitor^R and Tamaron^R). Mobay Report No. 87436. Study No. not specified. Report No. 84-T-066. Lab: Bayer AG Institut fuer Toxikologie Friedrich-Ebert-Str. 217 D 5600 Wuppertal, Germany. Sponsor: Agricultural Chemicals Division, Mobay Chemical Corporation, Kansas City, Missouri, USA. Study Duration: not specified. Report Date: 7 March 1984. Unpublished. (QA, GLP and Test Guidelines: not applicable).

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Note: this report is a review of an article by Senanayake & Johnson (1982), evaluated above.

These authors reviewed an article (Senanayake & Johnson, 1982) describing 10 cases of suspected acute methamodophos toxicity in Sri Lanka in humans who subsequently exhibited delayed neurotoxic effects. They referred to the evidence available at the time (Johnson, 1975a; Abou-Donia, 1981) that compounds structurally related to methamidophos had not induced neurotoxicity and that studies on the structure-effect relationship of organophosphates had shown that only those organophosphates which possess specific structural characteristics have a neurotoxic effect.

They also stated that while hens had shown reversible inhibition of NTE following methamidophos administration, no clinical or histopathological findings indicative of delayed neurotoxicity had ever been observed. They cited (Kimmerle & Lorke, 1967b; Kruckenberg *et al.*, 1979; Thyssen *et al.*, 1982).

The review authors emphasised that the diagnosis had been made solely on the basis of signs and symptoms of poisoning. ChE activity had not been determined and a positive identification of the substance involved was lacking. It was calculated that one patient who had survived treatment, consumed a dose of methamidophos 65 times greater than the known oral LD_{50}. The severity of the poisoning was estimable only from the observation that the patient was unconscious for more than 24 h and was administered very high doses of atropine (270 mg within 24 h). It was claimed that, in the absence of precise monitoring of the cardiovascular system, cardiovascular effects including cerebral oedema could not be ruled out. This can cause a change in muscle tone resulting in increased spasticity suggestive of pyramidal tract involvement. The electromyographic examination was not conclusive and did not permit a diagnosis of alkyl phosphate-induced polyneuropathy. The review authors further reported that individual cases of Tamaron™ poisoning in Germany had induced coma for up to 3 d from which the patients recovered without delayed effects.

Comment: (Abou-Donia, 1981), a studied a number of published observations to compare the delayed neurotoxic potential and potencies of a series of organophosphorus esters differing only in the structure of their hydrocarbon radicals. Methamidophos (a phosphoramidothiolate) is an aliphatic phosphorus ester and was not listed among aliphatic phosphorus esters known or not known to cause delayed neurotoxicity. The delayed neurotoxic activity and potency of aliphatic phosphorus esters was related to the degree of branching and substitution of the hydrocarbon radicals. The larger the size the less likely is the necessary adsorption of the organophosphorus ester onto the surface of the neurotoxicity protein. The hydrophobic areas on the neurotoxicity protein are of limited size and can accommodate only 3 or 4 methylene groups. No comment was made about methamidophos but its structure does not appear to preclude adequate linkage with the neurotoxicity active site.

Senanayake N (1985): Polyneuropathy following insecticide poisoning. A clinical and electrophysiological study. J Neurol, 232:Suppl:203, abstract.

This study described 27 isolated cases of acute polyneuropathy occurring after exposure to 3 organophosphorus compounds marketed in Sri Lanka. The cases were 22 males and 5 females aged 14-33 yr. They were encountered over a six yr period at the University Medical Unit at Peradeniya. Methamidophos was involved in 25 of the cases. The method of exposure was ingestion with suicidal intent (19), accidental ingestion (5) and accidents during spraying (3).

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The author did not distinguish between the compounds in providing data but since 25/27 of the cases involved methamidophos it is reasonable to consider the following signs as typical of methamidophos-induced delayed polyneuropathy.

Neuropathy developed following the acute cholinergic crisis after a delay of 15-22 (median: 20) d. The primary signs were acute weakness of feet and hands in 27 cases, with calf pain preceding the weakness in 20 and paraesthesia of limbs in 13. Physical signs were absent ankle jerk in 27 cases, foot drop in 22, claw hand in 11 and sensory (pain) impairment in 11. The dominant hand was more affected in 17. Two patients had external ocular palsies. Four patients, in late stages, had mild pyramidal signs.

Electromyography of distal muscles of the limbs showed varying degrees of denervation. Stimulation of common peroneal and/or tibial nerves failed to evoke motor action potentials (MAP) in 21 cases. Median nerve stimulation failed to evoke MAP in 4. Sensory conduction in the digital branches of the median nerve was impaired in 2. Signs of clinical improvement appeared any time after 2 wk from the onset. Four patients recovered completely in 6 wk to 2 yr. The other patients were under observation for signs of complete recovery at the time of the author's preparation of this abstract.

Senanayake N & Karalliede L (1987): Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. N Engl J Med, 316(13):761-763.

This study concerns 10 patients affected by organophosphorus poisoning and admitted to the University Medical Unit at Peradeniya, Sri Lanka, during the previous 3 yr. The study authors had recognised in the 10 patients an intermediate syndrome of neurotoxic effects that appeared after the acute cholinergic crisis but before the expected onset of delayed neuropathy. One of them, a 22 yr old male, had taken methamidophos with suicidal intent and had developed clinical signs characteristic of an intermediate syndrome. This was the only case involving methamidophos. The dose was not specified. The methamidophos-affected patient had exhibited well-defined cholinergic signs that included miosis, salivation, sweating and fasiculations and was unconscious on admission. Upon treatment with pralidoxime and atropine, the patient recovered from the cholinergic crisis within 24-48 h. Consciousness was restored. However, within 4 d, an intermediate syndrome had developed in the methamidophos-affected patient which lasted for 32 d and overlapped the development of polyneuropathy. The nature and time course of signs in this patient were similar to those caused by other organophosphates. The initial clinical signs were weakness and respiratory insufficiency, which required intermittant positive-pressure ventilation. It was respiratory insufficiency that drew attention to the syndrome in the methamidophos-affected patient and in 6 others.

The syndrome was characterised by weakness of the muscles innervated by cranial nerves. Marked weakness of neck flexion was a feature and was shown as an inability to raise the head from the pillow. Other features were weakness of the proximal limb muscles with normal strength in the distal muscles, weakness of shoulder abduction and hip flexion and the absence of knee and ankle reflexes. Sensory impairment was not observed. The regression of signs followed a characteristic pattern in those who survived (7/10). Cranial nerve palsies (palatal, facial and external ocular in that order), were the 1st to regress. Then followed an improvement of the respiratory impairment and recovery of strength in the proximal limb muscles. Neck flexion was the last function recovered. The period of recovery ranged from 5-18 d after the onset of weakness except in the methamidophos-affected patient whose neck

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flexion capacity took 32 d to recover. By that time he had developed weakness of the distal muscles of the limbs due to delayed neuropathy.

A comparison of the intermediate syndrome and delayed neuropathy is tabulated below.

Comparison of the intermediate syndrome and delayed neurotoxity in patients following poisoning by methamidophos and other organophosphates

Variable	Intermediate syndrome	Delayed neuropathy
Time of onset	1-4 d	2-3 wk
Sites of weakness		
Limb muscles	Proximal	Distal
Neck muscles	+	-
Cranial nerves	+	-
Respiratory muscles	+	-
Electromyogram	Tetanic fade	Denervation
Recovery, from time of onset	4-18 d	6-12 mo

Conclusion

Methamidophos at a presumed high oral dose, caused intermediate syndrome followed by delayed neuropathy in a 22 yr old male. The effects were similar to those caused by other organophosphates.

Karalliedde L, Senanayake N & Ariaratnam A (1988): Acute organophosphorus insecticide poisoning during pregnancy. Human Toxicology, 7(4):363-364.

This study concerned 2 female patients, 1 of which was a 22 yr old primigravada admitted to the Teaching Hospital, Peradeniya, Sri Lanka, 3 h after ingestion of methamidophos with suicidal intent. The amount ingested was not specified. The patient presented with mental confusion, pin point pupils and generalised muscle fasciculations. It was estimated that she was 36 wk pregnant. Treatment included gastric lavage, atropine and pralidoxime. The patient was rational and free of muscle fasiculations within 24 h. Eighty h after admission, she became restless, dyspnoeic and cyanosed with a respiratory rate of 50/min and tidal volume of 150 mL. Mechanical ventilation was required for 6 d. She was clinically normal afterwards. Forty d after poisoning, the patient delivered a male child with a bw of 2.85 kg and an Apgar score of 8 at 1 min. At birth the child was clinically free of congenital or neurological abnormalities.

The study author concluded that provided a prompt diagnosis and adequate management of cholinergic and intermediate phases occurs following poisoning with methamidophos during the 3rd trimester of pregnancy, normal progress of pregnancy to term and successful birth is possible.

Moretto A, Alvisi R, Capuzzo M, Davanzo F, Fioroni F, Tralli G & Lotti M (1994): A case of peripheral neuropathy by methamidophos in man. Society of Toxicology. Abstracts of the 33rd Annual Meeting. The Toxicologist, 14(1):390

A 32 y old male attempted suicide by swallowing 200 mL of Tamaron^{R} containing 228 g/L of methamidophos. The estimated dose was 600 mg/kg bw. The patient displayed severe

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cholinergic toxicity and became comatose within 2-3 h. Mechanical ventilation, treatment with atropine, pralidoxime and benzodiazepine were provided for 4 d. Plasma and erythrocyte ChE activities were < 10% unspecified normal values and began to recover from d 4. Lymphocytic neuropathy target esterase (LNTE) was 1.0, 1.6, 5.9, 16.4 and 12.5 nmoles/min/g of protein at 22, 43, 84 h and 9 and 37 d after intoxication, respectively. Methamidophos concentration in plasma peaked at 6.2 µg/mL at 22 h after ingestion. On d 10 the patient was well and neurological examination and electrophysiological parameters were normal. However, because of inhibition of LNTE, development of polyneuropathy was expected. On d 25 the patient complained of leg weakness and on d 36 neurologic examination showed discrete peripheral neuropathy in lower limbs. In tibial and peroneal nerves, motor nerve conduction velocities (NCV) were moderately reduced (40-44 m/sec) whereas amplitudes of motor action potential (MAP) were severely affected (0.1-1 mV). MAPs were also reduced in the median nerves (3-6 mV). Sensory NCV, somato-sensory and visual evoked potentials were normal.

Comment

The extent of LNTE inhibition was not determinable here since the study authors did not specify normal or baseline levels of LNTE. Using the normal values specified in (McConnell *et al.*, 1999) - also evaluated in this Section, LNTE was inhibited by approximately 89, 82 and 35% at 22, 43 and 84 h respectively.

Sun DH, Zhou HD & Xue SZ (1998): Epidemiologic survey on organophosphate-induced delayed polyneuropathy (OPIDP) among patients recovered from methamidophos poisoning. Medicina del Lavoro, 89(Suppl 2): S123-S128.

This study was concerned with 104 people poisoned with methamidophos. They were 63 patients admitted to the Wu-Xian county hospital and Heng-Jing, Pu-Zhaung, Du-Chun and Xu-Cou rural hospitals (Area 1) between 1987 and 1992, and 41 patients admitted to Shi-Qiu district hospital (Area 2) over the years 1989 to 1992. Each subject was selected from medical records, underwent a medical examination and completed a questionnaire.

The following criteria were used in the case selection process:-

a. history of ingesting or contamination with "large amounts of methamidophos";
b. typical clinical signs of a cholinergic crisis;
c. decreased blood ChE activity;
d. good response to treatment with atropine.

Diagnoses of OPIDP were made according to the following criteria:-

e. history of acute methamidophos poisoning;
f. onset of OPIDP 1-3 wk after acute methamidophos poisoning;
g. typical manifestations and course of the disease;
h. recovery 3-6 mo after the onset of OPIDP and complete normalisation wthin 2 y;
i. exclusion of other diseases.

Data were analysed with SPSS and EPI INFO statistical packages.

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The cases selected represented 73% of the organophosphate poisoning cases in the areas serviced by the hospitals concerned. Ingestion was the route of poisoning in 95% of cases. The circumstances of the ingestions were not specified. The other routes of exposure were not identified. The severity of poisoning was classified according to unspecified criteria of the Chinese Ministry of Health. Forty per cent. were classified as mild, 20% as moderate and 40% as severe.

Individual intake of methamidophos formulation(s) of methamidophos was determined in 90 cases. The relationship between volume of intake and severity of poisoning is tabulated below. Intake of 20 mL or more was associated with severe poisoning in the majority of cases. In the absence of information about the formulation(s) involved, the data provided cannot provide information on the achieved doses of methamidophos.

The amount of ingested product and severity of poisoning

Amount of ingested product (mL)	Mild and moderate	Severe	Total
0-19	34	9	43
20-49	9	15	24
50-99	5	4	9
100+	6	8	14
Total	54	36	90

$\chi^2 = 8.37$; $p < 0.05$

OPIDP: Among the Area 1 group of 63 cases of methamidophos poisoning, 7 cases of OPIDP were diagnosed (11% incidence). Seven of the Area 2 group of 41 cases also developed OPIDP (17% incidence). All 14 people with OPIDP complained of paralysis and reeling gait. Thirteen cases followed severe acute signs and 1 followed mild acute signs. There was no significant difference between the 2 areas in incidence of OPIDP ($\chi^2 = 0.52$; $p = 0.47$). The combined incidence was 13.5%.

In all 14 cases of OPIDP, poisoning occurred by the oral route. In 7 instances it followed the ingestion of methamidophos and "in the remaining 6 cases" it followed ingestion of product containing methamidophos and dimethoate (2:1). The intake volume ranged from 10-100 mL with a mean volume of 33 mL for methamidophos alone and 23 mL for the mixture. The mean time from intake to treatment in hospital was 43 min. The doses were not specified nor were they calculable from the data presented. The 14 affected persons had all shown signs of acute organophosphate toxicity. Eight had muscular tremors, 12 exhibited coma of 1-24 h duration. Three suffered from faecal and urinary incontinence. Blood ChE activity in all OPIDP cases had been severely inhibited. Data were not provided. The disappearance of cholinergic signs coincided with the restoration of ChE to "normal" levels.

The cases of OPIDP caused by methamidophos alone and those caused by a mixture of methamidophos and dimethoate were not distinguishable and the following data does not therefore relate specifically to methamidophos.

The study authors indicated that sex and age were not significant risk factors for OPIDP.

The clinical manifestations of OPIDP in the study populations were:-

a. sensory disturbances appeared earlier than dyskinesia. Twinge, numbness and paresthesia appeared in the distal limbs. In more severe cases, a symmetric "glove-sock" anaesthesia developed in the extremities.

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b. dyskinesia appeared at first in the distal portion of the limbs, after sensory disturbances. The main signs were weakness, difficulties in raising legs, dysbasia and loss of capacity to stand and walk. Difficulty in raising arms and with fine movements of hands and fingures.

c. functional disturbances of the autonomic nervous system were also noted e.g. chilling of the skin and excessive perspiration.

d. neurasthenic syndrome: insomnia, headache and dizziness.

Thirteen patients recovered, mostly within 2 y. One patient was still affected with OPIDP 64 mo after diagnosis.

Comment

These study authors have shown that among human cases of methamidophos toxicity occurring in two Chinese communities, the incidence of OPIDP is related to the severity of cholinergic signs experienced. Other studies in this review (Section 10) have shown that the severity of cholinergic signs in rats and hens exposed to methamidophos by ingestion is directly proportional to the dose administered and that the occurrence of OPIDP in antidote protected hens requires doses of methamidophos several times higher than LD_{50}. The absence of data on methamidophos doses and the coincident exposure with dimethoate reported in this study precludes comparisons.

McConnell R, Delgado-Tellez E, Cuadra R, Torres E, Keifer M, Almendarez J, Miranda J, El-Fawal HAN, Wolff M, Simpson D & Lundberg I (1999): Organophosphate neuropathy due to methamidophos: biochemical and neurophysiological markers. Archives of Toxicology, 73(6):296-300.

Method

A 16 y old hispanic male was brought to a Nicaraguan hospital shortly after intentionally ingesting approximately 100 mL of an unidentified formulation of pesticide. A diagnosis of methamidophos toxicity was made on the basis of the identification of methamidophos in a sample of ingested pesticide, the subsequent identification of a pesticide concentrate as methamidophos and the subsequent development of motor neuropathy in the patient. The patient was evaluated. Nerve conduction studies of ulnar sensory and sural nerves using orthodromic stimulation and of ulnar and tibial motor nerves were conducted using a TD 20 Mk1 and standard techniques. Quantitative vibrotactile threshold was measured using a Vibraton II and upper extremity grip and pinch strength were assessed using a Jamar dynanometer and a pinch gauge according to standard protocols (Gerr *et al.*, 1990) (Mathiowetz *et al.*, 1985). Twenty five mL of blood were obtained by venipuncture, heparinised and transported in plastic tubes at room temperature within 90 min for analysis of lymphocyte neuropathy target esterase (LNTE) according to the method of (Maroni & Bleecker, 1986). RBC and plasma ChE were measured using a modified Ellman assay (McConnell *et al.*, 1992). Serum from 5 mL of blood was frozen at -70^{o}C and subsequently thawed for measurement of autoantibodies to neurofilament triplet proteins (NF 68, NF 160 and NF 200) and to GFAP and MBP as described previously (El-Fawal *et al.*, 1996).

Results and discussion

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On admission the patient was unconscious, tachypneic and had diffuse rhonchi, miosis and prominent fasciculations of pectoral and abdominal musculature. The patient regained consciousness and his lung fluids cleared during the administration of 18 mg atropine iv in the 1st 1.5 h after admission. During his 7 d sojourn in hospital he was treated for recurrent sialorrhea, blurred vision, generalised weakness, fasciculations, nausea and vomiting with a total of 91 mg atropine iv and 1 g pralidoxime. At the time of his discharge from hospital (on d 8 after admission) his neurological examination was normal except that his pinch strength was reduced reflecting either residual cholinergic depolarisation blockade at the motor endplate as a result of acute ChE inhibition, or early neuropathy. Quantitative vibrotactile threshold was mildly elevated in the lower extremity. Vibrotactile threshold, pinch and grip strength measurements before and after the development of neuropathy are tabulated below.

Vibrotactile threshold, pinch and grip strength measurements

Parameters	**Measurements before and after clinical neuropathy induced by methamidophos**			
	Number of d after poisoning			**Normal range**
	7*	**24**	**52**	
Vibrotactile threshold[a]				
Right index finger	0.29	0.18	-0.19	< 0.81
Right first toe	1.85	0.71	-0.16	< 1.31
Key pinch strength[b]	14	7	9	> 21
Palmar pinch strength[b]	18	9	7	> 18
Grip strength[b]	64	52	52	> 91

* Discharged from hospital on d 8

a: log microns of peak vibration amplitude - normal range from (Gerr *et al.*, 1990)

b: Lbs - normal range from (Mathiowetz *et al.*, 1985)

Approximately 5 d after discharge, the patient began to experience pain in the gluteal region, thighs and calves. One d later he noticed weakness of his lower extremities and had difficulty walking, then weakness of grip. On examination he had bilateral foot drop and a steppage gait, weakness of wrist extension and flexion and of abductor pollicus brevis and there was marked weakness of the interossei of the hands. He had weakness, more marked distally, of iliopsoas, quadriceps and extensor and flexor muscles of the feet. He was unable to dorsiflex his feet against gravity, to stand on his toes or heels or to do deep knee bends without assistance. Deep tendon reflexes were normal in the upper extremities. The knee reflex was hyperactive without clonus and the ankle reflex was absent. The sensory examination was normal. Objective measurements of grip and pinch strength 52 d after poisoning demonstrated considerable deterioration from those obtained at the discharge examination. Quantitative vibrotactile threshold on subsequent measurements were normal. Nerve conduction studies demonstrated reduced amplitude of ulnar nerve distal compound muscle action potential (see Table). Sensory nerve conduction studies were normal.

Nerve conduction 52 d after acute poisoning

Parameters	**Conduction in each nerve examined at 52 d after acute methamidophos poisoning***		
	Ulnar motor	**Ulnar sensorial**	**Sural**
Distal amplitude in μV (normal)	3222 (> 5000)	25 (≥10)	19 (≥ 4)
Proximal amplitude in μV (normal)	2927 (≥ 5000	-	-

* Tibial motor nerve responses could not be elicited

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ChE and LNTE activity measured at 3 intervals after intake is tabulated below. ChE activity approximately 24 h after ingestion but before treatment with the ChE reactivator pralidoxime, was low in RBC and in plasma. RBC ChE was still mildly depressed at 52 d. LNTE activity was low at 3 d.

Plasma and RBC ChE and LNTE determinations

Esterase	**Plasma, RBC ChE and LNTE at three intervals after acute poisoning with methamidophos**			
	Number of d after poisoning			**Normal range**
	1	**3**	**52**	
Plasma ChE (IU/mL)	0.4	-	2.6	> 1.13[a]
RBC ChE (IU/g haemoglobin)	2.7	-	24.2	> 26[a]
Lymphocyte NTE (nmol/min/mg protein)	-	3.1	13.3	> 9.1[b]

a: Measured using Testmate OP field kit (EQM Research, Cincinnati, Ohio) in unexposed populations
b: normal value from (Maroni & Bleecker, 1986)

Autoantibody isotypes immunoglobulin M (IgM) and IgG against NF triplet proteins, GFAP (IgG only) and MBP were detected in serum collected 3 d and 52 d after poisoning. In the period 3-52 d there was a marked increase in IgG to GFAP and to NF 200 during this period. There was a moderate decrease in IgM to GFAP and of both isotypes to NF 68. The determined values were provided by the study authors but these are not reproduced here.

The study authors discussed their findings in the context of predicting the occurrence of OPIDP and establishing a reliable biomarker for it. They concluded that the inhibition of LNTE is predictive for OPIDP and that serum autoantibody titres to nervous system proteins may be useful markers of neuropathy. The development of autoantibody titres is however not specific to organophosphate induced injury.

Chan TYK (2001): Vegetable-borne methamidophos poisoning. Journal of Toxicology - Clinical Toxicology, 39(4):337-338.

This letter to the editor reported on methamidophos contamination of vegetables in Hong Kong. Because methamidophos was considered "highly toxic" and had a half life of 5 d (Lau, 1990), its use on vegetables was prohibited in Hong Kong and in Shenzhen, Dongguan and Huizhou, from which areas most of Hong Kong's green leafy vegetables were imported. Nevertheless medical experience in Hong Kong was suggesting that methamidophos was being used on vegetables.

Cholinergic stimulation attributed to the consumption of methamidophos contaminated vegetables was usually mild. The signs were mainly gastrointestinal and subsided in a "few" d. Protective measures noted were the elapse of time before consumption and the rinsing or soaking vegetables in water which removes all or part of the methamidophos.

In 1987, 64 patients presented to the emergency department of the Prince of Wales Hospital after eating vegetables contaminated with methamidophos. Sixty % developed signs within 1-3 h of exposure. Common features included nausea (95%), vomiting (86%), chills (75%), sweating (67%), dizziness (63%), abdominal pain (56%), diarrhoea (45%), headache (27%), increased lacrimation (27%), weakness (25%) and miosis (19%). These patients required symptomatic treatment only.

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After controls over organophosphate levels on vegetables were introduced and additional compliance measures such as labels identifying source and pesticides used were required, outbreaks of methamidophos poisoning declined significantly.

12. OTHER STUDIES

12.1 Biochemical, histochemical and histopathological studies

Hanafy MSM, Arbid MS & Afify MMH (1991): Biochemical and histopathological effects of the organophosphorus insecticide (Tamaron) in rats. Indian J Anim Sci, 61(1):43-47.

Test Substance:	Methamidophos (Tamaron: water-miscible concentrate). Purity: 60%. Source: Unspecified commercial origin
Test Species:	Rat: Strain, sex, bw, age or source were not specified.
Dose:	20 mg/kg bw single ig; 2 mg/kg bw/d for 14 d or 10 wk ig

Method

The housing, environmental conditions and diet of the rats were not described. Methamidophos (prepared in saline at 5 and 0.5 mg/mL) was given ig at doses of 20 and 2 mg/kg bw, chosen because they were reported to have been the LD_{50} and 1/10th LD_{50} of methamidophos in rats (Robinson & Beiergrohslein, 1980). The route of administration for the given LD_{50} was not specified. [In fact Robinson & Beiergrohslein (1980) determined an ip LD_{50} as 15 mg/kg bw]. The dosing solution was given orally using a stomach tube.

Biochemical effect of methamidophos: Fifteen rats were given a single dose of 20 mg/kg bw and 5 rats were given daily doses 2 mg/kg bw/d for 14 d. Two other groups of 5 rats were similarly treated with saline only. They acted as controls for the treated groups. Five surviving rats from the single dose group and five controls were blood sampled from the medial canthus of the eye at 24 h after dosing. The procedure was repeated at 15 d for the daily dose group and their controls. The blood samples were used to prepare serum for the determination of BUN, glucose, cholesterol, AST, ALT and AP. After collection of blood samples rats were killed and liver specimens taken immediately for determination of glutathione. No further methodological information was provided.

Effect of methamidophos on thiopentone sleeping time: Twenty rats were divided into 2 groups. The 1st group was given daily doses of 2 mg/kg bw for 3 wk. The other group was given daily doses of saline. At the end of 3 wk, sleeping time caused by 35 mg sodium thiopentone/kg bw ip, was determined in the treated and control rats.

Pathological effects of methamidophos: Forty rats were divided into 4 equal groups. One group was given a single dose of 20 mg/kg bw and another received daily doses of 2 mg/kg bw for 10 wk. The rats in the other 2 groups were similarly treated with saline only and acted as controls. Rats were kept under observation. The single dose rats and their controls were killed at 72 h and the daily dose rats and their controls were killed at 10 wk. Necropsy examinations were performed. Tissue specimens were removed, fixed in formalin, processed using standard paraffin embedding, stained with haematoxylin and eosin and subjected to histopathological examination. Tissue sections used to examine fatty degeneration were processed using freezing microtomy and stained with Sudan III stain.

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Statistical analysis: The standard student's 't' test was used to analyse differences in the concentration of different serum and tissue constituents and in thiopentone sleeping times.

Results and discussion

Biochemical effects of methamidophos: Observations made on biochemical constituents of blood and liver tissue glutathione are tabulated below. Administration of methamidophos caused a statistically significant ($p < 0.01$) rise in the serum concentrations of BUN, glucose, cholesterol, AST, ALT and AP compared to controls. Tissue glutathione showed a statistically significant ($p < 0.01$) decrease in livers of treated rats. The study authors did not comment on differences between the effects of single dosing and repeated dosing.

Concentrations of blood chemical constituents and liver tissue glutathione

Constituent (concentration)	**Concentrations of biochemical parameters following two regimes of treatment**		
	Control rats	**Treated rats***	
		Single dose	**Repeated doses**
Liver glutathione (mg/100g liver)	138.0	119.0	132.0
Urea (mg/dL)	7.8	71.3	146.7
Glucose (mg/dL)	56.5	120.0	120.0
Cholesterol (mg/dL)	40.8	64.5	71.2
GOT	127.3	150.0	140.0
GPT	50.4	124.3	71.0
Alkaline phospatase (K A units/dL)	6.0	20.0	13.8

Single dose: 20 mg/kg bw ig; Repeated doses: 2 mg/kg bw/d for 14 d ig

* The study authors stated that "All values were highly significant ($p \leq 0.01$)"

Effect of methamidophos on thiopentone sleeping time: Daily administration of methamidophos for 3 wk caused a statistically significant ($p < 0.01$) prolongation of thiopentone sleeping time. Mean sleeping time was 75 min in methamidophos treated rats and 30 min in controls.

Pathological effects: The reported effects (compared to control rats) are tabulated below. The detail shown in the table is the limit of the detail provided.

Pathological effects of methamidophos in rats

Clinical signs, mortality rates and gross and cellular pathological observations	
20 mg/kg bw in a single oral dose	**2 mg/kg bw orally daily for 10 wk**
Clinical signs: Salivation, diarrheoa, dyspnoea, paralysis.	*Clinical signs*: Loss of condition, persistent tremors.
Mortality: 6/10 deaths.	*Mortality*: 2/10 deaths.
General gross observations: Congestion of internal organs and brain.	*General gross observations*: None reported.
General histological observations: none reported.	*General histological observations*: Albuminous and fatty degenerative changes and hydropic vacuolation of the parenchymal cells of liver, kidney and heart.
Liver: Congestion of the central and portal blood vessels, hydropic and fatty degeneration; mononuclear cell infiltration.	*Liver*: Fibroblastic activity together with mononuclear inflammatory cells at the portal areas. Kupffer cells had prominent nuclei and swollen cytoplasm. Telengiectosis infrequently especially at the centrilobular areas.

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Kidney: Congestion and small foci of haemorrhages and degenerative changes of the tubular epithelium.	*Kidney*: Areas of intertubular haemorrhages and focal activation of fibroblasts at the corticomedullary junction, hyaline casts in some tubules.
Lung: Congestion, foci of haemorrhages, oedema and aggregates of lymphoid cells.	*Lung*: Enlarged mononuclear inflammatory cells mostly of lymphocytic type with variable sized phagocytic vacuoles, around the bronchioles and some perivascular areas.
Testes: Spermatogonia in the testes showed degenerative changes.	*Testes*: Speratogenic epithelium showed degenerative and necrobiotic changes. Nuclei of some cells showed pyknosis, fragmentation and complete lysis. Many of the tubules were devoid of sperm cells.
Brain: Chromatolysis and cellular oedema were present in some neurons.	*Brain*: Chromatolysis and cellular oedema were present, local and diffused gliosis and necrosis of a few neurons in different areas of the brain.
Other tissues: None reported.	*Other tissues*: Incidence and severity of mandibular abscesses were higher than controls

Conclusions

(1) Methamidophos administered as a large single dose (20 mg/kg bw) caused signs of cholinergic stimulation characterised by salivation, diarrhoea, dyspnoea and paralysis.

(2) Prolonged administration of small daily doses of methamidophos (2 mg/kg bw/d for 10 wk) caused persistent tremors. These were attributed by the study authors to widespread degenerative changes in different parts of the brain. It induced the development of numerous (mandibular) abscesses. These observations together with the inflammatory changes seen in lungs suggested that methamidophos treated rats had lowered immunity.

(3) A single dose (20 mg/kg bw) and daily doses (2 mg/kg bw/d for 14 d) of methamidophos:-
   a. caused elevation in AST, ALT, and AP, pathological changes in the liver of rats and depletion of liver glutathione. The study authors indicated that these changes were accompanied by evidence of decreased detoxifying activity as shown by the reduction in glutathione and significant prolongation of thiopentone sleeping time in rats receiving 2 mg/kg bw/d for 3 wk.
   b. reduced liver glutathione. It was suggested by the study authors that this depletion would increase the vulnerability of rats to many other toxic agents, and was indicative of possible involvement of glutathione in the biotransformation of methamidophos.

(4) Methamidophos caused nephrotoxicity and subsequent elevation of BUN. It increased the concentration of blood glucose and cholesterol. The study authors suggested that it therefore had potential to be a diabetogenic agent and a contributor to the development of atherosclerosis.

Comment

Decreased heaptic glutathione levels imply either decreased synthesis of glutathione or increased binding of glutathione (conjugation) by reactive metabolite(s). This feature is often seen when hepatotoxic agents are administered but it is not necessarily an indication that the detoxification (i.e. metabolism) of the chemical has become impaired. Although it is true that that thiopentone sleeping time may have been prolonged because hepatic metabolism of this drug was impaired by methamidophos, there is another possibility: the brain lesions (and

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AChE effects) might alter CNS function and so prolong sleeping time by an different mechanism than the one proposed.

12.2 Methamidophos technical grade impurities toxicity studies

Whenever available data permits, impurities have been examined for their possible contribution to the mammalian toxicity of methamidophos technical. The trimethyl phosphorothioates, present in methamidophos technical at < 1%, have been identified as impurities in a range of organophosphate compounds including malathion, phenthoate, acephate and fenthion. The trimethyl phosphorothioates are known potentiators of the toxicity of these compounds in the rat (Pellegrini & Santi, 1972; Umetsu *et al.*, 1977; Aldridge *et al.*, 1979; Toia *et al.*, 1980).

O,O-dimethyl phosphoramidothioate: an intermediate used in the manufacture of technical methamidophos and is the impurity in technical methamidophos with the highest concentration (≤ 9%). Median lethal dose determinations are tabulated below. The clinical signs reported by (Cavalli *et al.*, 1968j) were paresis, hyporeflexia, areflexia, anaesthesia and hypothermia. Death from respiratory failure occurred between 2-16 h after dosing. Survivors were severely depressed for as long as 48 h after dosing. No pathological abnormalities were noted at necropsy.

***O,O*-dimethyl phosphoramidothioate (≤ 9% of methamidophos technical)**

Species [strain]	Sex	Group Size	Route of administration & Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Mouse [SW]	ns	5	Oral;water at 1 mL/200 g rat	≥ 99.8	ns (6 doses used)	430 (24 h)	(Umetsu *et al.*, 1977)
Rat [SD]	M F	5/sex/ dose	Oral;water at 1 mL/100 g rat	ns	500, 563, 625, 675, 725, 780, 825	M 633 F 549 (14 d)	(Cavalli *et al.*, 1968j)
Rat [ns]	ns	ns	Oral;ns	ns	ns	410	(RTECS, 2003a)
Rat [ns]	ns	ns	Inhalation 4 h; ns	ns	ns	LC_{50} > 14.5 mg/m^3	(RTECS, 2003a)
Rat [ns]	ns	ns	Dermal;ns	ns	ns	4360	(RTECS, 2003a)

SD = Sprague-Dawley; ns = not specified

Monomethyl sulfate ion: An impurity present at ≤ 4%. In rats methyl sulfate has an oral LD_{50} of 205 mg/kg bw, an sc LD_{50} of 100 mg/kg bw, a 4 h inhalation LC_{50} of 45 mg/m^3 and in mice an oral LD_{50} of 140 mg/kg bw and an inhalation LC_{50} of 280 mg/m^3. Clinical signs of acute toxicity included somnolence and respiratory depression. Methyl sulfate is also a severe irritant to skin and eye of rabbits (RTECS, 2003b).

O,S-dimethyl methylphosphoroamidothioate (N-methyl methamidophos homolog) and O,O-dimethyl methylphosphoroamidothioate (N-methyl amidate homolog): These two homologs are present in methamidophos technical at ≤ 4 and 2% respectively. There are no available median lethal dose data.

O,O,S-trimethyl phosphorothioate (OOS-TMP): Present in methamidophos technical at < 1%. Median lethal dose data are tabulated below.

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***O,O,S*-trimethyl phosphorothioate (*OOS*-TMP) (< 1% of methamidophos technical)**

Species [strain]	Sex	Group Size	Route of administration & Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Mouse [SW]	ns	5	Oral;corn oil at 1 mL/200 g rat	98	ns (6 doses used)	530 (24 h)	(Umetsu *et al.*, 1977)
Mouse [L]	F	4	Oral;glycerol formal	98	ns	134	(Verschoyle & Cabral, 1982)
Mouse [L]	F	4	iv;glycerol formal	98	ns	123	(Verschoyle & Cabral, 1982)
Rat [SD]	F	4	Oral;corn oil at 1 mL/200 g rat	98	ns (5 doses used)	260 (24 h)	(Umetsu *et al.*, 1977)
Rat [P]	F	6	Oral;glycerol formal	98	ns	60 (4 d)	(Aldridge *et al.*, 1979)
Rat [SD]	F	4	Oral;corn oil at 0.2 mL/100g rat	ns (lab. grade)	0, 10, 15, 20, 30, 40, 60, 80, 200, 300	200 (48 h) 15-20 (25 d)	(Mallipudi *et al.*, 1979)
Rat [W]	F	4	Oral;glycerol formal	98	ns	60	(Verschoyle & Cabral, 1982)
Rat [SD]	M	6	Oral;arachis oil	98	ns	71	(Verschoyle & Cabral, 1982)
Rat [F344}	F	6	Oral;glycerol formal	98	ns	100	(Verschoyle & Cabral, 1982)
Rat [W]	F	4	iv;glycerol formal	98	ns	45	(Verschoyle & Cabral, 1982)
Rat [W]	F	4	ip;glycerol formal	98	ns	51	(Verschoyle & Cabral, 1982)
Rat [SD]	M	ns	Oral;10 mg TMP/mL corn oil	≥ 99.9	ns	60	(Imamura *et al.*, 1983)
Rat [SD]	F	4	Oral;corn oil at 2 mL/rat	≥ 99.8	0, 30, 60, 120, 240	85 (28 d)	(Koizumi *et al.*, 1986)

SW = Swiss White; L = LACa; SD = Sprague-Dawley; P = Lac:P; W = LAC:P (Porton-derived Wistar); ns = not specified;

Mallipudi NM, Umetsa RF, Toia RF, Talcott RE & Fukuto TR (1979): Toxicity of O,O,S-trimethyl and triethyl phosphorathioate to the rat. J Agric Food Chem, 27:463-466.

The study authors described the acute clinical signs as being: at 200 mg/kg bw po, bw loss, severe diarrhoea, excessive urination and bleeding from mouth, nose and ear. The signs were less severe but observable at 40 mg/kg bw po. At 15 mg/kg bw po the signs were absent except for bw loss.

Serum CE and serum ChE activities were significantly inhibited for extended periods. CE was initially more sensitive to inhibition than ChE. Mean serum CE at 4 h and 2 and 6 d after a single dose of 15 mg/kg bw po was 56, 76 and 61% control activity respectively. Activity was restored to 100% between 6-14 d in survivors. At the same dose, mean serum ChE at 4 h and 2 and 6 d was 67, 56 and 60% control activity respectively. Activity was restored to 100% between 6-14 d in survivors. The study authors considered that inhibition of CE was responsible for the toxicity potentiating property of this and other trialkyl esters present as impurities in malathion technical.

Verschoyle RD & Cabral JRP (1982): Investigation of the acute toxicity of some trimethyl and triethyl phosphorothioates with particular reference to those causing lung damage. Archives of Toxicology, 51(3):221-231.

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The study authors found that there were 2 phases of acute toxicity in rats. Within the 1st 30 min signs of poisoning were typically cholinergic and included fasciculations, salivation, urinary incontinence and occasionally chromodacryorrhea. Mortalities during this phase did not occur. Recovery from cholinergic signs was observed by 24 h but at this time rats began to show pilo-erection and panting respiration. They lost up to 20% of their bw in 4 d. The majority of deaths occurred within 3-6 d after dosing. In rats cholinergic signs occurred only at oral doses higher than the oral LD_{50} (60 mg/kg bw). The compound was less toxic to mice which showed a mixed response with deaths occurring both in the early cholinergic phase and in the later pulmonary phase.

At a dose of 50 mg/kg bw po female Wistar (LACp) rats showed a statistically significant ($p \leq 0.01$) doubling in wet and dry relative lung wt 4 d after dosing. There was no difference between treated and control rats in lung water content. The results were similar for Sprague-Dawley male rats given 60 mg/kg bw po and female F344 rats given 70 mg/kg bw po. Histological examination of enlarged lung tissue showed extensive cellular proliferation with progressive diffuse interstitial thickening which peaked at d 4 after dosing. This caused loss of alveolar space and consolidation of large areas of all lobes. In surviving rats the lesion had regressed by d 8. Pretreatment with the H_2 receptor antagonist cimetidine (SKF 525A) increased the oral LD_{50} of the compound from 50 to 252 mg/kg bw.

Imamura T, Gandy J, Fukuto TR & Talbot P (1983): An impurity of malathion alters the morphology of rat lung bronchiolar epithelium. Toxicology, 26:73-79.

The study authors reported that *O,O,S*-trimethyl phosphorothioate markedly alters the morphology of the bronchiolar epithelium of male Sprague-Dawley rats when given as a single oral dose in corn oil at 20 mg/kg bw.

The oral dose of 20 mg/kg bw resulted in few acute cholinergic signs and the rats survived for at least 28 d. Loss of bw and red staining around the nose and eyes were observed 24 h after treatment and were present for at least 7 d. Because signs were most apparent at 3 d after dosing, the morphology of the bronchiolar epithelium and extracellular bronchopulmonary lavage LDH activity were investigated at that time.

The morphology of the bronchiolar epithelium in treated rats was altered. The Clara cells were 50% fewer in number and 30% larger in diameter than control Clara cells. The number of ciliated cells also increased but the cilia appeared morphologically normal. Significantly higher ($p < 0.05$) mean LDH levels were found in tracheal lavage fluid from treated rats than from control rats (12 cf 6 U/100 mL respectively). The increase may have been due to LDH release from cells damaged by the compound in the bronchopulmonary tree.

Rodgers KE, Imamura T & Devens BH (1985): Effects of subacute treatment with O,O,S-trimethyl phosphorothioate. Toxicol Appl Pharmacol, 81:310-318.

These authors reported on the effects of 0.5 or 5.0 mg/kg bw/d *O,O,S*-trimethyl phosphorothioate for 14 d on the generation of cell-mediated and humoral immune responses in female C57B1/6 mice ($H\text{-}2^b$). The purity of the compound used was > 99.8%. The vehicle was corn oil at 2 mL/kg bw. Control mice were treated daily with corn oil only. Group size varied and is specified with the results. The bw of each mouse was measured daily. At termination the mice were killed by cervical dislocation and their thymus and spleen removed

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aseptically and utilised for a battery of tests. Comparison between groups was made by either Student's *t* test (2 groups) or one-way analysis of variance (> 2 groups) followed by a Duncan's multiple range test.

There was said to be no significant loss of bw in treated mice. The bw measurements were not provided.

Administration of 0.5 mg/kg bw/d of *O,O,S*-trimethyl phosphorothioate resulted in an increase ($p \leq 0.05$) in thymic lymphocyte number and wt and enhanced humoral immune responses ($p \leq 0.01$), mitogen responses ($p \leq 0.01$) and interleukin 2 (IL-2) production ($p \leq 0.05$). The capacity for generation of a cytotoxic T-lymphocyte (CTL) response was not changed. At a dose of 5 mg/kg bw/d, there was no change in humoral or CTL immune responses. There was an increase in IL-2 production ($p \leq 0.05$) and mitogenic responses ($p \leq 0.01$). Overall, treatment with low amounts of *O,O,S*-trimethyl phosphorothioate elevated immune responses in the mouse. The study authors emphasised that this was in contrast to the findings of their unpublished data which showed that unspecified acute amounts of this compound caused suppression of CTL and antibody responses.

Koizumi A, McCloud I & Imamura T (1986): Increased resorption of embryos in O,O,S-Trimethyl phosphorothioate-treated rats. Toxicology Letters, 32:185-194

The principal aim of this study was to establish a profile of reproductive toxicity in rats caused by *O,O,S*-trimethyl phosphorothioate at a relatively low oral dose when it is given early in gestation (GD 8-10). A pair-fed study was conducted to separate indirect effects on the gonadotrophic hormone system caused by decreased food consumption and loss of bw induced by *O,O,S*-trimethyl phosphorothioate, from other mechanisms of action.

For the reproductive study, virgin female Sprague-Dawley rats were mated and GD 0 determined. A control group of 5 rats was given corn oil (2 mL/kg bw po) on GD 8 and 10. A group of 7 mated rats were treated with 20 mg/kg bw po *O,O,S*-trimethyl phosphorothioate on GD 8 and 10. The dose was selected because toxic effects without mortality were expected based on the oral LD_{50} was 85 mg/kg bw. The dosing times were chosen to maximise adverse effects on pregnancy and foetuses. Food and water were freely available to treated and control rats. The pair-fed controls (7) were dosed with corn oil at 2 mL/kg bw po on GD 8 and 10 and fed the restricted amounts of food which had been determined by the mean food consumption in treated rats on the corresponding d of gestation i.e. 3.6 g from GD 8-12, 7.0 g from GD 13-15, 17 g from GD 16-18 and *ad libitum* from GD 19-21. Evaluation of the mean number of corpora lutea, mean number of implantations, mean number of live foetuses, mean foetal bw and the mean crown-rump length were performed using Student's *t*-test. Analysis of the frequency of termination of pregnancy, resorption rate and other incidence data was performed using the Fisher Exact Probability Test.

The effect of treatment on bw and food consumption is tabulated below. Severe emaciation was observed in the treated group (GD 12-15 or 21) and pair-fed groups from GD 12-21. The significant decreases ($p < 0.01$) in mean bw in treated group and pair-fed groups occurred in association with significant decreases ($p < 0.01$) in food consumption, amounting to an 88-66% decrement relative to controls over GD 8-15.

The effect of treatment on reproductive parameters is tabulated below. Of the 7 treated dams, 4 resorbed all their foetuses, while the resorption rate was approximately doubled in the other

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3 dams comared with controls. Resorption and foetal survival in the pair-fed control group were intermediate between the treated and control dams. The study authors considered that resorption in treated rats was not accounted for solely by a decrease in food consumption and was thus treatment-related.

Effects of *O,O,S*-trimethyl phosphorothioate on reproductive parameters in rats

Parameter	Reproductive parameters in each group (dose mg/kg bw)				
	Control (0)	Treated (20)			Pair-fed (0)
		Non-preg.*	Pregnant**	Total	
Number of dams[a]	5	4[b]	3	7	7
Total corpora lutea	67	62	44	106	97
Corpora lutea/dam	13	16	15	15	14
Total implantations	63	55	40	95	82
Implantations/dam	13	14	13	14	12
Total live foetuses	59	0	35	35	61
Live foetuses/dam	12	0	12	5[c]	9[c]
Total resorption	4	55	5	60	21
% Resorption	7	100[d]	13	63[d]	26[c]

*Non-pregnancy diagnosed by abdominal palpation on GD 15; no implantations observed at cesarean section on that d, **Pregnancy diagnosed by abdominal palpation at GD 15; cesarean section at GD 21 a: There were no deaths in any dams; b: Statistically significantly different from pair-fed rats, $p < 0.05$ c: Statistically significantly different from control rats, $p < 0.01$; d: Statistically significantly different from control and pair-fed rats, $p < 0.01$

The results of foetal measurements and the incidence of delayed ossification in the foetal skeleton are tabulated below. All foetuses were examined externally as were the soft tissues of 35% of foetuses from each group. No abnormalities were found. The foetal bw and crown-rump length of foetuses from treated dams were significantly smaller ($p \leq 0.01$) than that of controls or pair-fed rats. The incidence of delayed ossification in metacarpalis and metatarsalis was significantly higher ($p \leq 0.01$ or 0.05) than that of controls or pair-fed rats. Foetal growth was not affected in the pair-fed group.

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Effects of *O,O,S*-trimethyl phosphorothioate on foetal parameters in rats

Parameter	Foetal parameters in each group (dose mg/kg bw)		
	Control (0)	Treated (20)	Pair-fed (0)
Number of dams	5	3	7
Number of live foetuses	59	35	61
Foetal bw (g)	4.7	4.1*	5
Crown-rump length (cm)	4	3.8*	4
No foetuses given skeletal examinations	20	12	22
Number of foetuses (number of litters) showing delayed ossification in:-			
Sternebrae -	6 (4)	4 (2)	1 (1)
Centra-	7 (3)	6 (2)	2 (1)
Braincase	-	2 (1)	-
Metacarpalis	-	6 (2)*	-
Metatarsalis	-	3 (2)**	-

* Statistically significantly different from controls and pair-fed rats, $p \leq 0.01$, ** $p \leq 0.05$

In this study *O,O,S*-trimethyl phosphorothioate administered po as two doses at 20 mg/kg bw on GD 8 and 10 increased foetal resorption in rats 2.5-fold in comparison with pair-fed rats. It also induced growth retardation and delayed ossification in surviving foetuses.

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APPENDICES

APPENDIX I: Australian Registered Products Containing Methamidophos

NRA Product Code	Product Description	Product Name
33036	Emulsifiable Concentrate	Nitofol Insecticide Spray
47765	Suspension Concentrate	Monitor Insecticide

APPENDIX II: List of clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
Albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) Phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) Sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

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APPENDIX III: Organs for weight determination and histopathological examination

Organs Weighed	Tissues Examined		
Adrenals	Adrenals	heart	prostate
Brain	aorta	ileum	rectum
Gonads	blood smear	jejunum	salivary gland
Heart	bone	kidneys	seminal vesicle
Kidneys	bone marrow	lacrimal gland	skin
Liver	brain (3 levels)	liver	spinal cord (cervical
Spleen	cecum	lungs	thoracic, lumbar)
Thyroid	colon	lymph nodes	spleen
(w/parathyroid)	duodenum	mammary gland	sternum
	epididymes	muscle (smooth)	stomach
	eyes	muscle (skeletal)	testes
	eyes (optic nerve)	nerve (peripheral)	thymus
	gall bladder	oesophagus	thyroid
	Harderian glands	ovaries	(w/parathyroid)
	head - 3 sections	pancreas	trachea
	(nasal cavity, para-	pituitary	urinary bladder
	nasal sinus, tongue,		uterus
	oral cavity, naso-		vagina
	pharynx, inner-ear)		Zymbal's gland
			gross lesions

APPENDIX IV: 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 birth 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

$$\textbf{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

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$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of liveborn pups on the day of birth}} \times 100$$

* 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)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \times 100$$

$$\textbf{Preimplantation loss (\%)} = \frac{\text{number of corpora lutea – number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\textbf{Postimplantation loss (\%)} = \frac{\text{number of implantations – number of live foetuses}}{\text{number of implantation}} \times 100$$

APPENDIX V: Functional observation battery in neurotoxicity studies

I *Home cage observations*:

A. Posture
B. Piloerection
C. Gait abnormalities
D. Involuntary motor movements (Clonic and Tonic)
E. Vocalisations
F. Other

II *Observations during handling*

A. Ease of removal from its cage
B. Reaction to being handled
C. Muscle tone (if abnormal)
D. Palpebral closure (if abnormal)
E. Pupil size
F. Pupil response (test only if dilated).
G. Lacrimation
H. Salivation - indicate colour and score if present
I. Stains (if present, indicate colour and score)
J. Other

III *Open field observations*:

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A. Piloerection
B. Respiratory abnormalities
C. Posture
D. Involuntary motor movements (Clonic and Tonic)
E. Stereotypy (excessive or repetitive behaviour; if present)
F. Bizarre behaviour (if present)
G. Gait abnormalities
H. Vocalisation
I. Arousal (level of unprovoked activity and alertness; if abnormal)
J. Rearing
K. Other
L. Defecation - number of boluses, and Urination - number of pools

IV *Reflex/Physiologic observations and measurements*: Stimulus reactivity

A. Approach response
B. Touch response
C. Auditory response
D. Tail pinch response
E. Righting reflex
F. Grip strength
G. Landing foot splay
H. Body wt (g)
I. Body temperature

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