Australian Pesticides &
Veterinary Medicines Authority

The reconsideration of approvals of the active constituent azinphos-methyl, registrations of products containing azinphos-methyl and their approved labels

Preliminary Review Findings

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Toxicology

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FOREWORD

The Australian Pesticides & Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the Agvet Codes scheduled to the *Agricultural and Veterinary Chemicals Code Act 1994*.

The APVMA can reconsider the approval of an active constituent, the registration of a chemical product or the approval of a label for a container for a chemical product at any time. This is outlined in Part 2, Division 4 of the Agvet Codes.

The basis for the current reconsideration is whether the APVMA is satisfied that continued use of the active constituent azinphos-methyl and products containing azinphos-methyl in accordance with the instructions for their use:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues; and
- would not be likely to have an effect that is harmful to human beings; and
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment; and
- would not unduly prejudice trade or commerce between Australia and places outside Australia.

The APVMA also considered whether product labels carry adequate instructions and warning statements.

A reconsideration may be initiated when new research or evidence has raised concerns about the use or safety of a particular chemical, a product or its label.

The reconsideration process includes a call for information from a variety of sources, a review of that information and, following public consultation, a decision about the future use of the chemical or product.

In undertaking reconsiderations (hereafter referred to as reviews), the APVMA works in close cooperation with advisory agencies including the Office of Chemical Safety, the Department of the Environment and Heritage, and state departments of agriculture as well as other expert advisers as appropriate.

The APVMA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of review reports is a part of that process.

The APVMA also makes these reports available to the regulatory agencies of other countries as part of bilateral agreements. The APVMA recommends that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

This document sets out the preliminary review findings relating to the active constituent azinphos-methyl and products containing azinphos-methyl that have been nominated for review by the APVMA. The preliminary review findings and proposed recommendations are based on information collected from a variety of sources. The information and technical data

required by the APVMA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken.

The review summary (Volume 1) and the technical reports (Volume 2 & 3) for all registrations and approvals for azinphos-methyl are available from the APVMA web site: http://www.apvma.gov.au/chemrev/chemrev.html.

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ABBREVIATIONS

Ng	Nanogram	**nM**	Nanomolar
µg	Microgram	**mM**	Millimolar
Mg	Milligram	**s**	Second
Kg	Kilogram	**min**	Minute
ML	Millilitre	**h**	Hour
L	Litre	**m**	Metre
GI	Gastrointestinal	**sc**	Subcutaneous
Im	Intramuscular	**LH**	Luteinising hormone
Ip	Intraperitoneal	**mg/kg bw/d**	mg/kg bodyweight/day
Iv	Intravenous	**ppb**	Parts per billion
PO	Oral	**ppm**	Parts per million

ADI	Acceptable Daily Intake
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
ALT	Alanine aminotransferase (SGPT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatinine phosphatase
DDM	4,4'-Diaminodiphenylmethane
DMTP	dimethylthiophosphate
DMSO	Dimethylsulfoxide
EC	Emulsifiable concentrate
EUP	End Use Product
FL	Flowable liquid
GLP	Good Laboratory Practice
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LOEL	Lowest Observed Effect Level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MRL	Maximum Residue Limit
NOEL	No Observed Effect Level
NTE	Neurotoxic target esterase
Op	Organophosphorus pesticide
2-PAM	2-pralidoxime
P-2-S	2-pyridine-aldoxime methyl methanesulfonate
RBC	Red Blood Cells
SC	Spray concentrate
TGAC	Technical Grade Active Constituent
WP	Wettable powder

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

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of absorption	PO: almost complete absorption. Maximum plasma concentration 2-3 h after PO dosing. Majority of dermal absorption in the first h after application; 21-54% after 10 h in rats, 22-29% in humans
Distribution	Similar distribution following PO or iv administration; target organs include the kidneys, liver and lungs.
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	95% excreted in urine, bile and expired air within 48 h of PO or iv administration.
Metabolism	2 major urinary metabolites and 6 other products. 5 faecal metabolites (10-12% of administered dose).
Toxicologically significant compounds (animals, plants and environment)	Parent compound and benzazimide

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	4.4
Worst oral LD_{50} in other species	15 (ICR/SIM mice)
Rat dermal LD_{50} (mg/kg bw)	72.5
Worst dermal LD_{50} in other species	1380 (NZW rabbits)
Rat inhalation LC_{50} (mg/m^3)	132 (females)
Worst inhalation LC_{50} in other species	40 (CF mice; 1h exposure)
Skin irritation	Non irritant
Eye irritation	Irritant
Skin sensitization	Sensitiser
T-value	0.4

Degradation products

Benzazimide

Rat oral LD_{50} (mg/kg bw)	None reported
Rat dermal LD_{50} (mg/kg bw)	2000
Rat inhalation LC_{50} (mg/m^3)	1760

Short-term toxicity

Target/critical effect	Plasma ChE inhibition
Lowest relevant oral NOEL (mg/kg bw/d)	0.25 (28-d human study)
Lowest relevant dermal NOEL (mg/kg bw/d)	2.0 (3-wk rabbit study)
Lowest relevant inhalation NOEL (mg/m^3)	1.24 (12-wk rat study)

Genotoxicity	Non-genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect	Plasma ChE inhibition
Lowest relevant NOEL (mg/kg bw/d)	0.125 (52-wk and 2-y dietary dog studies)
Carcinogenicity	No evidence of oncogenic potential

Reproductive toxicity

Reproduction target/critical effect	Reduced pup viability and retardation of growth at maternally toxic doses
Developmental target/critical effect	Minor variations (skeletal development) at maternotoxic doses
Lowest relevant developmental NOEL (mg/kg bw/d)	1.2 (rats)

Delayed neurotoxicity	No evidence of delayed neurotoxicity
Immunotoxicity	No adequate evidence of immunotoxicity

Summary

	NOEL (mg/kg bw/d)	**Study**	**Safety factor**
Current ADI (0.001 mg/kg bw/d) [Plasma ChE inhibition]	0.125	2-y feeding dog	100
New ADI (0.025 mg/kg bw/d) [Plasma ChE inhibition]	0.25	28-d repeat-dose human	10
ARfD (acute reference dose)	0.075 mg/kg bw/d		

1 SUMMARY

1.1 INTRODUCTION

Azinphos-methyl is an organophosphorus insecticide and acaricide that has been widely used to control codling moth and light brown apple moth in pome fruit orchards. It is also used on a range of other food crops.

1.2 METABOLISM AND TOXICOKINETICS

1.2.1 Absorption

The gastrointestinal (GI) absorption of radiolabelled azinphos-methyl was judged to be rapid and almost complete following gavage administration to rats. This was based on the observation that the maximum plasma concentration occurred approximately 2-3 h after dosing, with the total radioactivity detected in the urine, bile and expired air similar irrespective of whether azinphos-methyl was administered intravenously or orally. Results from bile duct cannulation experiments indicated that at least a proportion of radioactivity excreted in the bile following iv administration was reabsorbed (enterohepatic circulation) and excreted in the urine (Patzschke *et al* 1976).

The absorption of gavage-administered radiolabelled benzazimide (a major metabolite of azinphos-methyl) in rats was also rapid as shown by the rapid appearance of total radioactivity in the whole body (minus the GI tract). In plasma, the C_{max} for radioactivity was achieved approximately 2 h after oral (PO) administration. At 3 h (the first sampling interval for measurement of whole-body radioactivity), approximately 82% of the administered dose was detected in the whole animal (minus the GI tract), whereas by 24 h this had decreased to approximately 1% (Weber *et al* 1980).

1.2.2 Percutaneous Absorption

Feldman and Maibach (1974), studied the urinary excretion of 12 dermally-administered ^{14}C-labeled pesticides and herbicides, including azinphos-methyl, in human subjects. Approximately 16% of the administered dose of azinphos-methyl was excreted in urine within 120 h. No urinary metabolites of azinphos-methyl were characterised or identified. However, this study had limited regulatory value as no radioactivity data for blood and faecal samples were provided, and the possible influence of the vehicle on absorption was unknown.

Percutaneous absorption and decontamination of a range of pesticides in humans, including azinphos-methyl, were reviewed by Wester and Maibach (1985), with particular attention directed toward the influence of factors such as the dose, application site, area of application, occlusion, skin condition and contact time, on absorption. Dermal absorption of azinphos-methyl by intact skin was 15.9%, with the applied dose, surface area of the skin, site of application and the time of exposure all influencing the rate of absorption. Occlusion of the site and application to abraded skin enhanced the rate of absorption by 3.5- and 3.8-fold respectively.

In a study by Schroeder (1992), ^{14}C-azinphos-methyl was mixed with unlabelled azinphos-methyl 35% WP and applied topically as a water-based suspension to groups of male rats. The

cumulative radioactivity excreted in urine and faeces after 168 h was 36%, 18% and 15% of the applied dose at 0.018, 0.185 and 2.08 mg/kg bw respectively. The majority of each dose was absorbed from the skin during the first hour after application, with approximately 21% and 54% absorption measured after 10 h at the high- and low-dose respectively.

In a study performed to determine the dermal absorption and excretion of azinphos-methyl in human volunteers (Selim, 1999), 6 males/group received ^{14}C-azinphos-methyl as a single dermal dose of 3 or 10 µg ai/cm^2, or as an aqueous suspension of a ^{14}C- Gusathion M WP 25 at 5 µg ai/cm^2. The dermal absorption of ^{14}C-azinphos-methyl at 3 and 10 µg ai/cm^2 was 29.3% and 23.5% of the administered dose respectively, while the mean absorption of ^{14}C-Gusathion M WP 25 formulation was 21.9%. There were no treatment-related effects on plasma and RBC ChE activities.

1.2.3 Distribution

Six hours after gavage administration of 2 mg/kg bw radiolabelled azinphos-methyl to rats, the only tissues containing more radioactivity (per gram) than plasma were the adrenals and those associated with excretion or metabolism, namely the kidneys and liver. The rate of decline in radioactivity in all tissues over 48 h mimicked that observed in plasma and was below the limit of detection for most tissues after 4 days, except in the blood, kidneys and adrenals. By day 8, radioactivity was only detectable in whole blood, plasma and erythrocytes. Autoradiography did not reveal any high levels of radioactivity in organs other than the liver and kidneys (Patzschke *et al* 1976).

In a study by Kao *et al* (1988), radiolabelled azinphos-methyl was administered by PO gavage to rats at 0.125 or 2.5 mg/kg bw. Apart from muscle, blood and fat, all other tissues had levels of radioactivity that were below the limit of detection after 72 h. However, when these results were expressed as a function of tissue weight, detectable concentrations of radioactivity that were at least twice the background level were found in the blood, kidneys and lungs after dosing at 0.125 mg/kg bw. At the higher dose of 2.5 mg/kg bw, the expected 20-fold increase in radioactivity was achieved in all tissues.

In lactating goats, approximately 17-18 h after the last of 3 consecutive daily doses (0.5 mg/kg bw/d), radioactivity levels detected in the harvested tissues were very low, with most detected in the liver followed by the kidneys, muscle and fat. However, total tissue radioactivity only accounted for a maximum of 0.25% of the administered dose (Murphy *et al* 1988).

Following gavage administration of radiolabelled benzazimide to rats, measurement of tissue radioactivity indicated that apart from the kidneys and liver, there did not appear to be any accumulation of radioactivity in tissues. The elimination half-life from tissues over 2-10 days was approximately 4 days, whereas from plasma (or blood), the elimination half-life over 24 h was estimated to be 2-3 h (Weber *et al* 1980).

1.2.4 Metabolism

Several published studies characterised the enzymes responsible for the metabolism of azinphos-methyl and some factors, which affect the rate of its conversion *in vitro* (Murphy & Dubois 1957; Dahm *et al* 1962; Hitchcock & Murphy 1971). Other published studies characterised metabolites formed *in vitro* or attempted to correlate metabolism *in vitro* with

organophosphate toxicity (Motoyama & Dauterman 1972; Levine & Murphy 1977; Lin *et al* 1980).

Incubation of radiolabelled azinphos-methyl with various subcellular fractions confirmed previous published observations that metabolism of azinphos-methyl in the liver was mediated by glutathione-S-transferase and cytochrome P-450 (Patzschke *et al* 1976).

Of the 10 identified urinary metabolites of intravenously-administered [carbonyl-^{14}C]-azinphos-methyl, none accounted for more than 30% of the total excreted radioactivity. Preliminary characterisation indicated that desmethyl-azinphos-methyl, dimethyl benzazimide sulfide and dimethyl benzazimide disulfide were the major metabolites. No unchanged azinphos-methyl was detected (Ecker 1976).

Gavage administration of [phenyl-UL-^{14}C]-azinphos-methyl to rats generated 2 major urinary metabolites, namely cysteinylmethylbenzazimide sulfone and methylsulfonylmethyl benzazimde. Both metabolites were present in approximately equal proportions and collectively accounted for an average of 57% urinary-excreted radioactivity. Other metabolites that were present at low urinary concentrations were cysteinylmethylbenzazimide, methylsulfinylmethylbenzazimide, benzazimide, glutathionyl-methylbenzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. Five faecal metabolites, accounting for 10-12% of the total administered dose (2.5 mg/kg bw) were characterised and included methylsulfonyl-methylbenzazimde, azinphos-methyl oxygen analogue, methylthio-methylbenzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. No unchanged azinphos-methyl was detected in urine or faeces (Kao *et al* 1988).

In a lactating cow, the maximum level of radioactivity detected in milk occurred at approximately 24 h, with less than 0.2% of this radioactivity present as unchanged azinphos-methyl or its oxon (Everett *et al* 1966).

In a study by Wargo and Waggoner (1978), very little unchanged azinphos-methyl or its oxon metabolite were detected in tissues or milk following dietary administration of azinhos-methyl to lactating cows at 11.5, 34.02 or 62.96 ppm. At all dietary levels, other uncharacterised metabolites were detected in the liver, kidneys, muscle and fat.

Metabolites of azinphos-methyl in goats milk partioned preferentially into an organic solvent (acetonitrile) so that approximately 2/3 of the radioactivity associated with the soluble fraction was present in this phase. No unchanged azinphos-methyl was found, however, the major metabolite found in this organic phase that accounted for nearly 40% of the total radioactivity was 3-[(methylsulfonyl) methyl]- 1, 2, 3-benzotriazin-4(3H)-one (methyl sulfonyl-methylbenzazimide). By contrast the radioactivity associated with the polar aqueous phase metabolites was distributed equally among four metabolites, namely S-methyl-S-[4-oxo-(3H)- 1, 2, 3-benzotriazin-3-yl) methyl] dithiophosphate (desmethylisoazinphos-methyl), O-methyl-S-[(4-oxo-3H- 1, 2, 3-benzotriazin-3-yl) methyl] thiophosphate (desmethyl azinphos-methyl oxygen analogue), 3-[(methylsulfinyl)methyl]-1, 2, 3-benzotriazin-4(3H)-1-(methylsulfinylmethylbenzazimide) and 3-[(methylsulfonyl) methyl]-1, 2, 3-benzotriazin-4-(3H)-1-(methylsulfonyl-methylbenzazimide). Judging by the partitioning of the radioactivity between the aqueous and organic phase, the metabolite profile in the liver, muscle and fat was similar to that found in milk except that appreciably more radioactivity was associated with the insoluble milk fraction (ie solids remaining after filtration). In milk, the insoluble fraction

accounted for approximately 10-15% of the total radioactivity whereas in tissues it ranged from 33% in fat to 87% in the liver. Release of much of the radioactivity in the insoluble fraction (up to 77%) was achieved by protease hydrolysis suggesting that azinphos-methyl is converted to a highly reactive mercaptomethylbenzazimide intermediate which in turn forms disulfide linkages with cystiene moieties in proteins. In the kidneys the extracted radioactivity distributed almost evenly between the organic and aqueous phase and this difference from the other tissues was mainly due to the relatively low concentration of the metabolite, 3-[(methylsulfonyl)-methyl]-1, 2, 3-benzotriazin-4-(3H)-1-methylsulfonylmethylbenzazimide. Unchanged azinphos-methyl was only detected in fat, accounting for less than 5% of the total radioactivity in that tissue (Gronberg *et al* 1988).

1.2.5 Excretion

Whether radiolabelled azinphos-methyl was administered by oral (PO) gavage or intravenously to rats, nearly all radioactivity was excreted in the urine, bile and expired air within 48 h. Less than 0.1% of the administered radioactivity (at both 0.1 and 2 mg/kg bw PO or iv) was excreted in air, with most being excreted in urine (62% and 68% at 0.1 and 2 mg/kg bw respectively after PO administration, or 63% and 68% at 0.1 and 2 mg/kg bw respectively after iv injection). The balance was found in bile/faeces (34% and 26% at 0.1 or 2 mg/kg bw respectively after PO administration or 29% and 26% at 0.1 and 2 mg/kg bw respectively after iv injection). These results suggest that the absorption of azinphos-methyl from the GI tract is almost complete (~100%). Radioactivity in bile at 24 h after dosing at 2 mg/kg bw accounted for 27% of the dose, whereas 54% of the administered dose was found in the urine, and about 6% in the faeces. It appears that at least a proportion of the radioactivity excreted in bile re-enters via enterohepatic circulation and is then excreted in the urine (Patzschke *et al* 1976).

In a review which considered the use of biological monitoring in the estimation of exposure during the application of pesticides (Franklin *et al* 1986), data showing the correlation between dermally- and intramuscularly-administered azinphos-methyl and urinary levels of dimethylthiophosphate (DMTP) in rats were presented with limited experimental details. Following dermal application of 100-800 μg of azinphos-methyl to the shaved intra-capular skin of male rats, a very strong positive correlation was found with the levels of DMTP excreted. ($r = 0.94$). The proportion of the amount of azinphos-methyl applied to the DMTP levels excreted was 10 to 1. When rats were intramuscularly dosed, about twice as much DMTP was excreted. The estimated dermal absorption of azinphos-methyl using urinary DMTP levels was approximately 50-60%.

Faecal and urinary excretion of radioactivity in male and female rats ranged from 93.8-96.5% following administration of ^{14}C-azinphos-methyl at 0.125 or 2.5 mg/kg bw, or following 14 days of conditioning at 0.125 mg/kg bw/d. For each of these groups, approximately 70% of the radioactivity was detected in the urine and 24% was found in the faeces. Generally, the majority of excretion (~95%) occurred within the first 48 h of administration (Kao *et al* 1988).

Following gavage administration of radiolabelled benzazimide to rats, the radioactivity excreted in the urine at 1, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42 and 48 h after dosing showed little difference in rate or quantity (54-58%) between the lowest (0.05 mg/kg bw) and highest (5 mg/kg bw) doses. Similarly, radioactivity excreted in faeces over the same time interval (0-48 h) ranged from 41-45%. Although only a single dose (1 mg/kg bw) of benzazimide was tested, females appeared to excrete somewhat more radioactivity in urine (66%) and slightly

less in faeces (33%) relative to males (ie 57% and 42% respectively). Intravenous administration of 1 mg/kg bw resulted in an almost equal ratio of radioactivity in urine (58%) and faeces (41%) in males. This result suggests almost all of the PO administered benzazimide is rapidly absorbed from the GI tract and excreted in urine and bile. This contention was confirmed with the observation that 55% and 38% of the radioactivity of a 1 mg/kg bw dose was recovered in the bile within 24 h following iv and intraduodenal administration respectively. However, the extent to which any bile-excreted radioactivity undergoes enterohepatic circulation was not investigated. Recovery of radioactivity from urine, faeces and expired air accounted for 99.34% of the 1 mg/kg bw gavage-administered dose (Weber *et al* 1980).

1.3 ACUTE TOXICITY

1.3.1 Acute toxicity

The acute toxicity of azinphos-methyl technical in mammals was high via all routes of administration. Consistent with the cholinergic effects observed with other organophosphates, signs of acute azinphos-methyl intoxication included diarrhoea, salivation, lacrimation and vomiting (muscarinic effects), muscular tremors and paralysis (nicotinic effects), and restlessness, ataxia and convulsions (CNS effects). The oral LD_{50} values in rats ranged from 4.4 to 26 mg/kg bw using a variety of vehicles, and was 15 mg/kg bw in mice. The oral LD_{50} in guinea pigs was 80 mg/kg bw, and > 10 mg/kg bw in Beagle dogs. By the ip route, the LD_{50} values for mice and rats ranged from 3.4 to 5.4 and 3.4 to 8.9 mg/kg bw, respectively.

Azinphos-methyl was highly toxic in rats via inhalation, with a LC_{50} value of 132 mg/m^3 in a head-only, 4-h exposure study. The acute dermal toxicity of azinphos-methyl was high in rats and moderate in rabbits, with LD_{50} values of 72.5 and 1380 mg/kg bw respectively. Azinphos-methyl technical was a slight eye irritant, but not a skin irritant in rabbits, and was a skin sensitiser in guinea pigs.

The acute toxicity of azinphos-methyl products was related to the percentage of active ingredient present in the formulation. Some azinphos-methyl products were found to cause ocular irritation and skin irritation in rabbits, and skin sensitisation in guinea pigs. However, not all of these products are currently registered in Australia.

1.3.2 Potentiation studies

No potentiation was observed following ip administration of azinphos-methyl and EPN (O-ethyl O-4-nitrophenyl phenylphosphonothioate) to an unspecified strain of female rats (Dubois 1956), however this study was of limited regulatory value due to the lack of adequate experimental detail. In contrast, evidence of potentiation was observed when azinphos-methyl and ethion were given to female SD rats (Dubois 1962). No potentiation was observed when azinphos-methyl and malathion technical were administered to rats (Kimmerle 1966). A study by Murphy *et al* (1976) showed that inhibition of carboxylesterases with DEF (defoliant S, S, S-tributylphosphorotrithioate) in male Holtzman rats potentiated the anti-ChE activity of azinphos-methyl by 11.2 and 10.2 fold following ip or PO administration respectively. A series of potentiation studies by Thyssen (1977a, b & c) in male Wistar rats revealed that azinphos-methyl administered by PO gavage in combination with methamindophos technical, propoxur, azinphos-ethyl, cytrolane or cyolane, had a slightly additive effect, while in combination with chlorpyrifos caused a super additive effect. In a study by Doull and Rehfus

(1957), a slight additive effect was apparent when a combination of azinphos-methyl and parathion, systox or malathion were administered to adult mongrel dogs in the diet, however, this study was of limited regulatory value due to the small group sizes and lack of experimental data. An *in vitro* study using the SH-SY5Y human neuroblastoma cell line (Marinovich *et al* 1996) showed that mixtures of azinphos-methyl and dimethoate or diazinon doubled the inhibitory effect of azinphos-methyl on protein synthesis.

1.3.3 Antidote studies

In mice, toxogonin (bis 4-hydroxyiminomethyl-pyridinium-1-methyl-ether chloride) administered intraperitoneally at 80 mg/kg bw doubled the LD_{50} of azinphos-methyl but had no effect on ChE inhibition (Sterri *et al* 1979). A study by Edery *et al* (1979) showed that Compound 30 [4-hydroxyiminomethyl-1(3-N, N-dimethylaminopropyl) pyridium chloride hydrochloride] or toxogonin, administered intraperitoneally at 300 and 90 mg/kg bw respectively with atropine sulphate (25 mg/kg bw), provided protection against sc poisoning with azinphos-methyl at 18.5, 27.75 or 46.25 mg/kg bw/d.

In adult SD rats, 2-PAM (pyridine-2-aldoxime), DAM (diacetyl monoxime) and TMB-4 (1, 1-trimethylene-bis(4-formyl-pyridinium bromide) dioxime) showed no antidotal action against acute ip azinphos-methyl poisoning (Dubois 1960). In contrast, a study by Sanderson (1961) reported that 2-PAM was effective against azinphos-methyl poisoning in rats, but in combination with atropine was less effective. However, a study by Lorke and Kimmerle (1969) reported that 2-PAM or toxogonin increased the LD_{50} of azinphos-methyl in rats by 50-200%, and this antidotal effect was enhanced with atropine. In another study (Crawford & Doull 1970), 2-PAM alone was ineffective against azinphos-methyl poisoning, while atropine and particularly scopalamine or propantheline yielded the greatest antidotal effect. A study in an unspecified rat strain (Kimmerle 1966) reported a 1.3-fold increase in the oral LD_{50} of azinphos-methyl following an ip injection of 50 mg/kg bw PAM (pralidoxime) or 50 mg/kg bw BH6 (Obidoxime chloride), and a 2-fold increase with 50 mg/kg bw atropine sulphate. In this same study, treatment with PAM and BH6 in combination with atropine sulphate increased the LD_{50} by 3.4- and 4-fold respectively. An ip injection of toxogonin or 4-hydroxyiminomethyl-1-(3-N,N-dimethylaminopropyl)pyridinium chloride hydrochloride (a synthetised oxime) each together with atropine, was reported to reduce posioning following a sc injection of azinphos-methyl (Edery *et al* 1970)

1.4 SHORT-TERM REPEAT-DOSE TOXICITY

In the study of Hecht (1955), azinphos-methyl of unspecified purity in water and emulsifier 233 was administered by PO gavage to a group of male rats at 2 mg/kg bw/d for 2 weeks (12 doses). No clinical signs were observed in any of the treated animals. The test animals gained about 14 g in body weight during the treatment period. The study, however, was considered inadequate for regulatory purposes due to lack of a control group, data limitations and the use of only one dose level.

In a 3-week study (Harper & Brown 1965), female weanling Wistar CD rats were fed azinphos-methyl of unspecified purity in the diet at 0 and 10 mg/kg bw/d for 3 weeks. A treatment-related deficit in group mean body weight of about 7% and a depression in food consumption of about 11% compared to the controls were seen in rats at 10 mg/kg bw/d at 3 weeks. At this observation time, plasma, RBC and brain ChE activities in treated rats were significantly depressed compared to the controls.

In a study by Loser (1966), male rats of unspecified strain and age were fed azinphos-methyl of unstated purity in the diet at 0, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg bw/d for 9 weeks. Average body weight at 2.5 and 5.0 mg/kg bw/d was depressed at termination by about 11% and 15%, respectively compared to the controls. The animals at 5.0 mg/kg bw/d did not gain any weight during the first 2 weeks, with their average body weight approximately 56% of the controls. A dose-related reduction in whole blood ChE activity was observed at all sampling times, exceeding 20% inhibition at 1.0, 2.5 and 5.0 mg/kg bw/d, compared to corresponding pre-treatment values. No treatment-related effects were seen at 0.5 mg/kg bw/d. The regulatory value of the findings, however, was limited due to the lack of experimental and observational details (eg clinical observations, haematology, clinical chemistry, pathology).

In a 28-day study (Eiben *et al* 1983), azinphos-methyl was fed to rats at 0, 5, 20 and 50 ppm in pulverized rat diet containing 1% peanut oil for 28 days. A dose-dependent inhibition of plasma and RBC ChE activity was seen at 20 ppm and above. Depressions in plasma ChE activity were seen only in mid-dose males and in both sexes at the high-dose at 4, 14 and 21 days. A statistically significant inhibition of RBC ChE activity was observed only in mid-dose females and in both sexes at the high-dose at 14 and 28 days. Brain ChE activity was inhibited in females at 50 ppm. The NOEL was 0.35 and 0.46 mg/kg bw/d for males and females, respectively.

In a dose range-finding study (Broadmeadow *et al* 1986), CD rats were treated with azinphos-methyl technical in corn oil by PO gavage at 0, 0.2, 0.8 and 3.2 mg/kg bw/d for 29 days. Significant depressions in plasma butyryl and acetyl ChE activities were seen in females at 3.2 mg/kg bw/d, in RBC ChE in males at 0.8 and 3.2 mg/kg bw/d, and in females at 3.2 mg/kg bw/d. Brain ChE activity in rats at 3.2 mg/kg bw/d was significantly reduced compared to the concurrent controls. Based on statistically significant RBC ChE inhibition in males at 0.8 mg/kg bw/d, the NOEL was 0.2 mg/kg bw/d. From this preliminary investigation, the dose levels of 0.2, 0.8 and 3.2 mg/kg bw/d were chosen for a 13-week study.

In the study of Anderson (1963), dairy cows were fed azinphos-methyl of unspecified purity in grain supplement at 0, 0.25 and 0.5 mg/kg bw/d for 28 days. Treatment-related and biologically significant reductions in average daily milk production and whole blood ChE inhibition were observed.

White *et al* (1968) administered azinphos-methyl of unstated purity to 12 crossbred heifers in gelatine capsules at 3.6 mg/kg bw/d for 6 days. Five animals died during treatment and paralysis was seen in 3 animals. Clinical signs such as diarrhoea, profuse salivation, muscle fasciculation and colic were seen in treated animals. Clinical signs in the surviving heifers persisted until day 5 post-treatment. Whole blood ChE activity was depressed by about 80% compared to the controls at 2 days. At 5 days, the ChE depression was about 60% compared to the controls. About 20% recovery of ChE activity was seen during the last 5 days of the 7-day recovery period. Necropsy examination of 3/5 animals that died revealed haemorrhagic lesions in the GI tract.

In a study by Crawford and Anderson (1973), Holstein bull calves were given azinphos-methyl in gelatin capsules orally at 0, 0.15 and 0.45 mg/kg bw/d for 30 days. No treatment-related effect on whole blood ChE activity, body weight gain or feed consumption were observed.

In the study of Giri *et al* (1974), crossbred, adult riding horses were fed azinphos-methyl in grain ration at 0, 0.1 and 0.3 for 30 days or at 0.5, 1, 1.5 and 2 mg/kg bw/d for 7 days. No treatment-related effects were seen at 0.5 mg/kg bw/d. Dose- and time-related decreases in plasma and RBC ChE activities were observed at 1 mg/kg bw/d and above. Except for RBC ChE activity in animals at 2 mg/kg bw/d, plasma and RBC ChE activities recovered to baseline values by the end of the 10-day recovery period.

In a dermal toxicity study (Dubois *et al* 1966), young SD rats were treated with azinphos-methyl SC at 50 (males) and 120 mg/kg bw/d (females), 5 days/week for 3 weeks. Test substance-related depression in average terminal body weight of about 23% was seen in males compared to the corresponding controls. Absolute liver, kidney, spleen, heart, lung, testis and thymus weights in males were reduced by about 20-30% compared to parallel controls. Brain ChE activity at termination was depressed by about 76% and 70% in males and females respectively. The regulatory value of the study was limited due to data limitations and the lack of clinical observations.

In a study by Fluke and Schilde (1980), technical grade azinphos-methyl in Cremophor EL and demineralised water was applied to the shaven, dorso-lateral intact or abraded skin of NZW rabbits at 0, 2 and 20 mg/kg bw/d day for 3 weeks. At 20 mg/kg bw/d, terminal body weights were depressed by about 70% and 36% in females for intact and abraded skin groups respectively compared to the corresponding controls. Plasma ChE depressions were noted in males at 20 mg/kg bw/d on application day 10, and in males with abraded skin at day 15. Depressions in plasma ChE activity of comparable magnitude were also seen at 20 mg/kg bw/d at both these sampling times in females with abraded skin sites. A similar effect on plasma ChE activity was seen in females at 2 mg/kg bw/d at day 15 in animals with abraded skin. RBC ChE activity in both sexes was inhibited at 20 mg/kg bw/d at both sampling times irrespective of whether the skin was intact or abraded. Brain ChE activity was unaffected by treatment. Because of reductions in terminal body weights in females and plasma and RBC ChE inhibition in males at 20 mg/kg bw/d, the NOEL for the animals with intact skin was 2 mg/kg bw/d. In animals with abraded skin, inhibition in plasma ChE was seen at 2 mg/kg bw/d and above.

In the study of Dubois and Murphy (1956), young, adult female SD rats were treated with azinphos-methyl of unstated purity in absolute alcohol and polyethylene glycol mixture ip at 0.5, 1, 2 and 3 mg/kg bw/d for 60 days. Mortality at 2 and 3 mg/kg bw/d were 80% and 100% respectively, and body weight was increased by about 25% and 21%, respectively. Depressions in brain, submaxillary gland and serum ChE activity were seen at both tested dose levels. The study was not adequate for regulatory purposes due to the lack of a control group, statistical analysis and clinical observations.

Dubois and Flynn (1969) exposed adult female rats of unspecified strain by inhalation to nominal azinphos-methyl concentrations of 10, 25 or 50 µg/L air as an aerosol in an unspecified volume of ethanol for 1 h/day for 5 or 10 days. Depressions in ChE activity of approximately 26%, 31% and 48% for serum, RBC and brain respectively were noted in all tissues at 50 µg/L after 10 days of exposure. However, the value of the study findings were limited due to the small group sizes, the lack of a vehicle control group, and the absence of haematological, clinical chemistry, gross necropsy and histopathological data. The duration of exposure was short (ie 1 h/day) compared to the exposure period of 6 h/day specified in OECD guidelines.

In an inhalational study conducted by Kimmerle (1976), Wistar rats were exposed in dynamic inhalation chambers to liquid aerosols of azinphos-methyl technical of unspecified purity in polyethylene glycol 400/ethanol at mean aerosol concentrations of 0, 0.195, 1.24 and 4.72 mg/m^3, 6 hours/day, 5 days/week for 12 weeks. At termination, the males treated with 4.72 mg/m^3 were about 20% lighter than the corresponding controls and this change was considered to be treatment-related. Plasma and RBC ChE activities were inhibited by about 30-40% at 4.72 mg/m^3, whereas the brain ChE activity was not significantly changed. Inhibition of plasma ChE activity was on occasion, about 20% at the highest dose compared with controls, but this effect was not considered to be biologically significant. The NOEL was 1.24 mg/m^3.

1.5 SUBCHRONIC TOXICITY

In a study by Doull & Rehfuss (1956), SD rats were fed diets containing azinphos-methyl at 0, 2, 5 and 20 ppm (estimated to be equivalent to 0, 0.1, 0.25 and 1.0 mg/kg bw/d) for 16 weeks (Doull & Rehfus 1956). At 1.0 mg/kg bw/d, brain ChE activity was inhibited by about 10%, and serum and RBC ChE activities by about 30% and 40% respectively. When treatment at 1 mg/kg bw/d was discontinued, inhibition of ChE activity persisted for less than 4 days in serum, for about 10 days in brain, and for more than 20 days in RBC. No appreciable changes in tissues were observed at necropsy or following histopathology, including central and peripheral nervous tissue. The NOEL was 5 ppm (0.25 g/kg bw/day), based on inhibition of RBC and serum ChE activities at 20 ppm (1 mg/kg bw/d).

In the study of Doull and Anido (1957a), male weanling SD rats were fed with diets containing azinphos-methyl at 0, 2.5 and 5 mg/kg/day for 16 weeks. The survival rates at 2.5 and 5 mg/kg bw/d at 8 weeks were about 89% and 78%, and at 16 weeks, about 55% and 44% respectively. At termination, the animals given 2.5 and 5.0 mg/kg bw/d were about 10% and 18% lighter respectively than the controls. Treatment-related inhibition of ChE activity in all tissues was seen at both 8 and 16 weeks. The study authors stated that at the end of the 3-week recovery period, the animals given 2.5 mg/kg bw/d showed only inhibition of RBC ChE activity, whereas the rats given 5 mg/kg bw/d exhibited about 25% and 52% inhibition in brain and RBC ChE activities respectively. A NOEL was not established (≤2.5 mg/kg bw/d) because ChE inhibition was observed at both dose levels. The regulatory value of the findings of this study were limited due to lack of statistical analyses, data limitations, and only male rats and two dose levels were used.

In a study by Broadmeadow (1987), azinphos-methyl was adminstered in corn oil by PO gavage to 10 CD rats/sex/group at 0, 0.2, 0.8 and 3.2 mg/kg bw/d for 13 weeks. The NOEL was 0.215 mg/kg bw/d based on a statistically significant inhibition of RBC ChE activity (males and females) and observations of salivation (males) at and above 0.86 mg/kg bw/d. At 3.44 mg/kg bw/d, inhibition of brain and plasma ChE activities (males and females), and the presence of a viscous yellow fluid (males) in the small intestine, were observed. There were no treatment-related mortalities or any treatment-related effect on body weight gain, food or water consumption, ophthalmoscopy, haematology, urinalysis or histopathology. The main deficiencies noted in the study were the absence of pre-treatment haematology, clinical chemistry or urinary parameter measurements, and that no historical control data was provided.

In a 12-week study, Doull and Anido (1957b) administered azinphos-methyl in the diet to adult mongrel dogs at 0.125, 0.25, 0.5 and 1.25 mg/kg bw/d. Serum ChE activity at 1.25

mg/kg bw/d was decreased gradually with time reaching about 20% inhibition at 10 weeks. It was further decreased during the remainder of the study period, showing about a 25% depression at termination. Inhibition of RBC ChE activity at 0.25, 0.5 and 1.25 mg/kg bw/d occurred in a dose- and time-related manner with the enzyme activity at 0.25 and 0.5 mg/kg bw/d reaching about 20% inhibition at 9 weeks. RBC ChE inhibition at 1.25 mg/kg bw/d, however, was more pronounced and was about 40% and 50% at 9 and 12 weeks, respectively. Although RBC ChE activity at 0.25 and 0.5 mg/kg bw/d showed a trend towards recovery from week 10, at 1.25 mg/kg bw/d RBC ChE activity remained depressed for the remaining study period. The NOEL was 0.25 mg/kg bw/d. The study was considered to have limited regulatory value due to lack of a control group, the small experimental group size, data limitations, and the large variation in body weights of experimental animals.

Beagle dogs were fed diets containing chemically pure azinphos-methyl technical at 0, 0.5, 1.25, 2.5, 5.0 and 10 mg/kg bw/d for 19 weeks. The general condition of the animals was impaired at 1.25 mg/kg bw/d and above, with cholinergic signs seen at and above 2.5 mg/kg bw/d. Doses of 1.25 mg/kg bw/d and above caused weight loss, and at 10 mg/kg bw/d dogs frequently refused to eat. At 10 mg/kg bw/d, the female animal died after 9 weeks. Dose-related and biologically significant inhibition of whole blood ChE activity was observed at all dose levels. The NOEL for this study was less than 0.5 mg/kg bw/d (Loser & Lorke 1967).

1.6 CHRONIC TOXICITY

A study conducted by the NCI (1978) evaluated the carcinogenic potential of azinphos-methyl in B6C3F1 mice. Groups of 50 mice/sex/group received Guthion® (azinphos-methyl) for 80 weeks in their diet at concentrations of 0, 31.2 and 62.5 ppm for males (estimated to be equivalent to 0, 4.7 and 9.4 mg/kg bw/d respectively) and 0, 62.5 and 125 ppm for females (estimated to be equivalent to 0, 9.4 and 18.75 mg/kg bw/d respectively). Animals were retained untreated but under observation for a further 12-13 weeks. A group of 10 males and 10 females served as matched controls. The pooled controls for statistical tests consisted of the matched controls combined with 130 male and 120 female untreated mice from similar bioassays of 11 other test chemicals. The study authors reported hyperactivity in all dosed females at week 49, and rough hair coats in low- and high-dose males from week 74 and 60 respectively (incidences unspecified). The mean body weight of the high-dose female group was up to 17% lower (no statistical analysis performed) than the control group throughout the treatment period, but was comparable to the controls after week 80.

There were no treatment-related effects on mortality, gross pathology or tumour incidences. The NOEL was 62.5 ppm (equivalent to 9.4 mg/kg bw/d) based on clinical signs and decreased body weight gain in females at 125 ppm (equivalent to 18.75 mg/kg bw/d). There was no evidence that azinphos-methyl up to 125 ppm (equivalent to 18.75 mg/kg bw/d) was carcinogenic. The usefulness of this study for regulatory purposes was limited by a number of aspects. The low sample size of the matched controls (n = 10) compared to the treatment groups (n = 50) was a considerable deficiency. Only 2 dose levels were investigated, with different doses given to each sex. Individual animals data were absent. The incidences of clinical signs were inadequately reported.

In an oncogenicity study in CD1 outbred strain mice (Hayes 1985), 50 mice/sex/group received azinphos-methyl in their diet for 104 weeks at nominal concentrations of 0, 5, 20 and 40 ppm (estimated to be equivalent to 0, 0.75, 3.0 and 6 mg/kg bw/d). The study was initially started with 80 ppm as the highest dose (estimated to be equivalent to 12 mg/kg bw/d) but this

was reduced to 40 ppm due to the high mortality. There were no treatment-related effects on clinical signs, mortality, body weight gain, food consumption, haematological parameters, organ weight, gross/microscopic abnormalities and tumour incidences up to 40 ppm. There was a clear treatment-related effect on the inhibition of brain, plasma and RBC ChE activities. At 5 ppm, a 22% inhibition of RBC ChE activity was observed in females at 2 years. At 20 ppm, inhibition of plasma ChE ranged from 17-31% in males and 22-53% in females, while at 40 ppm it ranged from 48-56% and 67-77% in males and females respectively. At 20 ppm, inhibition of RBC ChE ranged from 34-57% in males and 42-58% in females, while at 40 ppm it ranged from 50-81% and 46-77% in males and females respectively. At 20 ppm, the level of inhibition of brain ChE was 16% in males and 26% in females, while at 40 ppm it was 63 and 67% for males and females respectively. No statistical analysis was performed on the ChE data. The NOEL was 5 ppm (equivalent to 0.79 and 0.98 mg/kg bw/d in males and females respectively) based on inhibition of plasma, RBC, and brain ChE activities at 3.49 and 4.12 mg/kg bw/d for males and females respectively. There was no evidence that azinphos-methyl up to 40 ppm (estimated to be equivalent to 6 mg/kg bw/d) was tumorigenic in mice.

A 2-year chronic study (Harper *et al* 1966) was undertaken to assess the prolonged PO toxicity of Gusathion (azinphos-methyl) to Wistar rats. Thirty rats/sex/group received azinphos-methyl in their diet at nominal levels of 0, 2.5, 5, 20, and 50-100 ppm (estimated to be equivalent to 0, 0.125, 0.25, 1 and 2.5-5 mg/kg bw/d) for 2 years. Satellite groups consisting of 10 rats/sex were assigned to the same dose groups and used for laboratory tests (eg urinalysis). The dietary level of azinphos-methyl received by the high-dose group was increased from 50 to 100 ppm after week 47 due to the absence of any detectable toxicity. The low-dose group was commenced on its diet 5 months after all other treatment groups. There were no treatment-related effects on mortality. Convulsions were observed in 5/30 females at various times following the increase in dosing from 50 to 100 ppm. There was no treatment-related effect on food consumption, food conversion ratios, body weight, urinary and haematological parameters, gross pathology and histopathology. There was no evidence that azinphos-methyl was carcinogenic. There was a clear treatment-related effect on plasma, RBC and brain ChE activities. The high-dose group (50-100 ppm) had statistically lower plasma (13-49%) and RBC (25-60%) ChE activities throughout the study compared to the control.

The brain ChE activity of the high-dose group was inhibited by 19% in males, and by 49% in females. During the first 39 weeks, the 5 and 20 ppm groups had significantly lower plasma ChE activities than the control (up to 15 and 21 % respectively) after which time they generally recovered to control levels. At 20 ppm, males showed a transiently depressed (18%) RBC ChE activity at week 65 while females had a depressed RBC ChE until week 65 (24-26%) after which they recovered to control levels. The NOEL was 5 ppm (equivalent to 0.25 mg/kg bw/d) based on the inhibition of plasma and RBC ChE activities at 20 ppm (equivalent to 1 mg/kg bw/d). Deficiencies noted in this study included: the lowest dose group (2 ppm) commenced its diet 5 months after all other treatment groups; no indication of the variability of the data was provided (ie standard errors or deviations); it was unclear which control groups were utilised for statistical comparisons; urinalysis and haematology were not performed on all groups; the method for analysing ChE activity was changed at week 39; statistical analyses were inadequately described.

A published paper (Worden *et al* 1973) which summarised data from Harper *et al* (1966) reiterated the conclusion that the NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm (estimated to be equivalent to 0.25 mg/kg bw/d). A depression of

plasma and RBC ChE activity was observed at 20 ppm and above (estimated to be equivalent to 1 mg/kg bw/d) and clinical signs (convulsions) occurred at 100 ppm (estimated to be equivalent to 5 mg/kg bw/d).

A study conducted by the NCI (1978) evaluated the carcinogenic potential of azinphos-methyl in Osborne-Mendel rats. Groups of 50 rats/sex/group received Guthion® (azinphos-methyl) for 80 weeks in their diet at concentrations of 0, 125 and 250 ppm for males (estimated to be equivalent to 0, 6.25 and 12.5 mg/kg bw/d respectively) and 62.5 and 125 ppm for females (estimated to be equivalent to 0, 3.125 and 6.25 mg/kg bw/d respectively). The concentrations offered to male rats were reduced to 62.5 and 125 ppm from week 21, resulting in time-weighted average concentrations of 78 and 156 ppm (estimated to be equivalent to 3.9 and 7.8 mg/kg bw/d). Following 80 weeks of treatment, animals were retained untreated but under observation for a further 34-35 weeks. A group of 10 males and 10 females served as matched controls. Pooled controls for statistical tests consisted of the matched controls combined with 95 male and 95 female untreated rats from similar bioassays of 10 other test chemicals.

The mean body weights of low- and high-dose male and high-dose female rats were over 10% lower than that of the matched controls at most periods of time throughout the study. Body tremors were observed in 2 high-dose males and 2 high-dose females after 1 week. In females, a significant positive dose-related trend in mortality occurred ($p = 0.041$). Numerous tumours of the endocrine organs were observed in both dosed males and dosed females but when compared to the matched controls were not considered to be treatment-related. In male rats the results of a Cochran-Armitage test on the combined incidence of islet-cell adenomas or carcinomas of the pancreas was significant using either the pooled ($p = 0.008$) or matched ($p = 0.033$) controls [pooled controls (2%), matched controls (0%), low-dose (2%) and high-dose (13%)]. As the spontaneous incidence of these lesions in male Osborne-Mendel rats at the performing laboratory varied from 0 to 22% (mean = 2%) the incidence found in the high-dose males could not be clearly implicated as treatment-related. Similarly, although statistical analyses indicated a significant increase in the incidence of benign thyroid tumours (28% vs 8% in the control), malignant thyroid tumours (9% vs 0% in the control), or both (33% vs 8% in the control), in high-dose males, the spontaneous incidence of these neoplasms varied from 0-43% (mean = 7%) in the performing laboratory, and thus the incidence found in high-dose males could not be clearly implicated as a treatment-related effect.

The NOEL for dietary administration of azinphos-methyl to Osborne-Mendel rats over 80 weeks was < 78 ppm in males and 62.5 ppm in females (estimated to be equivalent to < 3.9 and 3.125 mg/kg bw/d respectively). Clinical signs, decreased body weight gain and mortality were observed at 7.8 mg/kg bw/d in males and 6.25 mg/kg bw/d in females. Under the conditions of the bioassay, neoplasms of the thyroid and pancreatic islets suggest but do not provide sufficient evidence for the carcinogenicity of azinphos-methyl in male Osborne-Mendel rats. The main weaknesses of this study were the absence of individual animal data, the use of only 2 dose levels and the relatively low sample size (n = 10) of the matched controls.

In a 2-year chronic toxicity study in Wistar rats (Schmidt 1987), 60 animals/sex/group were dosed with azinphos-methyl in their diet at 0, 5, 15 and 45 ppm (estimated to be equivalent to 0, 0.25, 0.75 and 2.25 mg/kg bw/d). High-dose females exhibited a higher (80%) incidence of alopecia than the controls (30%). Mortality was unaffected by treatment at all dose levels. Food consumption of high-dose females was approximately 10% higher than the controls. There was no treatment-related effect on water consumption. The mean body weight of high-

dose males was significantly lower than the control group throughout the study. There was no treatment-related effect on the incidence or location of palpable masses. There was no evidence of any treatment-related impairment or effect on clinical chemistry, haematology or urinary parameters with the exception of ChE activity. At 45 ppm, there was significant inhibition of ChE activity in plasma (38-66%) and RBC (23-31%) throughout the study, and in the brain (32-55%) at 12 and 24 months. At 15 ppm, RBC ChE activity was significantly decreased (12-22%) in both sexes at most intervals whereas ChE activity in plasma and brain was decreased significantly in females only (12-35 and 21% respectively). At 5 ppm plasma and RBC ChE activities were, on occasion, slightly reduced compared with the control.

Gross pathology and organ weight analysis revealed no treatment-related changes after 12 and 24 months. Histopathological examination of animals from the control and treated groups revealed predominantly inflammatory or degenerative changes after 12 and 24 months that were typical spontaneous lesions of conventionally housed rats of the age and strain employed. The nature, incidence and distribution of these non-neoplastic lesions did not suggest an effect of treatment. The nature, incidence and time of appearance of benign, malignant and multiple neoplastic changes in all test groups showed only a slight variation, and there was no shift in the normal spectrum of neoplasms in any of the treated groups. Thus, there was no indication of a carcinogenic effect.

The NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm, equivalent to 0.25 mg/kg bw/d in males and 0.31 mg/kg bw/d in females. A dose-related depression of plasma, RBC and brain ChE activities was observed at 15 ppm and above, and decreased body weight gain, increased food consumption and alopecia in females occurred at 45 ppm, with decreased body weights detected in males at 45 ppm. There was no evidence that azinphos-methyl was tumorigenic in rats up to 45 ppm (estimated to be equivalent to 2.25 mg/kg bw/d).

A 2-year feeding study in Cocker Spaniel dogs (Noel *et al* 1966) was undertaken to assess the chronic toxicity of Guthion (azinphos-methyl). Four dogs/sex/group were administered azinphos-methyl in their diets at 0, 5, 20-50 and 50-300 ppm (estimated to be equivalent to 0, 0.125, 0.5-1.25 and 1.25-7.5 mg/kg bw/d). The high-dose group received 50 ppm for 36 weeks, 100 ppm from weeks 37-57, 150 ppm from weeks 58-84 and 300 ppm for weeks 85-105. The mid-dose group received 20 ppm for the first 36 weeks and 50 ppm for the remainder of the study. Clinical signs were confined entirely to dogs given 300 ppm and included muscular tremor (3/4 males, 2/4 females), weakness in the neck (2/4 males, 0/4 females) and hind limbs (1/4 males, 1/4 females), and abnormal quietness (3/4 males, 1/4 females). These clinical signs appeared to be more frequent in males than in females. There was no statistically significant difference in body weight between treated and control dogs, however a transient loss of body weight was observed in both males and females when the dose given to the high-dose group was increased to 300 ppm at week 85. Additionally there was a transient loss of body weight in mid-dose males from weeks 37-57 which coincided with an increase in dosing from 20 to 50 ppm at week 36. Generally, treated dogs consumed more food than controls with the exception of high-dose males which showed reduced food consumption at weeks 85-104. There was no indication by the study authors whether food consumption by Gusathion-treated animals was statistically different to the control animals.

There was a treatment-related effect on plasma and RBC ChE activities. During the first 3 months, plasma and RBC ChE activities were higher across all treatment groups compared to the control groups. The study authors explanation for this apparent elevation was that control

levels fell from their pre-dose levels. From 3 months, plasma ChE activities were consistently depressed at 50-300 ppm (16-48%) with a greater effect observed toward the end of the study as the dose level was increased to 300 ppm. At 20-50 ppm, plasma ChE activity was comparable to the control until week 52 and then showed signs of depression for the remainder of the study. There was no effect of 5 ppm Gusathion on plasma ChE activity after 3 months. From 3 months, RBC ChE activity at and above 20 ppm was consistently lower than the control group with the effect substantially greater at 50-300 ppm (24-74%) than 20-50 ppm (25 –45%). There was no perturbation in RBC ChE activity at 5 ppm. Although an ANOVA indicated a statistical difference between groups, there was no further analysis to ascertain where these differences lay. There was no difference in any other clinical chemistry parameters between treated and control animals.

There was no treatment-related effect on any haematology or urinary parameters, organ weights, and occurrence of macroscopic abnormalities. Additionally, no tumours were observed. Histopathological examination indicated that splenic congestion was absent in controls but was present in high- (4/8) , mid- (8/8) and low-dose (8/8) groups. This evidence conflicts with the study authors statement that the incidence of extreme splenic congestion was 'similar throughout all groups'. Furthermore, the study authors concluded that this splenic congestion was the result of administration of large amounts of pentobarbitone to terminate the animals. In females, evidence of kidney inflammation was confined entirely to the treatment groups with 3/4, 1/4 and 2/4 high-, mid- and low-dose animals affected respectively. It was difficult to conclude that these histopathological abnormalities were treatment-related as the sample sizes were small and a clear dose-response effect was not evident.

The NOEL was 5 ppm (estimated to be equivalent to 0.125 mg/kg bw/d) based on depressed plasma and RBC ChE activities at 20-50 ppm (estimated to be equivalent to 0.5-1.25 mg/kg bw/d). Azinphos-methyl was non-carcinogenic as no effect on tumour incidence was observed up to 50-300 ppm (estimated to be equivalent to 1.25-7.5 mg/kg bw/d). The main deficiencies in this study were: the small group sizes (n = 4); the absence of standard deviation or errors from most data; statistical analyses were inadequately reported; two methods for determining ChE levels were utilised, with the method of Michel (1949) used until week 52 and then changed due to the insensitivity of this method to the method of Williams and Frawley (1957); the increased dose administered to the high and mid-dose group in the absence of any observable toxicological effects did not consider any delayed or cumulative effects of the test compound.

A published paper (Worden *et al* 1973), which summarised data from Noel *et al* (1966), reiterated the conclusion that the NOEL for dietary administration of azinphos-methyl to Cocker Spaniel Dogs over 2 years was 5 ppm (equivalent to 0.15-0.24 mg/kg bw/d in males and 0.16-0.26 mg/kg bw/d in females). A depression of plasma and erythrocyte ChE activity was observed at 50 ppm and above (estimated to be equivalent to > 1.25 mg/kg bw/d) and clinical signs were observed at 300 ppm (estimated to be equivalent to 7.5 mg/kg bw/d).

In a 52-week feeding study in pure-bred Beagle dogs (Allen *et al* 1990), 4 dogs/sex/group were given azinphos-methyl at dietary levels of 0, 5, 25 and 125 ppm (estimated to be equivalent to 0, 0.125, 0.625 and 3.125 mg/kg bw/d). There were no deaths throughout the study. There appeared to be an increase in the incidence of vomiting, vomiting mucus, diarrhoea, and faeces with mucus, in mid- and high-dose males although a clear dose-response trend was not evident as the high-dose group showed fewer clinical signs than the mid-dose

group. There was no clear treatment-related increase in clinical signs in females due to the high incidence of clinical signs displayed by the control group. The large increase in the incidence of diarrhoea at 125 ppm in females was attributed to a single dog with a chronic case of diarrhoea. There was no difference in food consumption, hearing performance and ophthalmoscopy parameters between treated and control animals. There were a variety of incidental anomalies in clinical chemistry parameters in treated dogs, however none of these were considered to be toxicologically relevant as they fell within the 95% confidence limits for age and sex-matched untreated Beagle dogs.

There was a clear treatment-related effect on RBC, plasma and/or brain ChE activity at 25 and/or 125 ppm. At 25 ppm, a significant transient 40% depression in RBC ChE activity was observed in males at week 13, while females showed consistently depressed RBC ChE activity (35-43%) from week 13. At 25 ppm, a 30-33% depression in plasma ChE activity occurred in females during the later half of the study though this effect was not statistically significant. At 125 ppm, male RBC and plasma ChE activities were significantly depressed (66-88 and 53-58% respectively) from week 4 and 13 respectively, while the RBC and plasma ChE activity of females was significantly depressed (86-92 and 52-57% respectively) from week 4. At 125 ppm, brain ChE activity was also significantly lower than the control (27% for males and 20% for females). Cytochrome P-450 levels were elevated in high-dose animals but only males were determined to be statistically higher than their controls.

There was no treatment-related effect on haematology or urinary parameters. Although there was no effect on absolute organ weights, there was evidence of a treatment-related effect on spleen:body weight and spleen:brain weight ratios in treated males The study authors concluded that the reduction in spleen weight in males could be attributed to a higher proportion of control males showing splenic congestion. Treated males also had statistically lower liver:brain weight ratios with high-dose males also having a statistically lower kidney:brain weight ratio compared to the control group. There was no treatment-related effect on organ weights, organ:body weight and organ:brain weight ratios in females. All pathology findings were within normal range of spontaneous background alterations, which may be encounted in this age and breed of dog. There was no evidence of any abnormal histopathological occurrences in azinphos-methyl-treated dogs.

The NOEL was 5 ppm (estimated to be equivalent to 0.125 mg/kg bw/d) based on a depression in RBC and plasma ChE activity at and above 25 ppm (estimated to be equivalent to 0.625 mg/kg bw/d). Azinphos-methyl was not tumorigenic in dogs fed diets containing up to 125 ppm (estimated to be equivalent to 3.125 mg/kg bw/d). The low sample size (n = 4) was a considerable deficiency of this study.

1.7 REPRODUCTIVE TOXICITY

In a 3-generation reproduction study (Root *et al* 1965), groups of 6 male and 24 female Carworth Farms CF_1 mice received Guthion (azinphos-methyl) in the diet at 0, 5, 10, 25 and 50 ppm (estimated to be equivalent to 0, 0.75, 1.5, 3.75 and 7.5 mg/kg bw/d). Parental mice were pre-treated for 30 days and then mated at a ratio of 1 male to 4 females for 10 days. Pregnant females were maintained on experimental diets until weaning with the offspring continued on the experimental diet until mating. This process was repeated to obtain 30-day old F_{3b} mice.

At 50 ppm there was a high incidence of mortality in F_0 females (15/24) compared to the control group (2/24) with the average litter size at weaning of this high-dose group (1.9) also substantially lower than the control (5.7). Due to this apparent toxicity, no further investigation was performed at a dietary level of 50 ppm. There was no treatment-related effect on fertility at 25 ppm in any generation of mice. At 25 ppm the relative lung weight of F_{3b} mice was lower than the control group, however it was unclear whether this result was significant as no statistical analysis was performed, and the next lowest dose appeared to show an increase in relative lung weight. There appeared to be a dose-response trend with regard to an increase in relative testis weight in F_{3b} males, however, the study authors indicated that the average testis weight of the control group was less than two thirds of that usually obtained with age-matched controls. At 5 ppm there was an incidental increase in the relative spleen weight of F_{3b} offspring but this was not considered to be treatment-related as there was no effect at the 2 higher dose levels. The study authors indicated that there was large variability in the organ weight data but no evidence was provided to support this statement. There were no gross pathological or histopathological abnormalities observed in F_{3b} offspring that could be attributed to the administration of azinphos-methyl.

The NOEL was 50 ppm (estimated to be equivalent to 7.5 mg/kg bw/d) for reproductive toxicity based on the absence of any effect on fertility at this dose. The NOEL was 25 ppm (estimated to be equivalent to 3.75 mg/kg bw/d) for parental and peri/post-natal toxicity based on mortality in both adults and offspring at 50 ppm (estimated to be equivalent to 7.5 mg/kg bw/d). This study had a number of deficiencies: the absence of statistical analyses; no indication of the variability of the data was provided (ie standard deviations or errors); mating behaviour, food consumption and pup sexes were unrecorded; body weight data was not provided; gross and microscopic examination was performed only on the F_{3b} offspring; ChE activity was not measured.

In a 2-generation reproduction study (Eiben & Janda 1987), groups of 12 male and 24 female Wistar rats received azinphos-methyl admixed in their diet at concentrations of 0, 5, 15 and 45 ppm (equivalent to 0, 0.33-0.42, 1.02-1.22 and 3.46-7.37 mg/kg bw/d in males and 0, 0.48-0.67, 1.48-2.02 and 4.84-10.27 mg/kg bw/d in females) throughout the entire test period (mating, gestation and raising of the pups). Both parental generations displayed signs of toxicity including unpreened hair coats, convulsion, tremors and alopecia at 45 ppm. A slight increase in mortalities were observed in F_0 females at 45 ppm. Reductions in body weight and/or body weight gain were confined to high-dose animals except for a reduced body weight in F_1 males at 15 ppm at week 13 only. No effect on gestational or fertility indices were observed.

In the first generation of offspring, a reduction in pup numbers was noted at 15 and 45 ppm, and an increase in the incidence of stillborn pups was seen at these doses in the first litter only. These findings were not statistically significant. Litter sizes were similar to controls on day 0 but were reduced by day 5 at 15 and 45 ppm, and reductions in viability and lactation indices were observed at these doses in one or both of the litters. Reduced birth weight (F_{1B} litter only) and reduced body weights during lactation (both litters) were seen at 45 ppm. In the second generation of offspring, no increase in the incidence of stillbirths was observed. Similar effects were seen to the first generation: there were decreases in the number of pups born at 15 and 45 ppm in the first litter, a decrease in mean litter size at days 0 and 5 (45 ppm), decreased viability index (45 ppm), decreased lactation index (15 and 45 ppm) and reductions in body weight during lactation (45 ppm). In both generations, the study authors stated that effects on lactation and/or viability indices were unrelated to treatment and were

within historical control values at doses below 45 ppm. No malformations were observed in either generation at any dose.

The reduction in viability and lactation indices at 45 ppm, and the reductions in pup body weight after parturition, were suggestive of a reduction in lactation or reduced maternal care at this dose, which also caused frank maternal toxicity. The effects at 15 ppm were not as marked but may be indicative of normal variability

The NOEL for parental toxicity was 15 ppm (equivalent to 1.02–1.22 mg/kg bw/d in males and 1.48-2.02 mg/kg bw/d in females) based on decreased body weight gain in both F_0 and F_{1B} parental animals at 45 ppm (equivalent to 3.46-7.37 and 4.84-10.27 mg/kg bw/d males and females respectively). The NOEL for peri/post-natal toxicity was also considered to be 15 ppm based on decreased pup body weight and viability at 45 ppm. The NOEL for reproductive toxicity was 45 ppm (equivalent to 3.46-7.37 and 4.84-10.27 mg/kg bw/d in males and females respectively), the highest dose tested, based on the absence of any reproductive effects at this dose.

The purpose of a summary report by Van Goethem (1987) was to correlate the findings in the previous 2-generation reproduction study (Eiben & Janda 1987) with ChE data from 28-day (Eiben *et al* 1983) and chronic toxicity studies (Schmidt 1987). It was probable that inhibition of plasma, RBC and brain ChE activities occurred in rats treated with 45 ppm azinphos-methyl during the previous 2-generation reproduction study. It was also probable that plasma ChE activity was inhibited in rats treated with 15 ppm azinphos-methyl in this same study.

In a one-generation study (Holzum 1990), groups of 18 male and 46 female Wistar rats received azinphos-methyl admixed in the diet at concentrations of 0, 5, 15 and 45 ppm (equivalent to 0, 0.43, 1.30 and 3.73 mg/kg bw/d in males and 0, 0.55, 1.54 and 4.87 mg/kg bw/d for females). After 14 weeks of treatment, females were mated with males on a 2:1 basis for a period of 16 days. Rearing of F_1 pups ended on day 28 post-partum. Additional groups of 10 treated males (5, 15 and 45 ppm) were paired with groups of 20 untreated females with rearing of F_1 pups ending on day 5 post-partum.

At 45 ppm, 5 F_0 females died during the pre-treatment period and 2 were killed in a moribund condition. None of the females that died during the study exhibited any abnormal clinical signs while the two that were sacrificed moribund had exhibited poor general condition, bloody noses, inertia and a stumbling gait. There were no deaths in F_0 males during the study. The study authors suggested that the observed effects in F_0 females were probably due to inhomogeneous distribution of azinphos-methyl in the diet. There was no treatment-related effect on body weight, food consumption or food efficiency in F_0 rats, though body weights and feed efficiency were marginally lower at 45 ppm during pre-treatment, and feed intake by females was significantly reduced during lactation at 45 ppm. Significant inhibition of plasma ChE activity was seen in males (15 and 45 ppm) and females (all doses), and RBC ChE activity was inhibited in both sexes at all doses at all sample intervals. Brain ChE activity was inhibited in males (19%), females (up to 69%) and pups (up to 46%) at 45 ppm, and in females at 15 ppm.

There were no treatment-related effects on fertility parameters (insemination, fertility and gestation indices, and gestation period), the number of pups at birth, the number of still born pups, the ratio of male:female pups, litter size, the lactation index and pup birth weight. The live birth index was statistically elevated across all treatment groups likely due to the high

number of stillborn pups in the control group. A dose-related decrease in the viability index of pups whose parents were both treated with azinphos-methyl was seen at 15 and 45 ppm. The viability index of pups whose male parent only was fed azinphos-methyl was the same or marginally higher than the control. The mean body weight of 5-day old pups whose parents were both treated with 45 ppm azinphos-methyl was statistically lower than the control. The mean body weight of most treated pups at day 14 and 28 was marginally lower than the control but no statistical significance was evident.

There were no treatment-related clinical signs observed in F_1 pups at birth or during the 4-wk rearing period. No treatment-related pathological changes were observed in routinely sacrificed male and female F_0 animals, and in pups, at doses up to and including 45 ppm. There were no treatment-related effects on the brain weight of F_0 rats, however, the brain weight of 45 ppm F_1 pups was lower than the control at day 5 post-partum but not at day 28. The study authors concluded that this was likely due to the relatively low body weight of this group.

The NOEL for parental toxicity was < 5 ppm (equivalent to < 0.43 mg/kg bw/d in males and < 0.55 mg/kg bw/d in females) based on inhibition of plasma ChE activity in females, and inhibition of RBC ChE activity in both males and females at this dose. The NOEL with respect to reproduction was 5 ppm (equivalent to 1.30 and 1.54 mg/kg bw/d in males and females respectively), based on a reduced viability index and retardation of growth in F_1 pups at 15 ppm when both F_0 parents were treated with azinphos-methyl. Treatment of F_0 males and subsequent mating with untreated females elicited no effects on reproduction parameters or on the progeny which suggested that the treatment of F_0 females with azinphos-methyl was responsible for the observed toxicological effects in F_1 pups. The slight effect on fertility (fertility index, number of delivered pups) observed in the previous 2-generation study (Eiben and Janda, 1987) at and above 15 ppm was not confirmed in this study. A number of deficiences were noted: although 3 extra groups were conscripted to determine whether any effect on fertility was attributable to the treatment of male or female animals, ChE measurements were not performed on these additional groups; rearing of pups whose parents were both treated ended at day 28, whereas rearing of pups whose father only was treated ended on day 5; relative organ weights (eg brain weight) were not calculated; besides brain, no other organ weights were recorded; only brain ChE activity was measured in F_1 pups.

1.8 DEVELOPMENTAL TOXICITY

In a study aimed at determining the effect of maternal toxicity on foetal development (Kavlock *et al* 1985), a single dose of azinphos-methyl was administered by intubation in corn oil to primiparous CD-1 mice on day 8 of gestation at dose levels of 0, 16 and 20 mg/kg bw/d. At day 18 of gestation, mice were sacrificed for examination of foetuses. A clear dose-response trend was evident with regard to maternal deaths. The percentage of viable litters was reduced at 20 mg/kg bw/d although no statistical significance was reported. There was no treatment-related effect on the incidence of whole litter resorptions, maternal body weight gain, the number of litters, prenatal mortality, the number of sternal and caudal ossifications, and the percentage of enlarged cerebral ventricles and renal pelvises. The mean foetal weight of the high-dose group was statistically lower than the control and a statistically significant increase in the incidence of supernumerary ribs (SNR) at both doses (approximately 23% at 16 mg/kg bw/d and 58% at 20 mg/kg bw/d compared to 17% in the control) was also recorded. The effect at 16 mg/kg bw/d fell within the historical control range of 3-25% and was not considered to be treatment-related. The effect at 20 mg/kg bw/d was considered to be

treatment-related and a consequence of maternal stress. There were no malformations detected at either dose.

These observations indicated that azinphos-methyl was maternally and foetally toxic at 20 and 16 mg/kg bw/d respectively. Maternal toxicity was evidenced by mortalities at both 16 and 20 mg/kg bw/d and thus the NOEL was < 16 mg/kg bw/d. The NOEL for foetal toxicity and developmental toxicity was 16 mg/kg bw/d due to a statistically significant decrease in foetal weight and an increased incidence of SNR at 20 mg/kg bw/d. The presence of SNR was a likely result of maternal stress due to the administration of a maternally toxic dose. This study had numerous deficiencies and deviations from OECD guideline 414 (adopted 12 May 1981): uneven sample sizes; the control group only contained 15 animals; animals were caged in groups of 5; 2 dose levels only were employed; the highest dose caused death in 50% of maternal animals; a single dose only was administered; no clinical observations were made on the maternal animals; food consumption was not measured; foetal sexes were unrecorded; statistical analyses were not performed on all data; housing conditions were not completely specified.

In a teratology study (Short *et al* 1978 & 1980), groups of 22 or 23 naturally inseminated CD®-1 mice received azinphos-methyl orally in a vehicle of corn oil at dose levels of 0, 1.25, 2.5 and 5.0 mg/kg bw/d from day 6 to day 15 of gestation. Animals were sacrificed on day 18 of gestation for examination of the foetuses. There were no treatment-related effects on maternal body weight or food consumption. Unspecified incidences of cholinergic signs and one death were observed at 5 mg/kg bw/d. There was no treatment-related effect on litter size, incidence of resorptions or foetal body weights. No external anomalies were observed and none of the individual soft-tissue anomalies increased in a dose-related fashion. The study authors ranked anomalies by severity and noted an increase in anomalies with a rank of 2 (anomalies with intermediate value in assessing teratogenic potential) or 3 (anomalies most valuable in assessing teratogenic potential) at 2.5 and 5 mg/kg bw/d. However, no dose-response was evident and the statistical significance of this result appeared to hinge on the incidence of haemorrhage in the pericardium which was only given intermediate value in assessing teratogenic potential. These two factors plus the high level of variability in the data made it difficult to conclude that this result was treatment-related. Additionally, skeletal anomalies with a rank of 2 and the incidence of malaligned sternebrae followed a dose-response trend and were significantly increased at 5 mg/kg bw/d. However, there was no pattern of characteristic defects. The NOEL for maternal toxicity was 2.5 mg/kg bw/d based on clinical signs of ChE inhibition at 5.0 mg/kg bw/d. In the absence of empirical data for these clinical signs or any other treatment-related effects, a definite NOEL for maternal toxicity could not be set for this study. The NOEL for developmental toxicity was 2.5 mg/kg bw/d, based on an increased incidence of malaligned sternebrae at 5 mg/kg bw/d. The main deficiencies of this study were that maternal animals did not appear to be macroscopically examined, incidences of clinical signs were inadequately recorded, individual animal data were not provided and no ChE measurements were taken.

In a combined teratology-peri/postnatal toxicity study (Short *et al* 1978 and 1980), groups of naturally inseminated CD rats received azinphos-methyl orally in a vehicle of corn oil at 0, 1.25, 2.5 and 5.0 mg/kg bw/d. In the teratology study, groups of 21 naturally inseminated rats were treated from day 6 to day 15 of gestation and sacrificed on day 20 of gestation for examination of the foetuses. In the peri-/postnatal toxicity study groups of 14 or 15 naturally inseminated rats were treated from day 6 of gestation until the pups were weaned, 21 days

after birth. After weaning, surviving pups of the control, intermediate and high dose group were sacrificed at 30-40 days of age.

In the teratology study, significantly reduced weight gain and food consumption during the treatment period, unspecified incidences of clinical signs (salivation, urination, lacrimation and tremors) and one death were observed at 5 mg/kg bw/d. The effects of azinphos-methyl on maternal food consumption and body weight gain were reversible and returned to control levels when treatment ended. There was an incidental increase of slight lateral and fourth ventricle hydrocephalus at 1.25 mg/kg bw/d, but in the absence of any effect at the next two doses this was not considered to be treatment-related. There was no treatment-related effect on reproduction parameters and no evidence of embryotoxicity, fetotoxicity or teratogenicity. The NOEL for PO administration of azinphos-methyl to pregnant rats from day 6 to day 15 of gestation was 2.5 mg/kg bw/d with respect to maternal toxicity, based on reduced weight gain and feed consumption and clinical signs of ChE inhibition at 5.0 mg/kg bw/d. No effects on development were seen at 5 mg/kg bw/d, the highest dose tested.

In the peri/post-natal toxicity study, treatment of the dams up to the end of the lactation period resulted in reduced weight gain and food consumption at 5 mg/kg bw/d. There was no treatment-related effect on the fertility index, the duration of pup survival and the number of total implants and pups delivered per dam. There was a reduced gestation index in the high dose group although this was not statistically significant. Pup weight and pup survival were significantly reduced at 5 mg/kg bw/d. There was a reduction in the number of viable litters from birth to day 21 in the high-dose group most of which were lost between days 0 and 7. At 5 mg/kg bw/d, pup weights were lower than the control group. One day after weaning, pups in the single surviving litter of the high-dose group were observed to maintain their rear legs at right angles to the body and to have muscular incoordination in the use of these legs, muscular tremors in the tail, and upturned snouts. In this litter of 5 pups, these effects were noticeable in 2 pups and of questionable incidence in 2 pups. However, similar signs were also observed in one pup from the control group, which complicated attempts to correlate these observations with azinphos-methyl treatment. The NOEL in pregnant rats treated from gestational day 6 to postpartum day 21 was 2.5 mg/kg bw/d with respect to maternal toxicity, based on mortality, clinical signs of ChE inhibition and reduced weight gain and feed consumption at 5.0 mg/kg bw/d. The NOEL for perinatal toxicity was 2.5 mg/kg bw/d, based on a reduced gestation index and reduced survival and weight gain of pups at 5 mg/kg bw/d. The main deficiencies in this study were that maternal animals did not appear to be macroscopically examined, incidences of clinical signs were inadequately recorded, no individual animal data were provided and no ChE measurements were taken.

In a rat teratology study (Kowalski *et al* 1987) groups of 33 naturally inseminated Crl:CD BR rats received azinphos-methyl orally, by daily intubation in a 6% aqueous Emulphor emulsion, from day 6-15 of gestation at 0, 0.5, 1.0 and 2.0 mg/kg bw/d. Five dams/group were sacrificed on gestation day 16 and the remaining dams on day 20. Appearance, behaviour, mortality, food consumption, and body weight gain of the dams were not adversely affected by treatment at any of the dose levels employed. There were no gross pathological abnormalities in dams that could be attributed to the administration of azinphos-methyl. Inhibition of plasma ChE (37%) occurred in the high-dose group at day 16 gestation. Both RBC and brain ChE activities were inhibited (79 and 39% respectively) at this same dose. Practically complete restitution of plasma ChE activity was found at day 20 while recovery of RBC and brain ChE activities was incomplete. Foetal brain ChE activity was not inhibited by treatment of the dams. There was no effect of treatment on reproduction parameters and no

evidence of embryotoxicity, foetotoxicity or teratogenicity. A slight increase in resorptions occurred but fell within the range for historical controls. An incidental decrease in the combined median weight of viable foetuses occurred at 0.5 mg/kg bw/d but was not seen at higher doses. The foetuses from maternal rats that were sacrificed at day 20 of gestation showed no treatment-related effects on mortality, foetal/placental weights, sex ratios or incidences of external and visceral anomalies. There were no treatment-related malformations, changes in the frequency of common variations, or delayed ossification of the foetal skeleton. There was a statistically higher incidence of incomplete ossification of certain vertebrae and sternebrae at 0.5 mg/kg bw/d compared to the control but not at higher doses. There was incomplete ossification in the 4th sternebrae in treated foetuses but in the absence of a clear dose-response effect and relevant historical data the toxicological significance of this result was unclear. The NOEL for maternal toxicity was 1.0 mg/kg bw/d based on the inhibition of RBC and brain ChE activity at 2.0 mg/kg bw/d. The NOEL for developmental toxicity was 2.0 mg/kg bw/d, the highest dose tested.

In a developmental study in Sprague-Dawley-derived CD rats (Rubin & Nyska 1988), pregnant females were given azinphos-methyl (92.7% purity) in a maize oil vehicle by PO gavage at 0, 0.4, 1.2, and 3.6 mg/kg bw/d from day 6 to day 15 post-coitum inclusive, with 22 animals per dose group. No deaths or treatment-related clinical signs were noted during the study. Food consumption during gestation was similar in control and treated females, and bodyweight and bodyweight gain were unaffected by treatment. Mean placental weights and foetal lengths and body weights were similar in control and treated groups. There were no treatment-related changes in the gravid uterine weights, the number or sex of live foetuses, or the number of resorptions. There was a slight increase in the number of litters with one or more resorption, but this effect was not statistically significant, and was not considered to be treatment-related. Free-hand sectioning of the foetuses did not reveal any findings that could be attributed to treatment. Skeletal observations revealed some retardation of ossification in foetuses at 3.6 mg/kg bw/d, and this reduced or absent ossification was seen in the supraoccipital, pubic and hyoid bones. An increased incidence of SNR (14th lumbar) was also observed at 3.6 mg/kg bw/d. These effects are consistent with treatment-related delayed development, and occurred in the absence of any notable signs of maternal toxicity. The NOEL for maternal toxicity was 3.6 mg/kg bw/d, with no effects seen at any dose in this study. The NOEL for developmental toxicity was 1.2 mg/kg bw/d, based on increases in the incidence of supernumerary ribs (14th, lumbar) and delayed ossification (pubic, hyoid, and supraoccipital bones) in foetuses at 3.6 mg/kg bw/d. No structural malformations were observed in foetuses at any dose. ChE activity was not measured in this study.

In a study designed to assess the embryotoxic and teratogenic effects of azinphos-methyl in rabbits (Machemer 1975), groups of 11 or 12 naturally inseminated Himalayan rabbits received azinphos-methyl orally, by intubation in a 0.5% aqueous Cremophor emulsion, from day 6-18 of gestation at 0, 0.3, 1.0 or 3.0 mg/kg bw/d. Caesarean section was performed on day 29 of gestation. Azinphos-methyl had no adverse effect on the appearance, behaviour, mortalities and body weight gain of maternal rabbits at any dose. At 1 mg/kg bw/d, 2 does resorbed all foetuses and at 3 mg/kg bw/d one doe also resorbed the foetuses and another doe aborted, but the the observed frequency was within the normal range for the rabbit strain used in this study. There were no treatment-related effects on the number of foetuses, number of resorptions, foetal weight, foetal sex ratios, placental weight, number of stunted foetuses, and number of foetuses with slight alterations in bone development or with malformations. The study authors indicated that the statistically higher mean foetus weight observed at 3 mg/kg bw/d (2.75 g higher than the control) was a random effect. No malformations were revealed

upon external examination, examination of the skeleton or dissection of the head. The NOEL for PO administration of azinphos-methyl to pregnant rabbits was 3.0 mg/kg bw/d. The tested dose levels did not induce maternal toxicity and had no detectable effects on embryonic nor foetal development, and thus the usefullness of this study for regulatory purposes was limited. A number of other deficiences were noted: a thorough macroscopic examination of maternal animals was not performed; maternal food consumption and the number of corpora lutea were unrecorded; ChE activity was not measured; the concentration, stability and homogeneity of azinphos-methyl in the vehicle was not determined.

In a study by Clemens *et al* (1987), groups of 20 artificially inseminated American Dutch rabbits received azinphos-methyl orally, by daily gavage in a 7% aqueous Emulphor emulsion, from day 6-18 of gestation at 0, 1, 2.5 and 6 mg/kg bw/d. Caesarean section was performed on day 28 of gestation. No animals were found dead during the study but several animals were sacrificed apparently due to broken backs. At 6 mg/kg bw/d, ataxia was noted in 4 does and tremors in 2 of these same animals. A single control doe had soft, little or no stools and a red-coloured discharge prior to aborting 10 progeny on day 27. Two mid-dose does aborted progeny on day 15 or day 27 and both exhibited pulmonary changes indicative of dosing trauma or a respiratory tract infection. Body weight and food consumption were unaffected by treatment. There were no gross pathological abnormalities observed in does at necropsy that could be attributed to the administration of azinphos-methyl. At 6 mg/kg bw/d, both plasma and RBC ChE activities in does were statistically (22 and 50% respectively) lower than the control group at day 19. The effect at 2.5 mg/kg bw/d was less definitive, with a statistically significant 13% depression in plasma ChE activity, and a 20% (non-statistical) depression in RBC ChE activity observed. ChE activities returned to normal near term at day 28 of pregnancy, with plasma and RBC ChE activities similar to controls at this interval. Brain ChE activity was statistically depressed at 6 mg/kg bw/d (12%). Administration of azinphos-methyl produced no adverse effect on any maternal reproductive or foetal parameter. The median litter size of the high-dose group was statistically lower than the control group but fell within the performing laboratory's historical control range. There was an increase in pre-implantation loss for all 3 treatment groups, when compared with the control, however the median values for the low- and mid-dose group fell within the laboratory's historical control range and pre-implantation losses would have occurred before treatment began. There were no external and visceral foetal abnormalities at termination on day 28 that could be attributed to the administration of azinphos-methyl. Additionally there were no treatment-related effects on the development of the foetal skeleton or on the incidence of malformations or variations. The NOEL for PO administration of azinphos-methyl to pregnant rabbits was 1.0 mg/kg bw/d with respect to maternal toxicity, based on inhibition of RBC and plasma ChE activity at 2.5 mg/kg bw/d and above and clinical signs (ataxia, tremor) at 6 mg/kg bw/d. The NOEL for developmental toxicity was 6 mg/kg bw/d, the highest dose tested.

In a study by Gal *et al* (1988), sexually-mature female New Zealand White rabbits were paired one-to-one with males and then given azinphos-methyl (92.7% purity) by PO gavage in a maize oil vehicle at 0, 1.5, 4.75, and 15.0 mg/kg bw/d from day 7 to day 19 post-coitum inclusive. Group mean bodyweights and food consumption were similar in all groups during the study. No consistent, treatment-related clinical signs were observed. Deaths occurred in all groups including controls and these were attributed to intercurrent disease or lung dosing. After 11 days of dosing, RBC ChE activity was statistically significantly decreased by 27% at 15 mg/kg bw/d compared with controls. Statistically significant decreases in plasma ChE activity compared with controls were seen at all dose levels after 11 days of treatment, but

there was no consistent dose-response relationship for this effect and so the toxicological significance of this finding is not clear. No statistically-significant, treatment-related effects were observed on gravid uterus weight, number of live foetuses, resorptions, post-implantation loss, foetal weights, placental weights or crown-rump lengths. A statistically-significant increase in the incidence of small foetuses was reported at 15 mg/kg bw/d, possibly indicative of delayed development resulting from treatment. No treatment-related major foetal malformations were observed. Minor structural variations occurred sporadically among treatment and control groups and in the absence of any dose-related increase in visceral malformations, these findings were considered to be incidental to treatment. Skeletal examination revealed increases in the incidence of reduced ossification of long bone epiphyses in foetuses at 4.75 and 15 mg/kg bw/d, and this finding may be indicative of delayed foetal development resulting from treatment with azinphos-methyl. There was also an increased incidence of high-dose foetuses with asymmetric pelvic articulation (ilium articulating with first or first and second sacral vertebra unilaterally), and while the incidence of this finding (8.2%) was not statistically significantly different to controls, it was outside of the range of historical control mean values (0.9-7.6%). An increased incidence of unossified pubic bones and reduced or incompletely ossified hyoid bones may also have been an indication of delayed foetal development at the high dose. The NOEL for developmental effects was 1.5 mg/kg bw/d, based on retarded ossification of the long bones at 4.75 and 15 mg/kg bw/d, and other skeletal variations (asymmetric pelvic articulation) and reduced foetal size at 15 mg/kg bw/d. No frank maternal toxicity was observed at any dose (NOEL 15 mg/kg bw/d). Statistically-significant decreases in plasma ChE activity were seen in dams at all dose levels, but the lack of a dose-response relationship and the magnitude of this effect (22-29% reduced compared with controls) makes the toxicological significance of this finding equivocal. The NOEL for inhibition of RBC ChE activity was 4.75 mg/kg bw/d, based on a 27% reduction in activity at 15 mg/kg bw/d after 11 days of treatment. The lack of any demonstrated signs of maternal toxicity (reductions in body weights, clinical signs) was a deficiency of this study.

1.9 GENOTOXICITY

Azinphos-methyl displayed some genotoxic potential *in vitro* but no evidence of genotoxicity *in vivo*. *In vitro* effects were confined to the induction of forward mutations and clastogenetic effects in mammalian cells, and recombination in yeast. A proportion of the evaluated studies, specifically the published studies, lacked sufficient methodological and observational data necessary for Australian regulatory purposes.

Azinphos-methyl showed no genotoxic potential in reverse mutation assays in *S. typhimurium* (Evenchik 1987; Herbold 1978 & 1988; Hrelia *et al* 1990; Lawlor 1987; Sandhu *et al* 1985; Simmon 1978; Waters *et al* 1982), *E. coli* (Sandhu *et al* 1985; Simmon 1978; Waters *et al* 1982) *and S. cerevisiae* (Hoorn 1983; Sandhu *et al* 1985; Waters *et al* 1982). In contrast, some genotoxic activity was determined in several forward mutation assays. A mutagenic effect was detected in *S. pombe* up to a concentration of 95 mM both in the presence and absence of metabolic activation, however, an approximately 2-fold more mutagenic effect occurred in the absence of metabolic activation (Gilot-Delhalle *et al* 1983). Two independent studies reported a positive response in mouse lymphoma L5178 cells (Sandhu *et al* 1985; Waters *et al* 1982) but neither were considered to contain adequate methodological or observational detail.

A variety of DNA damage and repair assays indicated that azinphos-methyl had some genotoxic potential. An *in vitro* ^{32}P-postlabelling assay (Shah *et al* 1997) showed that 1 mM azinphos-methyl damaged calf thymus DNA, with a total of 69.7 adducts/10^9 nucleotides detected in treated DNA as opposed to 5.6 adducts/10^9 nucleotides in control DNA. Differential toxicity assays in *E. coli* strains p3478 and W3110 (Herbold 1984; Simmon 1976) and *B. subtilus* strains H17 and M45 (Simmon 1978) revealed no mutagenic activity. A range of *in vitro* unscheduled DNA synthesis (UDS) assays (Hrelia *et al* 1990; Myhr 1983; Sandhu *et al* 1985; Waters *et al* 1982) were negative while a single study (Simmon 1978) reported a positive response at 10^{-4} and 10^{-5} M in WI-38 human lung fibroblasts in the presence of metabolic activation only. Recombination assays in *S. typhimurium* strains SL4700, SL4525, TA1978 and TA1538 (Sandhu *et al* 1985; Waters *et al* 1982) were negative while three independent experiments in *S. cerevisiae* strain D3 were positive both in the absence and presence of metabolic activation [Sandhu *et al* 1985 (up to 50 mg/mL); Simmon 1978 (up to 5%); Waters *et al* 1982 (unspecified)]. Two of these later studies (Sandhu *et al* 1985 and Waters *et al* 1982) did not contain adequate methodological or observational detail for regulatory purposes. Reversion, gene conversion and crossing over assays using *S. cerevisiae* strain D7 generally indicated a negative response (Hrelia *et al* 1990; Sandhu *et al* 1985; Waters *et al* 1982), with the exception being an experiment performed by Bianchi *et al* (1984) where a positive response was observed up to 25000 μg/mL only in the absence of metabolic activation.

In vitro chromosomal effect assays produced mixed findings. Azinphos-methyl did not cause sister chromatid exchange in chinese hamster lung (Chen *et al* 1982a and b) and ovary cells (Sandhu *et al* 1985; Waters *et al* 1982), or in human lymphocytes (Gomez-Arroyo *et al* 1987; Hrelia *et al* 1990). In the absence of metabolic activation, clastogenic effects were seen in CHO cells up to 120 μg/mL (Alam *et al* 1974), and WI-38 and HEp-2 cells up to 160 μg/mL (Alam & Kasatiya 1975 and 1976). In the presence of metabolic activation, a positive effect was seen up to 500 μg/mL in human lymphocytes (Herbold 1986). An *in vitro* micronucleus test in cytokinesis-blocked human lymphocytes (Bianchi-Santamaria *et al* 1997) did not reveal any biologically-significant effect.

Azinphos-methyl exhibited no genotoxic potential during *in vivo* chromosomal effect assays including mouse micronucleus tests (Herbold 1979a and 1995; Sandhu *et al* 1985; Waters *et al* 1982), mammalian bone marrow cytogenetic tests (Henderson *et al* 1988; Hrelia *et al* 1990), dominant lethal tests in mice (Arnold 1971; Herbold 1976b, Sandhu *et al* 1985; Simmon 1978; Waters *et al* 1982) and recessive lethal tests in *Drosophila melanogaster* (Sandhu *et al* 1985; Waters *et al* 1982).

1.10 SPECIAL STUDIES

1.10.1 Neurotoxicity

In the study of Sheets (1994), azinphos-methyl technical in 0.5% (w/v) methylcellulose and 0.4% (w/v) Tween 80 in deionised water was administered by PO gavage to overnight fasted Fischer rats at 0, 2, 6 and 12 mg/kg bw for males, and at 0, 1, 3 and 6 mg/kg bw for females. Twelve rats/sex/dose group were used for neurobehavioural testing, and 6 rats/sex/dose were used in satellite groups for ChE determinations. Single dose administration of the test substance resulted in mortalities in high-dose animals only (5 males: 1 in the main study and 4 in the satellite group, and 15 females: 9 in the main study and 6 in the satellite group). Treatment-related clinical signs included muscle fasciculations, tremors, incoordinated gait,

and oral (males), urinary and nasal staining (both sexes). Significant FOB findings were observed on day 0. No histopathological abnormalities attributable to treatment were observed. Significant inhibition of plasma and RBC ChE activities was observed about 90 minutes after dosing at and above 2 mg/kg bw in males, and at 3 mg/kg bw in females. Brain ChE activity was significantly lower in males at and above 6.0 mg/kg bw, and in females at 3 mg/kg bw.

In a subchronic neurotoxicity study (Sheets 1995), 18 Fischer 344 rats/sex/group were dosed with azinphos-methyl technical in the diet for 13 weeks at 0, 15, 45, or 120 ppm for males (0, 0.91, 2.81, and 7.87 mg/kg bw/d mean intake) and 0, 15, 45, or 90 ppm for females (0, 1.05, 3.23, and 6.99 mg/kg bw/d mean intake). Decreased forelimb grip strength, motor activity and locomotor activity were observed in both sexes at the high-dose, but did not correlate with any pathology of the nervous system. The NOAEL for neurotoxicity, including cholinergic signs, was 45 ppm for both sexes (2.81/3.23 mg/kg bw/d). A statistically significant inhibition of RBC ChE activity (37-98%) was observed at all dose levels, as was a statistically significant inhibition of plasma (14-83%) and brain (8-85%) ChE activities at the mid- and high-doses. The statistically significant inhibition of brain ChE activity in both sexes at the lowest dose level tested was considered to be a marginal adverse effect. The NOAEL based on ChE inhibition was < 15 ppm (< 1 mg/kg bw/d).

In a number of studies conducted in hens (Kimmerle 1959, 1964, 1965; Grundman 1965; Taylor 1965; Roberts *et al* 1988), azinphos-methyl was administered in the diet or by PO gavage at doses up to 500 or 225 mg/kg bw/d respectively for 30 days. No delayed neurotoxic effects were observed in hens in any of these studies. The regulatory value of these studies was limited due to inadequate reporting and/or poor study design.

Schumack (1995) investigated the neurotoxic effect/s of azinphos-methyl using cultured rat hippocampal neurones. Cell cultures were treated with azinphos-methyl in DMSO at concentrations of 0.1, 1.0, 10, 20 and 50 µg/mL. Cytotoxicity was observed at and above 5 µg/mL. AChE and ChAT activities were inhibited at 0.1 µg/mL and above in a dose and time related manner. Other study parameters were influenced only in the cytotoxic concentration range. The study was considered to be of limited regulatory value due to data limitations.

1.10.2 Porphyrin biosynthesis

Koeman *et al* (1980) reported that azinphos-methyl induced porphyria in chicken embryo hepatocytes in the presence of metabolic activation. However, the study was considered to be of limited regulatory value due to the lack of information on the purity of the test substance.

1.10.3 Haematoxicity

Parent-Massin and Thouvenot (1993) investigated the usefulness of human haematopoietic progenitor cultures in the predictive evaluation of pesticide myelotoxicity. Azinphos-methyl did not inhibit progenitor cell development at 0.2 and 20 µg/mL at 7 days post-treatment. However, significant inhibition in progenitor cell development was seen at both concentrations at 10 and 14 days post treatment. The study was considered to be of limited regulatory value as no information on the cytotoxicity or purity of azinphos-methyl was provided.

1.10.4 Immunotoxicity

Vos *et al* (1983) reported that azinphos-methyl caused a reduction in relative spleen, pituitary and mesenteric lymph node weights when administered to male Wistar rats in the diet. The study authors concluded that azinphos-methyl effected both immunological and general toxicological parameters at the same dose, however the study was considered to be of limited regulatory value due to the absence of key methodological and observational data.

1.11 HUMAN TOXICITY

1.11.1 Toxicity studies

In a study by McFarlane and Freestone (1999a), azinphos-methyl was administered to healthy volunteers as a single PO dose in gelatin capsules at 0, 0.25, 0.5, 0.75 and 1 mg/kg bw in males, and 0 and 0.75 mg/kg in females. Vital signs, ECG, haematology, clinical chemistry, urinalysis, plasma and RBC ChE activities, and adverse events were measured over 72 h. All volunteers were also examined 7 and 14 days post-dose for ChE sampling and adverse event reporting. There were no treatment-related effects observed for any study parameter, including any clinically relevant reduction in plasma or RBC ChE activities. The NOEL was 1 mg/kg bw for males and 0.75 mg/kg bw for females. The main deficiencies of this study were the use of a single dose level for females, absence of bioanalytical results for levels of azinphos-methyl or metabolites of azinphos-methyl in blood or urine samples, and the absence of historical control data.

In a short-term repeat dose study (McFarlane & Freestone 1999b), healthy male volunteers were given daily PO doses of azinphos-methyl for 28 days at 0.25 mg/kg bw/d. Vital signs, ECG, haematology, clinical chemistry, urinalysis, plasma and RBC ChE activities, and adverse events were measured throughout the study and 7 days after the final dose. There were no treatment-related effects on any experimental parameter including plasma and RBC ChE activities. The NOEL was 0.25 mg/kg bw/d based on the absence of any treatment-related effects at this dose. Deficiencies noted in this study included the absence of bioanalytical results for levels of azinphos-methyl or metabolites of azinphos-methyl in blood or urine samples, the fact that no female subjects were studied, the use of a single dose level, the variability of the ChE data and the absence of historical control data.

In a series of studies involving groups of 5 human volunteers (Rider *et al* 1967, 1970, 1971 & 1972; Rider & Puletti 1969), azinphos-methyl was administered orally at dose levels ranging from 1 to 20 mg/kg bw/d. No "significant changes" in plasma or RBC ChE activities were observed in any of the human subjects in any of these studies. Because the study reports were provided only in abstract form with no details on the study design, experimental methods, clinical signs and purity of the test chemical, the findings were of limited regulatory value.

To facilitate the development of a new urinary diagnostic method, correlations between the amount of azinphos-methyl ingested and the urinary levels of anthranilic acid precursors, and between the amount of urinary anthranilic acid precursors and depression of plasma ChE activity in human subjects was investigated by Thornton (1971). Five male volunteers were fed with azinphos-methyl at 16 mg/day for 30 days. Residues of azinphos-methyl, namely anthranilic acid derivatives, were measured in urine, and a correlation between the intake of azinphos-methyl and urinary residue levels was observed. No correlation between urinary

residue levels and blood ChE depression was seen. The findings of this study were of limited value due to lack of data on the purity of the test chemical, the extent of blood ChE depression, urinary metabolites, experimental methods, and the apparent lack of sensitivity of the assay method used.

A biological monitoring study (Franklin *et al* 1986) was conducted in azinphos-methyl applicators to investigate the usefulness of estimation of urinary metabolites in pesticide exposure assessments. The results of field and laboratory experiments were compared with dermal absorption data derived from animal and human studies. In general, the findings suggested that urinary metabolite data could be used more reliably and accurately to estimate exposure compared to data obtained from skin patch experiments. However, in the absence of adequate knowledge on absorption and metabolism of the parent compound, the usefulness of the urinary metabolite data was limited.

In a study by Lisi *et al* (1987), 36 pesticides including azinphos-methyl were patch tested in human subjects for 48 and 72 h. No irritant or allergic skin reactions were noticed in any of the individuals exposed to azinphos-methyl.

1.11.2 Occupational exposure

Simpson (1965) reported the findings of an unannounced visit by the Division of Occupational Health, New South Wales Department of Public Health, to an azinphos-methyl and azinphos-ethyl formulation plant which produced these chemicals under "primitive conditions". Azinphos-methyl levels in the operators' breathing zone ranged from 0.5 to 1.0 mg/m^3, which was well above the tentative limit of 0.3 mg/m^3 set by NSW at that time. A normal level of plasma ChE activity was considered to be 78–110 units. During a processing season, 13 workers were removed from contact as their ChE activity fell below 60 units or 60% of the pre-exposure level. Two operators showed symptoms of organophosphate poisoning, and one of them had to be hospitalised. The ChE levels of these 2 persons were 25 and 30 units respectively, and that of the remaining 13 persons ranged from 32 to 110 units. Whole blood ChE activity appeared to have recovered to approximately 80-90 units in about 15 days after removing these workers from the plant completely. A similar recovery of ChE activity in workers who remained in the plant doing other jobs, was noticed only after about 35 days. The health effects reported here could be attributed to the fact that levels of azinphos-methyl existed in the formulating plant. However, due to data limitations and lack of data on atmospheric levels of azinphos-ethyl, it was not possible to comment as to whether the effects observed were due to azinphos-methyl or azinphos-ethyl, or a combined effect of both of these chemicals.

Waggoner *et al* (1970a) examined plasma and RBC ChE levels in 19 employees who worked in an orange grove sprayed with azinphos-methyl at 4.2 kg ai/ha. No biologically significant inhibition of plasma or RBC ChE activity related to azinphos-methyl exposure was noticed in workers re-entered at 7, 8, 9 and 11 days after spraying. However, the regulatory value of this study was limited due to wide variability in individual pre-exposure plasma ChE data, lack of statistical analysis, data limitations and possible insensitivity of the ChE assay.

Waggoner *et al* (1970b) measured plasma and RBC ChE activities in 15 individuals who worked in an orange grove sprayed with azinphos-methyl at 4.2 kg ai/ha. Greater than 20% inhibition in plasma and/or RBC ChE activities, probably attributable to azinphos-methyl exposure was noticed in some workers at 8 and 11 days after spraying. In comparison to the

study author's previous investigation with azinphos-methyl SC, the exposure duration in the present study was longer (6-7 h vs 3-4 h). The validity of these findings was reduced due to the lack of statistical analysis, data limitations (clinical observations, urinary data) and the lack of sensitivity of the ChE assay.

A study by Lamb (1980) monitored plasma and RBC ChE activities in a group of citrus pickers in California, USA, which combined with foliar and patch residue analyses were used to determine a re-entry interval for azinphos-methyl. The main study consisted of 3 separate monitoring experiments with workers entering the treated fields at 7 days post-spray. In excess of 20% inhibition in plasma ChE activity was seen in workers exposed to azinphos-methyl WP at the 2nd and 4th re-entry days. Greater than 20% inhibition in RBC ChE activity was seen in workers exposed to azinphos-methyl SC at the 2nd, 5th and 6th re-entry days. The depressions in plasma and RBC ChE activities appeared to be related to azinphos-methyl exposure and were considered to be toxicologically significant. RBC ChE inhibition appeared to have occurred later compared to plasma ChE inhibition.

Franklin *et al* (1981) reported exposure to, and absorption of, azinphos-methyl 50 WP in a group of 17 orchardists in British Columbia, Canada. Exposure was estimated by air monitoring and patch techniques, and by measuring urinary alkylphosphate levels and serum and RBC ChE activities. The majority of workers excreted greater than 60% of total azinphos-methyl metabolites in urine at 16 h on the spray day. Forty-eight hour composite urine samples appeared to be a better indicator of exposure than 24-h composite samples. Furthermore, the total amount of azinphos-methyl sprayed was a more reliable gross index of exposure than the total time sprayed.

To evaluate their usefulness in exposure assessments, urinary alkylphosphate metabolite levels and oxime induced reactivation of plasma and RBC ChE activities in 20 peach orchard workers exposed to azinphos-methyl were measured (McCurdy *et al* 1994). Additionly, measurements of foliar residues, skin and clothing contamination, and plasma and RBC ChE activities were made. Significant reductions in median plasma and RBC ChE activities (approximately 9% and 7% respectively) were seen on re-entry day 3. On re-entry day 44, the level of inhibition was 12% and 19% for median plasma and RBC ChE activities, respectively, with statistical significance limited to RBC ChE activity. Urinary metabolite levels of azinphos-methyl showed a better correlation (r_s = 0.77) with RBC ChE activity in blood drawn on re-entry day 3 than with plasma ChE activity on that sampling day (r_s = 0. 09).

Drevenkar *et al* (1991) assessed whether urinary metabolites should be used as an alternative to measurement of ChE activity in organophosphorus pesticide exposure assessments. Orchard workers were monitored following exposure to 3 organophosphorus pesticides, including azinphos-methyl 25% WP. Urinary metabolite levels were monitored, and serum ChE, paraoxonase and arylesterase activities were measured. The findings demonstrated that urinary azinphos-methyl metabolites could be considered as indicators in exposure assessments. However, no correlation was observed between inhibition of serum ChE activity and the total urinary concentrations of dialkylphosphorus metabolite. Paraoxonase and arylesterase activities also showed no correlation with either inhibition of serum ChE activity or urinary dialkylphosphorous metabolite levels. Lack of correlation between inhibition of serum ChE activity and urinary metabolite concentrations suggested that both these parameters need to be monitored during exposure assessments.

In a report by Hernadez *et al* (1992), data collected from an occupational exposure assessment study conducted by the California Department of Pesticide Regulation (CDPR), as part of its exposure method validation process, were presented in abstract form. Peach and apple harvesters were monitored for exposure to azinphos-methyl for over 2 years by measuring dermal residue and dialkyl phosphate levels in urine. ChE activity was measured as an additional monitoring tool. Based on urinary dialkyl phosphate data, the mean dermal absorption of azinphos-methyl in peach and apple harvesters ranged from 1.8 mg/d (19%) and 15.7 mg/d (55%) respectively. No significant perturbation in ChE activity was observed. Furthermore, no correlation between dermal dosimetry and urinary metabolites was seen.

An epidemiological study of 90 male pesticide applicators in New York, USA was conducted by Stokes *et al* (1995) to investigate the effects of exposure to organophosphate pesticides on the peripheral nervous system. A clear exposure-related increase in urinary dimethylthiophosphate (DMTP) levels were seen in workers who sprayed azinphos-methyl. However, urinary DMTP level did not increase with the increasing number of exposure symptoms reported.

Heimann (2000) conducted an operator exposure study involving 8 male operators in Italy and France with the objective of determining real exposure under practical conditions. Although reporting was limited, no relevant differences between pre- or post-exposure plasma or RBC ChE activities was noticed in any of the workers compared to the reference values of the laboratory. The usefulness of the findings, however, was reduced due to lack of details on the test substance, exposure conditions and experimental methods.

Two in-company communications (Miksche 1981; Kehrig1999) reported that regular medical examination of employees who worked in azinphos-methyl production or formulation plants, under the prevailing industrial hygiene conditions, plant and personal safety precautions did not reveal any clinically relevant sensitisation or health impairments to this chemical. The conclusions in one of these communications were based on the findings of annual occupational medical examinations, experience gained by the physicians during their regular visits and information from the management of the relevant production or formulation plant. Reporting however, was limited and the details on the chemical, workers involved, the types of medical tests conducted or the work environment were not provided.

1.12 DISCUSSION

1.12.1 Metabolism and Toxicokinetics

The GI absorption of azinphos-methyl in rats was rapid and almost complete following gavage administration. Experiments conducted with radiolabelled azinphos-methyl indicated that the maximum plasma concentration was achieved approximately 2-3 h after dosing, and the concentration present in the whole animal (minus the GI tract) was similar irrespective of whether it was administered intravenously or orally. Bile duct cannulation experiments indicated that a proportion of azinphos-methyl enters enterohepatic circulation and is excreted in urine following iv administration.

The distribution of azinphos-methyl in rat tissues was similar following PO or iv administration. Six hours after gavage administration to rats, azinphos-methyl was predominantly detected in the adrenals and those organs associated with its excretion or

metabolism, namely the kidneys and liver. The rate of decline of azinphos-methyl in all tissues over 48 h mimicked that observed in plasma and was below the limit of detection for most tissues after 4 days, except in blood, the kidneys and adrenals.

Approximately 62-72% of azinphos-methyl was excreted via the urine following PO administration to rats, and at least two major urinary metabolites (namely, cysteinylmethylbenzazimide sulfone and methylsulfonylmethyl-benzazimide) and up to 6 other products (formed at low concentrations) have been identified. Five faecal metabolites, accounting for approximately 10-12% of the administered dose, have also been characterised. In rats, the initial steps of the metabolism of orally-administered azinphos-methyl appear to be sulfoxidation, hydroxylation of the ester bond or conjugation with glutathione. *In vitro* studies utilising various subcellular fractions suggested that azinphos-methyl metabolism is mediated by glutathione-S-transferase and cytochrome P-450. The major metabolite found in the organosoluble fraction of goat milk (accounting for about 40% of the administered dose) was 3-[(methylsulfonyl)-methyl]-1,2,3,benzotriazin-4-(3H)-1, methylsulfonylmethylbenzazimide).

Whether dosed by PO gavage or intravenously, over 95% of azinphos-methyl was excreted in the urine, bile and expired air within 48 h in rats. Approximately 26-34% of orally-administered azinphos-methyl was excreted in bile/faces, whilst less than 0.1% was found in expired air. In rats, no unchanged azinphos-methyl has been identified in urine or faeces.

Dermal absorption of azinphos-methyl technical in rats was approximately 21-54% after 10 h when applied as a water-based suspension, with the majority of absorption occurring within the first hour of application. In a recent study, the dermal absorption of a single dose of azinphos-methyl in human volunteers was approximately 22-29%. Earlier published studies reported dermal absorption of 16% over 36 h in human subjects. Factors such as occlusion and application to the abraded skin increased the rate of absorption to approximately 56% and 60%, respectively.

The absorption of gavage-administered benzazimide, a major metabolite of azinphos-methyl in rats, was also rapid with approximately 82% detected at 3 h in the whole animal. Other studies also indicated that apart from the liver and kidneys, there was no tissue accumulation of benzazimide. In rats, approximately 54-58% of gavage-administered benzazimide was excreted in urine over 48 h, and about 41-45% was eliminated in faeces over the same time period. Generally, the toxicokinetic characteristics of benzazimide were similar to its parent molecule, azinphos-methyl.

Acute toxicity

Azinphos-methyl was highly acutely toxic when administered orally in both aqueous and non-aqueous vehicles, and its profile of clinical signs was similar to those of other ChE inhibiting organophosphorus pesticides. Clinical signs commonly observed in experimental animals following acute exposure were salivation, lacrimation, vomiting, diarrhoea, anorexia, reduced locomotor activity, piloerection, staggering gait and muscular tremors. These signs were qualitatively similar irrespective of the route of administration and were generally evident within 5-20 minutes after treatment in the lethal dose range, except after dermal exposure, where the signs occurred between 1-24 h after treatment. Acute oral toxicity studies in rats reported LD_{50} values of 4.4-26 mg/kg bw. The lowest acute 1-h and 4-h inhalational LC_{50} values in rats were 310 and 132 mg/m^3, respectively. Acute toxicity was slightly increased by ip administration, with LD_{50} values in rats ranging from 3.9 to 11.6 mg/kg bw.

The acute dermal toxicity of azinphos-methyl in rats was high, with LD_{50} values ranging from 72.5-200 mg/kg bw when applied to intact skin under non-occluded conditions for 24 h. Technical grade azinphos-methyl was not a skin irritant in rabbits, but was a slight eye irritant and skin sensitiser.

Amongst azinphos-methyl metabolites, benzazimide was toxicologically significant, however no other metabolites have been investigated. Although, there were no acute oral toxicity studies in the toxicology database, an acute inhalational study in rats indicated that the 4-h inhalational LC_{50} of benzazimide is in excess of 1760 mg/m^3. The acute dermal toxicity of benzazimide in rabbits was low (LD_{50} > 2000 mg/kg bw).

Reported LD_{50} values for end-use products containing azinphos-methyl were generally representative of the percentage of active ingredient present in the formulation.

Antidote studies

Studies conducted in rats have investigated the antidotal activity of compounds such as toxogonin, pyridine-2-aldoxime (2-PAM), diacetyl monoxime (DAM), 1, 1-trimethylene-bis(4-formyl-pyridinium bromide) dioxime (TMB-4), atropine sulphate, pralidoxime (PAM) and obidoxime chloride (BH6). Amongst these compounds, toxogonin, 2-PAM, BH6 and atropine sulphate appeared to be effective at providing some protection against acute azinphos-methyl toxicity. Treatment with atropine sulphate alone increased the oral LD_{50} by approximately 2-fold, while this effect was increased when atropine sulphate was used in combination with either toxogonin, 2-PAM or BH6.

ChE Inhibition – Short-term studies

A summary of NOEL and LOEL findings for plasma, RBC and brain ChE activities in different species in a range of repeat-dose studies is presented in the following Table.

Summary of NOELs and LOELs (mg/kg bw/day or mg/m^3) for plasma, RBC and brain ChE activities during short-term repeat-dose studies in various laboratory animals.

Species	Duration	Route	Plasma	Erythrocyte	Brain	LOEL
Rat	28 days	PO	0.35/0.46 M/F	1.3/0.46 M/F	>3.37/1.54 M/F	1.3/1.54 M/F
	29 days	PO	> 3.2/0.8 M/F	0.2/0.8 M/F	0.8/0.8 M/F	3.2/0.8 M/F
	13 weeks	PO	0.86	0.215	0.86	0.86
	16 weeks	PO	0.25	0.25	>1.0	1.0
	16 weeks	PO	< 2.5	< 2.5	< 2.5	2.5
	60 days	ip	< 0.5	ND	< 0.5	0.5
	10 days	Inhalation	25.0	ND	25.0	50
	12 weeks	Inhalation	1.24	1.24	ND	4.72
Rabbit	3 weeks	Dermal	2.0	2.0	ND	20.0

Dog	12 weeks	PO	0.5	0.125	ND	1.25
Human	4 weeks	PO	> 0.25	>0.25	ND	>0.25

ND = not determined.

The data presented in the above Table indicates that the inhibition of plasma and/or RBC ChE activities occurs at relatively low dose levels. There was little intra- and inter-species variability in the NOELs for inhibition of plasma ChE activity when azinphos-methyl was administered orally. In the recent studies of McFarlane and Freestone (1999a & b) in human volunteers, azinphos-methyl was tolerated (without effect on vital signs, ECG, haematology, clinical chemistry, urinary parameters and plasma and RBC ChE activity), by males as a single PO dose up to 1.0 mg/kg bw and in females at 0.75 mg/kg bw/d, or as a 28-day repeat PO dose in males at 0.25 mg/kg bw/d.

Neurotoxicity

The delayed neurotoxic potential of azinphos-methyl has been studied in a series of experiments in hens. Although these studies provided limited methodological information and/or data, the findings suggested that technical grade azinphos-methyl does not induce delayed neurotoxicity in hens after single or repeated PO administration.

Genotoxicity

Consistent with the absence of any detectable carcinogenicity during chronic studies, azinphos-methyl did not show any genotoxic potential during a variety of *in vivo* chromosomal effect assays (mouse micronucleus test, mammalian bone marrow cytogenetic test, mouse dominant lethal assay, recessive lethal test in *D. melanogaster*). Although some *in vitro* clastogenetic activity was detected in CHO cells at 60-120 μg/mL, and in WI-38 and HEp-2 cells at 120-160 μg/mL, no effect was observed in human lymphocytes up to 500 μg/mL. A battery of other *in vitro* genotoxicity assays indicated that azinphos-methyl had no or minimal genotoxic potential. Studies that described mutations in *S. pombe* up to 95 mM, and in mouse lymphoma cells, were discounted due to the inadequacy of the reports. Several studies reported recombination in *S. cerevisiae* D3 but only one of these, which showed an effect at 4.5 and 5.5%, was considered to be adequate for regulatory purposes. Reversion, gene conversion and cross overs in *S. cerevisiae* D7 were reported in 2 studies but only one of these, which showed an effect at 10000 μg/mL and above, was considered to be adequate for regulatory purposes. Unscheduled DNA synthesis (UDS) was detected in human lung fibroblasts up to 10^{-4} M, however, other studies using the same cell line or rat hepatocytes, were negative. On the weight of evidence azinphos-methyl was not considered to be genotoxic.

Reproduction and Development

There was no evidence that azinphos-methyl caused structural malformations in offspring, or affected reproductive parameters in mice, rats or rabbits. However, at maternotoxic doses azinphos-methyl was toxic to both parental animals and their offspring. The toxic effect on offspring was observed as reduced pup weight and viability during lactation, and was possibly the consequence of reduced maternal care or lactation.

In mice, an approximately 80% reduction in mean litter size at weaning (d 21) occurred while in rats, pup growth and viability were reduced at doses that caused marked inhibition of ChE activity, clinical signs of intoxication, and/or deaths in maternal animals. These effects on pups were possibly the result of maternal toxicity (ie lack of care or lactation due to poor maternal health).

In developmental studies, no major malformations were observed in mice, rats or rabbits. In mice, findings including malaligned sternebrae, reduced foetal weight and supernumerary ribs (SNR) were observed at maternally toxic doses. As SNR are known to be caused by maternal stress in CD1 mice, it was concluded that this treatment-related effect was due to poor maternal health. In the majority of studies using rats, signs of maternal toxicity (significantly reduced weight gain and food consumption, an increase in cholinergic signs) were noted at dose levels that also affected pups (reduced gestation index, pup survival and weight gain) which again supports the premise that poor maternal health leads to reduced maternal care and thus reduced pup health.

In a single rat study (Rubin & Nyska 1988), an increased incidence of SNR and some retardation of ossification were observed at an apparently non-maternally toxic dose (3.6 mg/kg bw/d), however no maternal ChE activity was measured. Other rat studies had reported LOELs for plasma/RBC/brain ChE activity of approximately 1 mg/kg bw/d and thus it was probable that ChE inhibition would have occurred in this study at the dose where developmental variations were observed. No frank maternal toxicity or teratogenicity were observed in rabbits. In a single rabbit study (Gal *et al* 1988), retarded ossification of the long bones, asymmetric pelvic articulation and reduced foetal size were observed at doses that did not cause clinical signs of toxicity in dams but caused significant inhibition of RBC ChE activity. It should be noted that these developmental variations seen in rats and rabbits either fell within or just outside the historical control range of the performing laboratory. Although this single rat study and rabbit study suggested that delayed developmental effects occurred at doses lower than those causing frank maternal toxicity, the weight-of-evidence supports the conclusion that minor developmental variations occur in laboratory animals at maternotoxic doses of azinphos-methyl.

Long-term toxicity and carcinogenicity

As with other organophosphorus pesticides, the typical toxicological effects of azinphos-methyl during chronic studies in mice, rats and dogs included dose-related ChE inhibition (plasma, RBC and brain) and classic cholinergic signs (body tremors, convulsions, muscle weakness, reduced weight gain) and mortality. There have been no reported effects of azinphos-methyl on gross pathology, histopathology or tumour incidences in mice, rats or dogs, and thus there is no evidence of any carcinogenic potential.

In long-term studies, ChE inhibition which is the most sensitive indicator of toxicity, has been observed at and above 3.49, 0.75 and 0.5 mg/kg bw/d in mice, rats and dogs respectively (inhibition of plasma, RBC and brain ChE activities in mice and rats, inhibition of plasma and RBC activities in dogs). Other effects including decreased body weight gain were seen at higher doses.

Human Studies

In recent human studies, a single PO dose of azinphos-methyl was well tolerated by male volunteers up to 1 mg/kg bw, and in female volunteers at 0.75 mg/kg bw, the highest doses tested. No effect on any vital signs, ECG, haematology, clinical chemistry, urinalysis, plasma and RBC ChE activities were detected. In a subsequent 28-day repeat dose study, no effects were observed in male volunteers who were given daily PO doses of azinphos-methyl at 0.25 mg/kg bw/d. Although these experiments indicated that azinphos-methyl could be tolerated by males, either as a single PO dose up to 1 mg/kg bw, or as a 28 day repeat PO dose at 0.25 mg/kg bw, neither study addressed the acute or short term effects in females, or the long term or cumulative effects of azinphos-methyl in humans. However, based on results in animal studies and in the absence of any toxicological effects, it is unlikely that longer-term administration to humans would lead to adverse effects at the dose used in the 28-day study.

In general, no adverse health effects have been observed in male or female workers involved in azinphos-methyl production and formulation under normal safety precautions. A single report indicated that azinphos-methyl caused generalised dermatosis in an individual with apparently hypersensitive and dry skin, however, no effects on any internal organs such as the liver could be attributed to azinphos-methyl with adequate certainty. Additionally, no further details on the chemical, affected individual, or the severity of the skin reaction were discussed.

A small number of occupational studies conducted in agricultural workers demonstrated greater than 20% inhibition of plasma and/or RBC ChE activity, which was probably attributable to azinphos-methyl exposure. However, due to the lack of methodological details, data limitations and clinical observations, the findings of these studies had limited value in establishing regulatory standards. In general, RBC ChE inhibition appeared to have occurred later compared to plasma ChE inhibition. The measurement of urinary dimethylthiophosphate (DMTP) levels appears to be a useful tool to for determining human exposure to azinphos-methyl.

NOEL considerations

To establish the lowest NOEL for azinphos-methyl, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is tabulated below.

Study type	NOEL (mg/kg bw/d)	LOEL and toxic effect
Chronic Studies		
B6C3F1 mice 80-wk dietary	9.4	Clinical signs (hyperactivity, rough hair coats) and decreased body weight gain in females at 18.75 mg/kg bw/d.
CD1 mice 2-y dietary	0.79 males 0.98 females	Inhibition of plasma, RBC and brain ChE activities at 3.49 and 4.19 mg/kg bw/d in males and females respectively.
Wistar rats 2-y dietary	0.25	Inhibition of plasma and RBC ChE activities at 1 mg/kg bw/d.
Osborne-Mendel rats 80-wk dietary	< 3.9 males 3.125 females	Clinical signs (body tremors), decreased body weight gain and/or increased mortality at 3.9 and 6.25 mg/kg bw/d in males and females respectively.
Wistar rats 2-y dietary	0.25 males 0.31 females	Inhibition of plasma, RBC and brain ChE activities at 0.75 and 0.96 mg/kg bw/d in males and females

Study type	NOEL (mg/kg bw/d)	LOEL and toxic effect
		respectively.
Cocker Spaniel dogs 2-y dietary	0.125	Inhibition of plasma and RBC ChE activities at 0.5-1.25 mg/kg bw/d.
Beagle dogs 52-wk dietary	0.125	Inhibition of plasma and RBC ChE activities at 0.625 mg/kg bw/d.
Reproduction Studies		
CF1 mice 3-generation	> 7.5	No effect on reproduction at 7.5 mg/kg bw/d.
	3.75	Maternal mortality and reduced litter size at weaning (day 21) at 7.5 mg/kg bw/d.
Wistar rats 2-generation	> 3.46-7.37 males > 4.84-10.27 females	No effect on reproduction at the highest dose.
	1.02-1.22 males 1.48-2.02 females	Reduction in parental body weight gain and pup viability at 3.46-7.37 and 4.84-10.27 mg/kg bw/d in parental males and females respectively.
Wistar rats 1-generation	> 3.73 males > 4.87 females	No reproductive effects at the highest dose
	< 0.43 males < 0.55 females	Inhibition of plasma and RBC ChE at 0.43 and 0.55 mg/kg bw/d in parental males and females respectively.
	0.43-0.55	Reduced pup viability and retardation of growth at 1.30-1.54 mg/kg bw/d.

Study type	NOEL (mg/kg bw/d)	LOEL and toxic effect
Teratology Studies		
CD-1 mice gavage	< 16	Maternal mortalities at 16 and 20 mg/kg bw/d.
	16	Decreased foetal weight and increased incidence of SNR at 20 mg/kg bw/d.
CD-1 mice gavage	2.5	Cholinergic signs at 5 mg/kg bw/d.
	2.5	Increased incidence of malaligned sternebrae at 5 mg/kg bw/d.
CD rats gavage Teratology and peri/post-natal toxicity	2.5	Reduced weight gain and feed consumption, and clinical signs of ChE inhibition at 5 mg/kg bw/d in pregnant rats treated from day 6-15 of gestation.
	> 5	No developmental effects at the highest dose.
	2.5	Mortalities, cholinergic signs, reduced weight gain and feed consumption at 5 mg/kg bw/d in pregnant rats treated from day 6 of gestation to day 21 post-partum.
	2.5	Reduced gestation index, pup survival and weight gain at 5 mg/kg bw/d.
Crl:CD BR rats gavage	1.0	Inhibition of RBC and brain ChE activities at 2 mg/kg bw/d.
	> 2.0	No embryotoxic and teratogenic effects at the highest dose tested.
SD-derived CD rats gavage	3.6	No maternal toxicity at the highest dose tested
	1.2	Increased incidence of SNR (14^{th}, lumbar) and delayed ossification of the pubic, hyoid and supraoccipital bones at 3.6 mg/kg bw/d
Himalayan	> 3.0	No maternal toxicity, embryotoxicity or

rabbits gavage		teratogenicity detected.
American Dutch rabbits gavage	1.0	Inhibition of plasma and RBC ChE in does at 2.5 mg/kg bw/d.
	> 6.0	No developmental effects at the highest dose.
NZW rabbits gavage	4.75	Inhibition of maternal RBC ChE activity at 15 mg/kg bw/d
	1.5	Retarded ossification of the long bones at and above 4.75 mg/kg bw/d, skeletal variations (asymmetric pelvic articulation) and reduced foetal size at 15 mg/kg bw/d
Human Studies		
Single PO dose	> 1.0	No effect in males at the highest dose.
	> 0.75	No effect in females at the highest dose.
28-d repeat PO dose (daily)	> 0.25	No effect in males at the single dose tested.

1.12.2 Determination of Public Health Standards

Acceptable Daily Intake (ADI)

The current acceptable daily intake (ADI) is 0.001 mg/kg bw/d, derived by applying a 100-fold safety factor to the NOEL of 0.125 mg/kg bw/d for inhibition of plasma and RBC ChE activities in a 2-year dog study (Noel *et al* 1966). This ADI was established in 1988.

A 28-day human study has been reviewed as part of the ECRP, in which azinphos-methyl was administered orally at 0.25 mg/kg bw/d to male volunteers, with no clinical signs or any treatment-related effects observed. The ADI, based on this study, is 0.025 mg/kg bw/d, using the NOEL of 0.25 mg/kg bw/d and a 10-fold safety factor for inter-individual variability.

Market Basket Survey

The Australian Market Basket Survey (now called the Australian Total Diet Survey) conducted in 1992, 1994 and 1996 under the auspices of the National Food Authority (NFA) [now called the Australia New Zealand Food Authority (ANZFA)] and the National Health and Medical Research Council (NHMRC) of Australia monitored azinphos-methyl residue levels. Azinphos-methyl was detected in foods in all these surveys with residues being detected in chicken nuggets and pears in 1992, in apples, peaches and pears in 1994, and in apples, grapes, pears and plums in 1996. According to the 1996 survey, the highest dietary exposure to azinphos-methyl in the groups studied, based on the 95th percentile energy intake, was 192.9 ng/kg bw/day in 2-year old toddlers (average body weight of 12.3 kg). This exposure level accounts for 19.3% of the existing Australian ADI of 0.001 mg/kg bw/d. The lowest dietary exposure was 47.9 ng/kg bw/day recorded for adult males (average body weight of 75 kg) and is estimated to be about 5% of the existing ADI. Except for apples, the estimated consumption of azinphos residues in peaches and pears varied considerably between surveys. This variability may be a reflection of changes in use patterns or of the sampling protocols used in the surveys.

In 1992 and 1994, the highest estimateed azinphos-methyl intakes were also calculated for 2-year old toddlers, which recorded average values of 47 and 238 ng/kg bw/day, accounting for about 5% and 24% of the existing ADI respectively. Thus, based on the 1994 and 1996 survey

results, the group with the highest estimated intake value appears to be 2-year old toddlers with average intake values of about 19-24% of the current ADI. Nevertheless, these values provided about a 4- to 5-fold additional safety factor between the ADI and maximum daily intake values. If the ADI were changed to 0.025 mg/kg bw/d, the highest dietary exposure to azinphos-methyl based on the 95th percentile energy intake in 2-year old toddlers (1996 intake data) would be less than 1% of this ADI.

Acute Reference Dose (ARfD)

To derive the ARfD, a safety factor of 10 was applied to the NOEL of 0.75 mg/kg bw/d, based on the absence of any treatment-related effects in female volunteers in a single-dose human study (McFarlane & Freestone 1999a), giving an ARfD of 0.075 mg/kg bw/d.

[It is worth noting that in the German BgVV assessment, the ARfD was established on the basis of the NOEL of 0.25 mg/kg bw/d in the 28-day human study (McFarlane & Freestone 1999b). The NOEL from the single-dose human study was not used as it was considered that the acute dietary risk is not restricted to high intakes on a single day, but also to high intakes in short-term, repeat-dose situations (eg 2-3 days). Acute dietary studies in rats showing irreversible inhibition of plasma, RBC and brain ChE activities within 24 hours ($\geq$ 2.0 mg/kg bw) were also considered. Therefore the German BgVV reasoned that the use of an ARfD of 0.025 mg/kg bw would provide an adequate safety margin for possible cumulative effects following repeat-dose exposure situations.]

2 MAIN TOXICOLOGY REPORT

2.1 INTRODUCTION

2.1.1 Regulatory History of Health Considerations in Australia

Azinphos-methyl is an organophosphorus insecticide and acaracide that has been widely used in Australia to control pests such as codling moth, light brown apple moth, oriental fruit moth, pear and cherry slug, apple leaf hopper, spring beetle, woolly aphid, san jose scale, oyster shell scale, soft brown scale, red scale and black or olive scale in a range of fruits (eg pome fruits, stone fruits, citrus fruits, blueberries, grapes, kiwifruit, litchis, macadamias

In Australia, public health standards for agricultural and veterinary chemicals, such as the first aid and safety directions and acceptable daily intake (ADI) are established by the Department of Health and Aged Care. Poisons Schedules are set by the National Drugs and Poisons Schedule Committee (NDPSC). In the case of maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Committee (PACC) of the National Health and Medical Research Council (NHMRC). This function was subsequently transferred to the Department of Health in 1992, and then to the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) in June 1994.

A history of the public health considerations of azinphos-methyl by regulatory committees in Australia is detailed in the Table below. It should be noted that MRLs for azinphos-methyl have been considered by the Department of Health and Aged Care or by NHMRC committees since 1966.

History of Public Health Consideration of Azinphos-methyl in Australia

Date	Decision
June 1988	PACC: Within current MRLs. Translocation of chemicals from trunk to fruit is unlikely. Minor use in citrus as a trunk barrier would not result in residues above the MRL.
August 1988	NDPSC: Confirmed TGAC clearance with the existing Schedule 7 classification. The company was asked to supply formulation details. Toxicity data sheet to be amended
December 1988	PACC: The total organophosphate residue situation is to be reviewed rather than an examination of azinphos-methyl or any other organophosphate in isolation. Should be reviewed with all other organophosphates.
August 1989	PACC: Studies noted. Request full details of human study.
May 1993	NDPSC: Considered a request for review of the packaging of azinphos-methyl/GUSATHION 350 INSECTICIDE in a resealable 700 g foil bag. The Committee agreed that the packaging was not acceptable for an S3 poison as a primary pack or as a measure pack.

PACC - Pesticide and Agricultural Chemicals Committee; NDPSC - National Drugs and Poisons Scheduling Committee.

2.1.2 International Toxicology Assessments

JMPR

Azinphos-methyl has been reviewed by the Joint FAO/WHO Meeting of Pesticide Residues (JMPR) in 1965, 1968, 1973 and 1991. In 1965, the JMPR established an ADI for azinphos-methyl of 0.0025 mg/kg bw/d based on plasma ChE inhibition in a 17-week rat study. In

1968, the ADI was reaffirmed despite there being long-term studies (2 yr) in rats and dogs with lower NOELs (ie at 0.125 mg/kg bw/d).

The most recent review, in 1991, incorporating the change in JMPR policy to use inhibition of brain ChE (or RBC ChE inhibition as a surrogate) as the toxicologically-relevant endpoint, identified reduced ChE activity in the brain of parental animals together with reduced fertility in females and diminished pup viability at 15 ppm (1.3 mg/kg bw/d) in a 2-generation rat reproduction study. These results were confirmed in a subsequent 1-generation study with the NOEL being 5 ppm (0.43 mg/kg bw/d) (Holzum 1990). Hence, using a 100-fold safety factor, a new ADI was established at 0.005 mg/kg bw/d. In other species, the NOELs were:

Mouse: 5 ppm, equal to 0.88 mg/kg bw/d
Rat: 5 ppm, equal to 0.86 mg/kg bw/d in a carcinogenicity study
Dog: 25 ppm (equal to 0.74 mg/kg bw/d)
Human: 0.3 mg/kg bw/d

US EPA

The US EPA, which supports the use of inhibition of plasma ChE as an appropriate toxicological endpoint, has set a slightly lower reference dose RfD (equivalent to an ADI) at 0.0015 mg/kg bw/d (as at Aug 1998) based on the NOEL (0.149 mg/kg bw/d for males) from a 1-year dog study. The NOEL in this study was based on a significant decrease in RBC ChE activity (Allen *et al* 1990). [At the time of writing this review, no determination had been made on the inclusion of an additional safety factor for FQPA requirements, although the Hazard Identification Assessment Review Committee (HIARC) recommended that no additional factor was warranted because there was sufficient evidence to indicate no increased susceptibility for children and infants.]

IARC

Azinphos-methyl has not been evaluated by the International Agency for Research on Cancer (IARC).

2.2 METABOLISM AND TOXICOKINETICS

The following table summarises results for the dermal absorption of azinphos-methyl.

Dermal absorption of azinphos-methyl (AM)

Study	Dose	Method of analysis	Absorption	Author
Rat	100 – 800 µg ai/cm^2	Urinary DMTP (24 h exposure)	50-60%	Franklin *et al* (1986) (review)*
	Unspecified (with ^{14}C-AM)		100%	
	0.018, 0.185, 2.08 ai mg/kg bw (with ^{14}C-AM, aqueous) intact, unoccluded, residues removed)	Urinary and faecal excretion of radioactivity (after 1, 4 & 10 h exposure)	20-46% (1-h) 26-49% (4-h) 21-54% (10-h)	Schroeder (1992) (GLP)
Rabbit	Unspecified (with ^{14}C-AM), in acetone	Urinary DMTP (24 h exposure)	100%	Franklin *et al* (1986) (review)*
Monkey			32-47%	

Human	4 µg/cm^2 (in acetone), 24-h, unoccluded (with ^{14}C-AM)	Urinary excretion of radioactivity (corrected for incomplete excretion after iv dose)	16% (in 120 h)	Feldman & Maibach, 1974)
	4 µg/cm^2 (in acetone), 24-h, unoccluded (with ^{14}C-AM)		16% (unoccluded) 56% (occluded) 60.5% (damaged skin)	Wester & Maibach (1985), (review)*
	3 or 10 µg ai/cm^2 , aqueous (with ^{14}C-AM) or 5 µg/cm^2 (Gusathion M 25 WP), 8-h	Urinary and faecal excretion of radioactivity plus tape stripping	23-29% 22%	Selim (1999) (GLP)

*Original data were not reviewed by the Australian regulatory authorities.

2.2.1 Rats

Patzschke K, Wegner LA & Weber H (1976) ^{14}C-azinphos-methyl (^{14}C-Gusathion M). Biokinetic investigations on rats. Lab: Bayer AG. Institute for Pharmacokinetics, Isotope Laboratory, Wuppertal, Germany. Study duration: not stated. Report nos. 1054 & 6419. Report date: October 11, 1976.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: The absorption, distribution and excretion of [carbonyl-^{14}C]-azinphos-methyl [synthesized in house, purity not stated, specific activity 27.6 µCi/mg (1 Mbq/mg)] in Cremophor EL (5% v/v in the end volume) was investigated in male SD rats (Mus Rattus AG, Munich, Germany) following either PO or iv administration. Group size was generally limited to 5 except for whole body autoradiography, where only one rat was used at each time interval after both iv and gavage administration, and for the bile cannulation experiment where 3 rats were used.

For the duration of the study, rats were housed in metabolism cages to facilitate the collection and measurement of radioactivity in expired gases, urine and faeces. Radioactivity in the whole rat (minus GI tract), liver, kidneys, brain, muscle, skin, testes, adrenals, whole blood, erythrocytes, plasma and fat was measured by liquid scintillation counting after combustion in a sample oxidizer 3, 6, 24, 48, 72, 144 and 240 h after treatment. Whole-body autoradiography was performed 2 and 60 min after iv administration at 2 mg/kg bw or 24 h after gavage administration at 6 mg/kg bw. Radioactivity in excreta (urine and faeces) was monitored for 48 h following gavage or iv dosing at 0.1 or 2 mg/kg bw. Radioactivity in expired air was monitored for 24 h after both gavage and iv dosing at 2 mg/kg bw. Excretion of radioactivity in bile was measured for 24 h following iv dosing at 2 mg/kg bw.

Findings: Following gavage administration, the absorption of azinphos-methyl was judged to be rapid since the maximum radioactivity in plasma (C_{max}, 4 µg eq/g at 2 mg/kg bw) was achieved approximately 2-3 h after dosing. After 6 h (the first sampling interval for tissue radioactivity), the only tissues containing more radioactivity (per gram) than plasma were those associated with its excretion or metabolism, namely kidneys and liver, and somewhat unexpectedly, the adrenal gland. The rate of decline in radioactivity in all tissues over 48 h mimicked that observed in plasma and was below the level of detection for most tissues after 4 days except in blood, kidneys and adrenal glands. By day 8, radioactivity was only detectable in whole blood, plasma and erythrocytes. Autoradiography did not reveal any high levels of radioactivity in organs other than those involved in metabolism or excretion (ie liver

and kidneys). Irrespective of the route of administration, nearly all the radioactivity was excreted within 48 h in the urine, bile and expired air. Whilst less than 0.1% of the administered radioactivity (at both 0.1 and 2 mg/kg bw PO or iv) was excreted in air, most was excreted in urine (ie 62% and 68% at 0.1 and 2 mg/kg bw respectively after PO administration or 63% and 68% at 0.1 and 2 mg/kg bw respectively after iv injection) with the balance being found in bile/faeces (ie 34% and 26% at 0.1 or 2 mg/kg bw, respectively, after PO administration, or 29% and 26% at 0.1 and 2 mg/kg bw respectively after iv injection).

Conclusions: These results suggest that the absorption from the GI tract is almost complete (~100%). Radioactivity in bile up 24 h after dosing at 2 mg/kg bw accounted for 27% of the dose, whereas 54% was found in the urine and about 6% in the faeces. At least a proportion of bile excreted radioactivity re-enters via enterohepatic circulation, and is then excreted in the urine.

Ecker W (1976) ^{14}C-azinphos-methyl. Metabolism studies on rats; preliminary results. Lab: Bayer AG. Institute for Pharmacokinetics. Isotope Laboratory, Wuppertal, Germany. Study duration: not stated. Report nos. 1034 & 6106. Report date: May 17, 1976.

Pre GLP, non-quality assured study.

Study and observations: Metabolites of [carbonyl-^{14}C]-azinphos-methyl (synthesized in house, purity > 99%, specific activity approx. 30 μCi/mg (1.1 Mbq/mg)) in Cremophor EL (5% v/v in the end volume) excreted in the urine of male SD rats (group size unspecified; Mus Rattus AG, Munich, Germany) following iv administration were quantified by liquid scintillation counting. The metabolites were isolated from 0-48 h pooled urine by using either column chromatography (with different media, ie Amberlite XAD-2, Sephadex G10 or LH20) or partitioning between immiscible solvents. Metabolites were characterised by co-migration of radioactivity with reference standards on silica gel TLC. The radioactivity of the spots on the TLC plates was detected by autoradiography and/or a scanner. Glucuronidated and sulfated conjugates were identified following treatment with β-glucuronidase/arylsulfatase

Findings and conclusions: TLC analysis revealed 10 radioactive spots of which none had an activity in excess of 30% of the total radioactivity excreted in the urine. However, preliminary results indicated desmethyl-azinphos-methyl, dimethyl benzazimide sulfide, and dimethyl benzazimide disulfide, were the major metabolites. No unchanged azinphos-methyl was detected.

Kao LR, Murphy JJ & Flint DR (1988) Disposition and metabolism of azinphos-methyl in rats. Study no: GU4R. Lab: Mobay Corporation, Mobay Research Park, Stilwell, Kansas, USA. Sponsor: Ciba-Geigy Corp, Greensboro, NC, USA. Study duration: November, 1987 – August, 1988. Sponsor: Mobay Corporation, Report no. 98327. Report date: Sep, 1988.

GLP, quality assured study.

Study and observations: [Phenyl-UL-^{14}C]azinphos-methyl (Mobay Chemical Corp., lot no. C-108, purity: approximately 99%, specific activity: 70.3 μCi/mg) in Cremophor EL was administered at 0.125 or 2.5 mg/kg bw to 2 groups of SD rats (Sasco Inc, Nebraska, USA; 5/sex/group, bw: about 200 g) by gavage. A third group of 7 rats/sex was conditioned by daily treatment with 0.125 mg/kg bw of unlabelled azinphos-methyl for 14 days and then 5/sex of this group were selected and treated with 0.125 mg/kg bw of labelled azinphos-methyl (as for

group 1) on day 15. After dosing, all rats were housed in metabolism cages for 72 h, after which they were sacrificed, and the liver, kidneys, lung, brain, muscle, testes, heart, spleen, GI tract, ovaries, bone, blood, plasma and fat were collected for residue analysis and metabolite identification. Mortality and clinical signs were monitored daily. Metabolites excreted in urine and faeces were isolated and identified using TLC and HPLC.

Findings: Clinical signs such as salivation, lacrimation and minor tremors were seen in animals at 2.5 mg/kg bw for several hours after treatment. They recovered and excreted very little faeces for at least 8 h after dosing. No clinical signs were observed in rats treated at 0.125 mg/kg bw.

No sex difference in the excretion of radioactivity in urine and faeces was observed. Excretion of radioactivity in faeces and urine ranged between 95.9-96.5, 94.5-94.8 and 93.8-94.2% for the low, high and the pre-conditioned low-dose treatment, respectively. For each of these groups an average of 71.8%, 70.7% and 70.3% of the radioactivity, respectively, was found in urine and the difference, namely 24.3%, 23.9% and 23.6%, respectively, was found in the faeces. Excretion of most of the administered radioactivity (approximately 95%) generally occurred during the first 48 h after exposure. Recoveries were complete (92.2-107.7%) with some 3-5% of the radioactivity not in the urine and faeces being detected in tissues and a further 0.8-1.3% in the cage wash. A supplementary experiment with a small group of rats (3/sex) revealed that air expired over 24 h contained less than 0.2% of the administered radioactivity, suggesting that very little phenyl ring cleavage occurred.

Apart from muscle (1.2-1.6%), blood (1.0-1.4%) and fat (0.1-0.2%), all other collected tissues had levels of radioactivity that were less than the level of detection (< 0.1%) after 72 h. However, when these results are expressed as a function of tissue weight, detectable radioactivity concentrations that were at least twice background were found in blood (0.13-0.02 µg eq/g), kidney (0.008-0.018 µg eq/g) and lungs (0.08-0.012 µg eq/g) after dosing at 0.125 mg/kg bw. At the higher dose of 2.5 mg/kg bw, the expected 20-fold increase in radioactivity concentration was achieved in all tissues.

Two metabolites, namely cysteinylmethylbenzazimide sulfone and methylsulfonylmethylbenzazimide were identified in approximately equal proportions and collectively accounted for an average of 57% of the radioactivity excreted in urine. Other metabolites characterised and present at low concentrations in urine were cysteinylmethylbenzazimide, methylsulfinylmethylbenzazimide, benzazimide, glutathionylmethylbenzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. The remaining radioactivity was associated with four uncharacterised metabolites but none of these accounted for more than 5% of total radioactivity in urine. Incubating all the urinary metabolites with β-glucuronidase or aryl sulfatase did not yield any additional metabolites suggesting the absence of any glucuronidated or sulfated conjugates. Owing to the low extractability of the radioactivity (approx. 16-29%), metabolites in faeces were not characterised in the low dose groups but for the 2.5 mg/kg bw group five metabolites which accounted for 10-12% of the total dose administered were characterised, namely methylsulfonylmethylbenzazimde, azinphos-methyl oxygen analogue, methylthiomethyl benzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. No unchanged azinphos-methyl was found in urine or faeces.

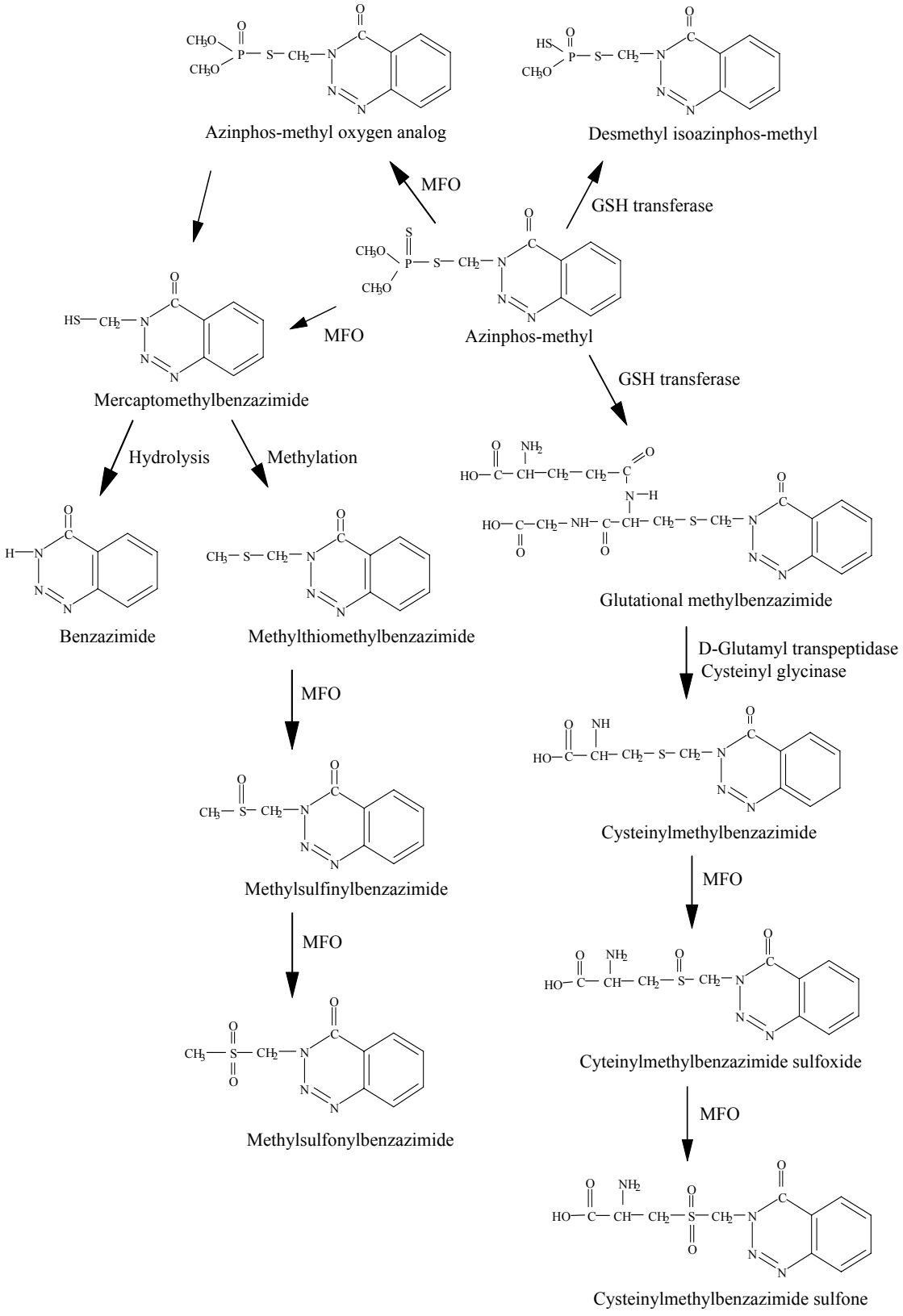
In vitro incubation of radiolabelled azinphos-methyl in the presence of various subcellular fractions confirmed results observed in similar experiments by other investigators, namely

that metabolism of azinphos-methyl in the liver was mediated by glutathione-S-transferase and cytochrome P-450 enzymes.

Based on the metabolites identified in this study, a proposed metabolic pathway in rats is shown in the Figure overleaf.

Conclusions: Following administration of [phenyl-UL-^{14}C]azinphos-methyl by PO gavage to rats two major urinary metabolites, namely cysteinylmethylbenzazimide sulfone and methylsulfonylmethylbenzazimide were identified. Both metabolites were present in approximately equal proportions and accounted for about 57% of the radioactivity excreted in urine. Other characterised metabolites and present at low concentrations in urine were cysteinylmethylbenzazimide, methylsulfinylmethylbenzazimide, benzazimide, glutathionylmethylbenzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. In faeces, 5 metabolites which accounted for 10-12% of the total dose administered (2.5 mg/kg bw) were able to be characterised, namely methylsulfonylmethylbenzazimide, azinphos-methyl oxygen analogue, methylthiomethylbenzazimide, cysteinylmethylbenzazimide sulfoxide and desmethyl isoazinphos-methyl. No unchanged azinphos-methyl was found in urine or faeces.

Metabolic Pathway of Azinphos-methyl in Rats



Franklin CA, Muir NI & Moody (1986) The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicol Lett 33: 127-136.

[This paper also presented the data on DMTP excretion following dermal exposure to azinphos-methyl obtained from human volunteer and occupational exposure studies. Evaluations of these data are presented under the "Humans" sub-section of the Toxicokinetics and Metabolism and Occupational Exposure sections, respectively.]

In this review, the data showing the correlation between dermally and intramuscularly administered azinphos-methyl and the urinary of dimethylthiophosphate (DMTP) in rats were presented with limited details on experimental methods. It was stated that following dermal application of 100-800 µg of azinphos-methyl to the shaved intra-capular skin of male rats, a very strong positive correlation was found with the levels of DMTP excreted. (r = 0.94). Over this dose range, the proportion of the amount of azinphos-methyl applied to the DMTP levels excreted was 10 to 1. When the rats were dosed intramuscularly, approximately twice as much DMTP was excreted. The estimated dermal absorption of azinphos-methyl using urinary DMTP levels was about 50-60%. The data are presented in the following Table.

Excretion of DMTP following intramuscular (im) and dermal administration of azinphos-methyl to rats

Dose (µg/rat)	Route	Cumulative total (µg DMTP)					DMTP /AM[a]
		24 h	48 h	72 h	96 h	120 h	
100 (n=7)	im	15.8					16
200 (n=6)		23.8	24				12
400 (n=4)		74.1	75				19
800 (n=1)		123.7	136	137.7	139		17
100 (n=8)	dermal	6.9	8.3	8.8			9
200 (n=5)		12.2	15.5	16			8
400 (n=5)		23	34.8	36.4	37.3		9
800 (n=3)		18.9	73.6	81.7	86.1	91.2	11

[a]Ratio of DMTP/azinphos-methyl applied

Schroeder RS (1992) Dermal absorption of azinphos-methyl by rats from a Guthion 35% wettable powder formulation using ^{14}C-azinphos-methyl. Study no: 90-722-GE. Lab: Miles Inc., Agriculture Division, Stilwell, Kansas, USA. Sponsor: Miles Inc, Agriculture Division, Kansas City, Missouri, USA. Study duration: October 1990 - March 1991. Report no. 90-722-GE. Report date: March, 1992.

GLP, quality-assured study.

Study and observations: Radiolabelled [phenyl-UL-^{14}C]-azinphos-methyl (lot no. C-107, purity; 99.3%) mixed with unlabelled azinphos-methyl 35% WP was applied topically as a water-based suspension to groups of male SD-derived rats (24/group except for 4/group untreated controls and 4/group for sham-treated controls; Sasco Inc, Missouri, USA) at a nominal dose of 0, 0.056, 0.56 or 5.6 mg/kg bw (0, 0.0196, 0.196 or 1.96 mg/kg bw as ai). The actual administered dose, calculated by subtracting the radioactivity remaining on the applicator, ranged between 90-106% of the nominal dose (ie 0.051, 0.53 or 5.9 mg/kg bw as the WP, or 0, 0.018, 0.185 or 2.08 mg/kg bw calculated as ai). Dose selection was based on the anticipated maximum dermal exposure for field workers. The formulation was applied to a shaved dorsal site measuring 15 cm^2 and then partially dried with an air blower and left unoccluded for the duration of treatment. After 1, 4 and 10 h in metabolism cages, 4

rats/group were removed, residual formulation wiped off with a moistened swab, and then killed. All remaining rats had the residual formulation removed after 10 h, and then continued to be monitored for excretion of radioactivity in urine and faeces for up to 168 h after dosing. At 24, 72 and 168 h after treatment, another 4 rats from each group were sacrificed to enable radioactivity balance monitoring.

Rats were weighed before treatment and observed twice daily except for weekends and holidays when they were only observed once daily. Urine and faeces were collected daily. At sacrifice, any bladder urine collected was added to the daily urine pool for each rat, respectively, and the application site excised and dissected to enable quantification of radioactivity present in the underlying subcutaneous fat and muscle layers. Blood was also collected at sacrifice for the determination of ChE activity in plasma and erythrocytes.

Findings: There were no deaths, body weight changes or treatment-related clinical signs observed. Oedema, red lacrimation and a red nasal discharge observed with similar incidence among treated and control groups were attributed to the fitting of a protective neck collar to prevent PO ingestion of the applied formulation. Based on the actual administered ai dose, the cumulative radioactivity excreted in urine and faeces after 168 h was 26.2% and 9.8%, respectively, at 0.018 mg/kg bw; 11.77% and 5.83%, respectively, at 0.185 mg/kg bw and 10.14% and 5.07%, respectively, at 2.08 mg/kg bw. The cumulative mean recovery of the administered radioactivity in urine, faeces, blood and at the application site skin wash for all sacrifices was 100.1, 80.8 and 94.1% at the actual administered doses of 0.018, 0.185 or 2.08 mg/kg bw, respectively. The non-absorbed radioactivity measured by swabbing the application site after 1, 4 and 10 h was largely unchanged at all doses (by ANOVA and t-test, see Table below). Hence, the majority of the dose was absorbed during the first hour after application. The reduced absorption at the highest tested dose of 5.6 mg/kg bw was attributed to the thickness of the dried residue from the formulation.

Percutaneous absorption of azinphos-methyl in male SD-derived rats

Actual administered dose (mg ai/kg bw/d)	Absorption (%)	
	Direct	**Indirect†**
		1 h exposure (n=4)
0.018	45.8	44.5
0.185	22.8	41.6
2.08	20.2	23.9
		4 h exposure (n=4)
0.018	48.8	47.3
0.185	26.5	39.9
2.08	15.9	21.3
		10 h exposure (n=16)
0.018	54.1	54.8
0.185	31.7	52.4
2.08	20.7	27.3

† The indirect calculation method assumes all unrecovered material is absorbed, hence the generally higher values relative to the direct calculation method.

As shown in the Table below, significant inhibition of ChE activity was only observed in erythrocytes at 10 and 24 h after treatment at 1.96 mg/kg bw ai It should be noted that the group size for treated animals for ChE assays was 4, whereas the controls numbered 8 (4 untreated and 4 sham-treated).

ChE Inhibition (mean % reduction relative to the control) in male SD-derived rats following dermal application of radiolabelled azinphos-methyl (n=4)

Dose (as ai)		Interval (h)	Plasma ChE	Erythrocyte ChE
Actual administered (mg/kg bw)	Nominal (µg/cm^2)			
0.018	0.93	1	[20]	[4]
		4	[25]	[8]
		10	[23]	0
		24	[31]	2
		72	[23]	[2]
		168	[20]	3
0.185	9.3	1	[23]	[16]
		4	[19]	[10]
		10	[40]	[51]
		24	[14]	[4]
		72	[15]	12
		168	[36]	5
2.08	93	1	[16]	14
		4	0	9
		10	[13]	17*
		24	0	16*
		72	[10]	13
		168	1	15

* $p < 0.05$. Values in square brackets indicate the extent (%) to which the measured activity was greater than untreated or sham-treated controls.

The investigators recommended that based on the absorption at the highest dose, a value of 21% be used for dermal absorption in Occupational Health and Safety risk assessment, however, given that the additives in the formulation (see Appendix VII for composition) have an effect on absorption rate. It is likely that other commercial formulations, which contain either different additives, or at different concentrations may result in different absorption rates. The dermal absorption of azinphos-methyl decreased with dose, and ranged from 21% after 10-h exposure at the high-dose to 54% absorption after 10 h exposure at the low-dose, and did not vary considerably with the exposure duration.

Conclusions: Of the radiolabelled azinphos-methyl that was mixed with unlabelled azinphos-methyl 35% WP and applied topically as a water based suspension to groups of male rats at doses of 0, 0.018, 0.185 or 2.08 mg/kg bw ai, the cumulative radioactivity excreted in urine and faeces after 168 h was 36%, 18% and 15.2% at 0.018, 0.185 and 2.08 mg/kg bw, respectively. The majority of each dose was absorbed from the skin during the first hour after application. The dermal absorption decreased with dose, and ranged from 21% after 10 h exposure at the high-dose to 54% absorption after 10 h exposure at the low-dose.

Summary of urinary and faecal excretion of azinphos-methyl in rats following PO or iv administration

Species [strain]	Dose (mg/kg bw)	Label Site	Collection Interval (h)	Faeces (% dose)	Urine (% dose)	Total (% dose)	Reference
Rat [SD]	0.1	carbonyl	0-48	34	62	96	Patzschke *et al* (1976)
				29§	63§	92§	
	2			26	68	94	
				26§	68§	94§	
Rat [SD]	0.125	phenyl	0-72	24.3	71.8	96.1	Kao *et al* (1988)
	2.5			23.9	70.7	94.6	
	Precond‡ 0.125			23.6	70.3	93.9	

‡ Preconditioned at 0.125 mg/kg bw/d for 14 days prior to treatment; § iv administration.

2.2.2 Goats

Gronberg RR, Lemke VJ & Lasley MB (1988) Metabolism of azinphos-methyl in lactating goats. Lab: Mobay Corporation, Mobay Research Park, Stilwell, Kansas, USA. Sponsor: Ciba-Geigy Corp, Greensboro, NC, USA. Study duration: July 1987 – March 1988. Report no. 95649. Report date: March, 1988.

GLP, non-quality assured study.

Study and observations: Radiolabelled [phenyl-UL-^{14}C]-azinphos-methyl (Mobay Chemical Corp., lot no. C-108, purity: 99%, specific activity 70.3 µCi/mg) was administered to 2 lactating goats (Alpine strain, source not reported) in capsules. The amount in each capsule was adjusted for bodyweight difference so that each goat, given 1 capsule/day for 3 days, had a daily dose of 0.5 mg/kg bw/d. Goats were housed in metabolism cages for the duration of dosing after which they were sacrificed (about 17-18 h after the last dose). At the terminal kill, liver, kidney, composite muscle and composite fat were collected and washed free of excess blood for residue analysis and metabolite identification. Milk was collected twice daily, at approx. 8000 h and before dosing at 1600 h. Metabolites in tissues and milk were extracted and then identified using an array of different techniques, ie TLC, HPLC, GC/MS and fast atom bombardment (FAB) mass spectrometry. Radioactivity was quantified by liquid scintillation spectrophotometry after treatment in a sample oxidiser.

Findings: Radioactivity levels detected in the harvested tissues of both goats were low with the most being detected in the liver (0.71 and 0.8 µg eq/g respectively) followed by kidneys (0.28 and 0.32 µg eq/g respectively), muscle (0.06 and 0.08 µg eq/g respectively) and fat (0.03 and 0.04 µg eq/g respectively). Thus, the total tissue radioactivity accounted for a maximum of 0.25% of the administered dose. As expected, levels of radioactivity in the milk on all three days were higher after the first sampling time (0.7-0.8 µg eq/g at 0800 h) than the second (0.03-0.4 µg eq/g at 1600 h).

Metabolites in milk displayed a strong preference for separation into an organic solvent (acetonitrile) so that approx. 2/3 of the radioactivity associated with the soluble fraction was present in this phase. No unchanged azinphos-methyl was found, however, the major metabolite found in this organic phase, accounting for nearly 40% of the total radioactivity, was 3-[(methylsulfonyl) methyl]- 1, 2, 3-benzotriazin-4(3H)-one (methylsulfonylmethylbenzazimide). By contrast, the radioactivity associated with the polar aqueous phase metabolites was distributed equally among four metabolites, namely S-methyl-

S-[4-oxo-(3H)- 1, 2, 3-benzotriazin-3-yl) methyl] dithiophosphate (desmethylisoazinphos-methyl), O-methyl-S-[(4-oxo-3H- 1, 2, 3-benzotriazin-3-yl) methyl] thiophosphate (desmethyl azinphos-methyl oxygen analogue), 3-[(methylsulfinyl)methyl] 1, 2, 3-benzotriazin-4(3H)-one (methylsulfinylmethylbenzazimide) and 3-[(methylsulfonyl) methyl]- 1, 2, 3-benzotriazin-4(3H)-one (methylsulfonylmethylbenzazimide). Judging by the partitioning of the radioactivity between the aqueous and organic phase, the metabolite profile in liver, muscle and fat was similar to that found in milk except that appreciably more radioactivity was associated with the insoluble fraction (solids remaining after vacumn filtration). In milk, the insoluble fraction accounted for about 10-15% of the total radioactivity, whereas in tissues it ranged between 33% in fat to 87% in the liver. Release of much of the radioactivity in the insoluble fraction (up to 77%) was achieved by protease hydrolysis suggesting that azinphos-methyl is converted to a highly reactive mercaptomethylbenzazimide intermediate, which in turn forms disulfide linkages with cystiene moieties in proteins. In the kidneys the extracted radioactivity distributed almost evenly between the organic and aqueous phase and this difference from the other tissues was mainly due to the relatively low concentration of the metabolite, 3-[(methylsulfonyl) methyl]- 1, 2, 3-benzotriazin-4(3H)-one (methylsulfonylmethylbenzazimide). Unchanged azinphos-methyl was only detected in fat accounting for less than 5% of the total radioactivity in that tissue.

Conclusions: Following PO administration of [phenyl-UL-^{14}C]-azinphos-methyl to 2 lactating goats at 0.5 mg/kg bw/d, the radioactivity levels detected in the tissues were low, with the most being detected in the liver followed by kidneys, muscle and fat, accounting for a maximum of 0.25% of the administered radioactivity. About 2/3 of the radioactivity in milk was found in the organosoluble fraction. The major metabolite found in the milk organic phase, accounting for nearly 40% of the total radioactivity, was 3-[(methylsulfonyl) methyl]-1, 2, 3-benzotriazin-4-(3H)-one (methylsulfonylmethylbenzazimide). The radioactivity associated with the aqueous phase metabolites was distributed equally among four metabolites. No unchanged azinphos-methyl was found in milk. The insoluble fraction in milk accounted for about 10-15% of the total radioactivity, whereas in tissues it ranged between 33% in fat to 87% in the liver. The metabolite profile in liver, muscle and fat was similar to that found in milk except that appreciably more radioactivity was associated with the insoluble fraction. In the kidneys the extracted radioactivity distributed almost evenly between the organic and aqueous phase. Unchanged azinphos-methyl was only detected in fat, accounting for less than 5% of the total radioactivity in that tissue.

2.2.3 Cows

Everett LJ, Anderson CA & Macdougall D (1966) Nature and extent of gluthion residues in milk and tissues resulting from treated forage. Chemago Corp., Kansas City, Missouri, USA. J Agric Food Chem 14: 47 - 82

Study and observations: [^{32}P]-Azinphos-methyl (specific activity 1.23 μCi/mg; Bayer AG, Leverkusen, Germany) was administered orally (although the form was not specified, it is presumed from the report title to be in forage) to a lactating Holstein cow at a dose of 5 mg/kg bw.

Findings: Judging from the graphical presentation of the data, the maximum radioactivity in blood and urine was detected between 4 and 6 h after dosing. On the basis of a lack of any phase partitioning of radioactivity into an organic solvent (chloroform), it was reasoned that no unchanged azinphos-methyl or its oxon were present in blood. The appearance of

radioactivity in urine appeared to mimic the decline of radioactivity in plasma and the total amount of radioactivity collected over 88 h in faeces (17%) and urine (40%) accounted for approximately 57% of the administered dose. Similar kinetics, albeit somewhat delayed, were observed in milk where the maximal radioactivity occurred at approximately 24 h (2.16 µg eq/mL) and less than 0.2% of the radioactivity in the 24 h sample was present as unchanged azinphos-methyl or its oxon. Five days after dosing when the cow was killed and samples of brain, fat (omental, renal and subcutaneous), heart, kidneys, liver, muscle and udder taken, the radioactivity was highest in liver (1.46 µg eq/g) followed by kidneys (0.57 µg eq/g) and omental fat (0.56 µg eq/g). Other tissues had levels less than 0.14 µg eq/g. None of the metabolites in blood, urine, faeces, tissues or milk were characterised.

Conclusions: Following PO administration of [^{32}P]-Azinphos-methyl to a lactating cow at 5 mg/kg bw, the radioactivity in urine appeared to mimic the decline of radioactivity in plasma. The total radioactivity collected over 88 h in faeces (17%) and urine (40%) accounted for about 57% of the administered dose. Although somewhat delayed, similar kinetics were observed in milk where the maximal radioactivity occurred at about 24 h. Less than 0.2% of the radioactivity in the 24 h sample was present as unchanged azinphos-methyl or its oxon. Five days after dosing, the highest tissue radioactivity was found in the liver.

Wargo JP & Waggoner T (1978) The effect of feeding Guthion to dairy cattle. Study reference no: 78-R-183. Lab: Mobay Chemical Corporation, Agricultural Division, Kansas, USA. Study duration: Not reported. Report no. 66448. Report date: Jun, 1978.

Pre GLP, non-quality assured study.

Feeding alfalfa pellets containing residues of azinphos-methyl (Guthion; Bayer AG, Leverkusen, Germany) at 11, 33 or 77 ppm (actual concentrations 11.5, 34.02 or 62.96 ppm respectively) to groups of lactating Holstein cows (3/sex/group) for 28.5 days caused no deaths or changes in behaviour, body weight, food consumption or milk production. However, judging from a graph showing the inhibition of ChE in whole blood (relative to pre-test), inhibition in excess of 20% was observed at 77 ppm (equal to 1.2 mg/kg bw/d) on each day of sampling (ie 7, 11, 21 and 28) and on days 11, 21 and 28 for the cattle at 33 ppm (equal to 0.57 mg/kg bw/d). Although very little unchanged azinphos-methyl or its oxon metabolite were detected (ie all below the detection limit of 0.01 ppm) in all collected tissues (ie liver, kidney, muscle and fat) and milk at any dietary concentration, other uncharacterised metabolites (measured by fluorescence) were detected. At 11, 33 and 77 ppm, liver contained 0.25, 0.37 and 0.8 ppm respectively, muscle, 0.08, 0.15 and 0.21 ppm respectively, kidney, 0.29, 0.37 and 0.47 ppm respectively and fat, 0.06, 0.10 and 0.14 ppm respectively.

2.2.4 Humans

Feldman RJ & Maibach HI (1974) Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Phamacol 28: 126-132.

This study also reported dermal absorption data for eleven other pesticides and herbicides. Only the data pertaining to azinphos-methyl are included in the following evaluation.

Study and observations: This study investigated the urinary excretion of twelve dermally administered ^{14}C-labeled pesticides and herbicides in human subjects to quantify their absorption from the skin surface. One of these compounds was Guthion (azinphos-methyl,

radio chemical purity or specific activity, and batch unspecified, source: New England Nuclear Boston, Massachusets or Amersham Searle Corporation, Skokie, IL, USA). Azinphos-methyl in an unspecified volume of acetone was applied on the skin of the ventral surface of forearms of 6 normal, male volunteers (body weight, age unspecified). The dose applied was nominally 4 µg of azinphos-methyl/cm^2 (1 µCi). The study authors stated that this amount was equivalent to the amount that would be deposited by a thin film of 0.25% solution. However, because of variation in the specific radioactivity, the total area of application was changed in order to administer the desired dose. The study authors stated that although the usual dose of radioactivity administered was 1µCi, it was increased to 2 or 5 µCi when the data from iv studies (discussed below) showed slow or low (terms were not defined in the report) urinary excretion. Therefore, the area of application was based on the dose used. Circular skin areas of 1½ to 5 cm diameters were used. When the specific activity of the test chemical was found to be low, several sites were tested, with the chemical being applied on both forearms. The concentration of acetone was also adjusted so that each application site received 0.1 mL of the solution. This volume was pipetted onto the marked application site on the skin and evaporated by gentle blowing during application. It was stated that the solvent remained on the skin for "only a few seconds". The application sites were not covered, and the volunteers were asked not to wash the area for 24 h (ie. application time was 24 h).

Urinary excretion of metabolites following dermal application was compared with the data obtained following iv administration of each chemical. For this purpose, azinphos-methyl in ethylene glycol (1 Ci/mL) was administered slowly into the antecubital vein of each volunteer. The radioactivity dose each person received was 1 µCi.

The 5-day urine collection procedure was the same for both iv and topical studies. Samples were collected every 3-4 h for 12 h, at 24 h post treatment, and then at 24 h intervals for 4 days (8 samples/person/study). The radioactivity in a 5 mL aliquot of each urine sample was determined using the wet ashing method previously described by Maibach and Feldman (1967). The radioactivity was measured by liquid scintillation spectrophotometry, and the results were calculated as a percentage of the administered dose. In calculations, the results of topical studies were corrected for incomplete urinary excretion; ie if 50% of test compound was excreted following iv administration, all values for topical administration were doubled.

Findings: The percentage of radioactivity (mean values, corrected for incomplete urinary excretion) excreted following iv and topical administration is given in the following Table.

Urinary excretion of ^{14}C-labeled azinphos-methyl by human subjects after iv and topical administration

Percent of administered dose/h (sampling period in h)*										
0-4	**4-8**	**8-12**	**12-24**	**24-48**	**48-72**	**72-96**	**96-120**	**% dose**	**SD**	**T½ (h)**
Intravenous administration										
1.51	1.20	1.60	1.04	0.81	0.46	0.26	0.13	69.5	6.9	30
Topical administration										
0.04	0.20	0.29	0.28	0.21	0.13	0.06	0.04	15.9	7.9	-

*Includes the 50% correction factorr (see text).

The total azinphos-methyl radioactivity recovered in the urine over the 120 h sampling period following iv administration was about 69% of the administered dose. Following topical application of azinphos-methyl, the total radioactivity excreted in urine during the same

sampling period was about 16% of the administered dose. No urinary metabolites of azinphos-methyl were identified, nor were any data for faecal excretion of radioactivity provided.

Conclusions: The data presented in this study demonstrated some dermal absorption of azinphos-methyl following application to the skin of ventral forearm of human subjects. About 16% of the dermally administered dose was excreted in urine within 120 h, after a 24 h application period. No urinary metabolites of azinphos-methyl were characterised or identified. Because the radioactivity data in blood and faeces for this single dose study are lacking, and the influence of the vehicle used cannot be established with the limited information provided, the value of the study findings is limited.

Franklin CA, Muir NI & Moody (1986) The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicol Lett 33: 127-136.

Study & observations: This review report also included data from a pilot study conducted in human volunteers (age body weight unspecified) in which dermal doses of 500 to 6000 µg of azinphos-methyl of unspecified purity were applied on the forehead of volunteers. No further details on experimental methods were provided.

Findings: Urinary dimethylthiophosphate (DMTP) levels at different sampling times following dermal application of azinphos-methyl on the forehead of volunteers are given in the following Table.

Excretion of DMTP following dermal application of azinphos-methyl to the forehead of human volunteers

Dose[a] (µg/person)	Cumulative total (µg DMTP)			Ratio[b]
	24 h	48 h	72 h	
500	54	76	85	0.17
1000	96	130	152	0.15
2000	56	90	119	0.06
4000	154	267	404	0.10
6000	153	284	323	0.05

[a]Two replicates/dose; [b]Ratio of DMTP/azinphos-methyl applied.

Conclusions: According to the data, the ratio between DMTP excreted at 72 h and azinphos-methyl applied was about 1:10, and was similar to the ratio observed in rat dermal studies by the study authors (data presented under rat metabolism studies).

Selim S (1999) Absorption, excretion, balance and pharmacokinetics of ^{14}C radioactivity after single dose dermal application of three dose levels of ^{14}C labelled guthion to healthy volunteers. Study no: XBL 98052. Lab: Xenobiotic Laboratories Inc (XBL), 107 Morgan Lane, Plainsboro, NJ, USA and Bayer AG, PH-PDT Toxicology, Elberfeld 0514, D-42096, Wuppertal, Germany (analytical phase), and Pharma Bio-Research Clinics, BV (PBR), Beilerstraat 16, 9401 PK Assen, The Netherlands (clinical phase). Sponsor: Bayer Corporation, Stilwell, KS 66085, USA. Study duration: May 7 1998 to February 17 1999. Report no: 108891. Report date: March 24 1999.

Quality assured, GLP (21 CFR, Part 58, FIFRA, 40 CFR Part 160 and OECD) study. All aspects of the clinical portion were conducted in accordance with Good Clinical Practice Regulations.

Study and observations: This study was performed to determine the dermal absorption and excretion of azinphos-methyl in healthy human volunteers. Groups of 6 male volunteers [18-45 years old, body weight: within ± 15% deviation from normal range] each received ^{14}C labelled azinphos-methyl (radiochemical purity: 98.9% in isopropyl alcohol (IPA) as single dermal doses of 3 and 10 µg ai/cm^2 or an aqueous suspension of a 14C labelled Gusathion M WP 25 (radiochemical purity: 97.5%, formulation details provided) at a dose of 5 µg ai/cm^2. [The study author stated that these doses represent the range of exposure levels typically encountered by agricultural workers and the doses selected together with the study design were discussed with, and approved by California EPA prior to study initiation]. A 100 µL aliquot of each preparation was applied over a 4 x 6 cm intact, non-shaven area of the volar aspect of the non-dominant forearm. [The test substance was applied using a calibrated micropipette and spread evenly over the skin area. The amount of residual activity remaining in the micropipette was subtracted from the theoretical amount administered to each volunteer to determine the actual amount administered].

The application site was covered with a non-occlusive dome [aluminium domes with air holes were secured in place with an adhesive bandage] and the applied materials were allowed to remain in contact with the skin site for approximately 8 h. [An indwelling venous catheter was placed in both arms for blood collection]. After removal of the protective enclosures, the skin sites of the first 2 groups (ai) were cleansed with cotton swabs and IPA, while for the third group (25 WP), 2% soapy water was used instead of IPA. One third of the dosed site was also "stripped" with tape [adhesive cellophane tape] approximately 45 h after the removal of the protective enclosures to determine the amount of residual radioactivity associated with the surface layer of the skin.

All urine and faeces excreted by the volunteers were collected for a 5 to 8 day period following dose administration. Venous blood samples were collected from the ipsilateral and contralateral veins during and after the exposure period [at 0 (pre-dose), 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h after application of the dose] to obtain information on the onset, rate and duration of absorption. Plasma samples from all groups and red blood cells [RBC] for the high-dose group were analysed for total radioactivity [Liquid Scintillation Counting]. Additional blood samples were collected [at the above sampling times and from groups that received azinphos-methyl at 3 and 10 µg ai/cm^2] for the determination of ChE [in plasma and RBC (as AChE/erythrocyte and reported as CHEE and AChE/erythrocyte/gram of haemoglobin and referred to as CEHB) using a method modified from Ellman *et al* of (1961), reference values provided].

Findings: Data of venous plasma samples for both the ipsilateral and contralateral veins showed an increase in the level of radioactivity at the first 8 h time intervals, while the test material was still on the skin of all subjects. The concentration of ipsilateral radioactivity was higher than the corresponding value in the contralateral plasma [prior to removal of the applied test substance]. After removal of the test material, plasma radioactivity from the ipsilateral and contralateral arms were comparable indicating complete removal of radioactivity. Peak radioactivity in the contralateral plasma was about 10 h. Plasma radioactivity plattaued from 10 to 36 h, then slowly decreased with time, indicating a slow elimination of radioactivity. The half-life of plasma radioactivity for the ipsilateral and

contralateral arm was similar and averaged 94.0, 90.5 and 72.5 h for groups 1, 2 and 3, respectively. [In volunteers dosed with Gusathion M WP 25, peak plasma radioactivity was recorded at 6 h post treatment.]

The analysis of the RBCs showed that some of the radioactivity binds to RBCs, however, the radioactivity is lower than in plasma.

A mean 25.7%, 21.0% and 19.2% of the administered radioactivity was recovered in the urine of volunteers in groups 1, 2 and 3, respectively. The urinary excretion of radioactivity during the 8 h exposure time was low and the predominant amount of radioactivity was slowly excreted during the 24 to 96 h period. Faecal radioactivity represented 3.3, 2.4 and 2.6% of dosed radioactivity for groups 1, 2 and 3, respectively.

Recovery of radioactivity (%)

Formulation Dose Level	ai in IPA 3 µg a./cm^2 (0.79 µg/kg bw)	ai in IPA 10 µg ai/cm2 (3.0 µg/kg bw)	25 WP 5 µg ai/cm^2 (1.49 µg/kg bw)
Urine	25.72	20.99	19.22
Faeces	3.30	2.36	2.59
Tape stripping	0.30	0.15	0.08
Swabs	72.39	73.13	78.01
Skin rinsate	2.47	2.12	0.88
Dome	0.24	2.46	0.74
Duoderm	0.61	1.02	0.27
Gauze	0.33	0.25	0.08
Total	105.36	102.46	101.88

The majority of applied radioactivity was accounted for in the swabs, with 72.4, 73.1 and 78.0% of radioactivity for groups 1, 2 and 3, respectively. Soapy water was equally effective as IPA in removing the surface radioactivity. The mean total recovery of administered radioactivity was 105.4, 102.5 and 101.9% for groups 1, 2 and 3, respectively. For all groups, tape stripping contained less than or equal to 0.3% of applied radioactivity, indicating that the active ingredient does not accumulate in the superficial layers of the skin.

Plasma ChE activity and the RBC AChE activity for volunteer groups 1 and 2 were within the normal range and similar to the pre-dose values [with the exception of group 2 CHEB values at 0 to 12 h, which were around 21 U/g, but were comparable to the pre-dose data].

Plasma ChE and RBC AChE values (kU/L)

Formulation Dose level	ai in IPA 3 µg ai/cm^2 (0.79 µg/kg bw)		ai in IPA 10 µg ai/cm^2 (3.0 µg/kg bw)	
Time	**Plasma ChE**	**RBC AchE**	**Plasma ChE**	**RBC AChE**
Pre-dose	5.37	7.09	5.65	6.28
2 h	5.21	7.34	5.46	6.47
4 h	5.26	7.05	5.42	6.19
8 h	5.10	7.34	5.34	6.67
12 h	5.11	7.11	5.43	6.28
24 h	5.20	7.38	5.28	7.45
48 h	5.22	7.78	5.26	7.14
120 h	5.03	7.99	5.66	7.59
Normal range	3.5-8.5	5.26-9.62	3.5-8.5	5.26-9.62

The mean dermal absorption of radioactivity (sum of urine, faeces and tape stripping) for volunteers in groups 1 and 2, receiving the ai in IPA at doses of 3 and 10 µg ai/cm^2, were 29.3% and 23.5% respectively. The mean dermal absorption of radioactivity for volunteers in group 3, receiving the 25 WP formulation at a dose of 5 µg ai/cm^2, was 21.9%.

Conclusions: The mean dermal absorption of radioactivity for volunteers receiving azinphos-methyl (in isopropyl alcohol) at single doses of 3 and 10 µg ai/cm^2 was 29.3% and 23.5%, respectively. The mean dermal absorption of radioactivity for volunteers receiving an aqueous suspension of a Gusathion M WP 25 formulation at a single dose of 5 µg ai/cm^2 was 21.9%. There were no treatment-related effects on plasma and RBC ChE activity.

2.2.5 *In vitro* Studies

Motoyama N & Dauterman WC (1972) The in vitro metabolism of azinphosmethyl by mouse liver. Department of Entomology, North Carolina State University, Raleigh, North Carolina, USA. Pesticide Biochem Physiol 2: 170-177.

Subcellular fractions prepared from the livers of white mice were incubated in the presence of 0.16 mM of [carbonyl-^{14}C]-azinphos-methyl or its oxygen analogue (both synthesized in house, purity > 99%, specific activity approximately 0.91 µCi/mg). The formed metabolites were identified by co-migration with non-radioactive standards on silica gel TLC and paper chromatography. Radioactivity on the chromatographs was detected using a scanner.

The major activity occurred in the microsomal and the soluble fractions. Microsomes catalysed the formation of dimethyl phosphorothionic acid, the oxygen derivative of azinphos-methyl and subsequently dimethyl phosphoric acid. By contrast, the only metabolite observed after incubation of azinphos-methyl with the soluble fraction was desmethyl azinphos-methyl. No product was detected after incubation with the oxygen analogue of azinphos-methyl (oxon).

Murphy SD & DuBois KP (1957) Enzymatic conversion of the dimethoxy ester of benzotriazine dithiophosphoric acid to an anticholinestrase agent. Department of Pharmacology, University of Chicago, Chicago, Illinois, USA. J Pharmacol Exp Therap 119: 572-583.

This somewhat dated report compared the rate of enzymatic conversion of azinphos-methyl by liver homogenates to an uncharacterised metabolite that had the ability to inhibit ChE activity. The rate of conversion was greatest in the presence of crude liver homogenates from the rat followed in order by that from mice and guinea pigs, ie male rats,11.2; female rats, 4.7; male mice, 3.0; female mice, 3.8; male guinea pigs, 2.5; female guinea pigs, 2.1; all measured as units of metabolite formed/5 mg of liver/h. Fractions taken after differential centrifugation indicated that the activity was confined to the microsomal fraction and that this activity could be readily inactivated if incubated at 38°C.

Dahm PA, Kopecky BE & Walker CB (1962) Activation of organophosphorus insecticides by rat liver microsomes. Departments of Zoology and Entomology, Iowa State University, Ames, Iowa, USA. Toxicol Appl Pharmacol 4: 683-696.

The rate of activation of a number of different organophosphate insecticides by male rat liver microsomes was compared by assaying the degree of ChE inhibition achieved in rat brain and fly head preparations. Whilst no effect was observed for demeton, phorate or dimethoate strong inhibition occurred with methyl parathion, diazinon and azinphos-methyl. Fluoride ions and SKF-525A slowed the rate of activation of azinphos-methyl.

Hitchcock M & Murphy SD (1971) Activation of parathion and guthion by mammalian, avian, and piscine liver homogenates and cell fractions. Department of Physiology, Harvard University, Boston, Massachusetts, USA. Toxicol Appl Pharmacol 19: 37-45.

The rate of enzymatic conversion of methyl parathion and azinphos-methyl to metabolites capable of inhibiting rat brain ChE activity was compared. In the presence of NADP and glucose-6-phosphate, crude liver homogenates from mammals (male mice, rats, guinea pigs) catalysed the reaction more quickly than from birds (cockerels, quails) or fish (bullhead and flounder). However, the rate of conversion of azinphos-methyl was unchanged in the presence of NAD suggesting that activation is not a NADPH specific reaction.

Levine BS & Murphy SD (1977) Effect of piperonyl butoxide on the metabolism of dimethyl and diethyl phosphorothionate insecticides. Department of Physiology, Harvard University, Boston, Massachusetts, USA. Toxicol Appl Pharmacol 40: 393-406.

In an attempt to correlate the metabolism with the observed toxicity of organophosphates, the effect of inhibiting the *in vitro* activity of male mouse mixed-function oxidase activity with piperonyl butoxide was investigated. Microsomal mixed function oxidases are known to control the rate of conversion of a number of biologically inactive organophosphate insecticides to their active oxon analogues. In the current study, mixed function oxidases were shown to potentiate the toxicity of parathion-methyl and azinphos ethyl but to antagonize that for parathion and azinphos-methyl in the presence of piperonyl butoxide. Furthermore, although *in vitro* glutathione-dependent detoxification of parathion methyl occurred irrespective of the mixed-function oxidase activity, the detoxification of azinphos-methyl was dependent on its activity. Similarly, the oxygen analogue of parathion methyl (ie methyl paraoxon) was rapidly metabolised by the glutathione-dependent enzyme whereas the oxygen analogue of azinphos-methyl was not. The rate of conversion of methyl paraoxon was shown to be more rapid than for parathion methyl. These results were consistent with the observation of a 40-fold antagonism of parathion methyl toxicity by piperonyl butoxide compared with only 3-fold for azinphos-methyl.

Lin S-N, Chen C-Y, Murphy SD & Caprioli RM (1980) Quantitative high performance liquid chromatography and mass spectroscopy for the analysis of the in vitro metabolism of the insecticide azinphos-methyl (Guthion) by rat liver homogenates. Division of Toxicology and Analytical Chemistry Center, University of Texas Medical School at Houston, Houston, Texas, USA. J Agric Food Chem 28: 85 - 88.

This report described the use of HPLC coupled with mass spectrometry (which overcomes the thermal decomposition associated with GC analysis) to positively identify the oxygen analogue of azinphos-methyl (oxon) following *in vitro* incubation of azinphos-methyl with male rat liver homogenates. The subsequent formation of benzazimide was shown to arise either directly from azinphos-methyl or indirectly via the oxon metabolite, however, the rate of formation was much more rapid via the direct route.

2.2.6 Benzazimide

Weber H, Patzschke K & Wegner LA (1980) [Phenyl-UL-^{14}C]-Benzazimide. Biokinetic study on rats. Report no. 9005. Bayer AG. Institute for Pharmacokinetics. Isotope Laboratory, Wuppertal, Germany. Report date: 10 March 1980.

Pre GLP, non-quality assured study.

Study and observations: The distribution and excretion of an azinphos-methyl metabolite [phenyl-UL-^{14}C]-benzazimide (Mobay Chemical Corp, lot no. C-235, purity: 99%, specific activity 136 μCi/mg (5 Mbq/mg)) in Cremophor EL (5% v/v in the end volume) was investigated in SD rats (72 males and 5 females, Mus Rattus AG, Munich, Germany) following both PO and iv administration. Apart from a comparison of the radioactivity excreted in urine and faeces between male and female rats following gavage administration at 1mg/kg bw, only male rats were used for the other investigations in the study. Group size was generally limited to 5 except for the whole body autoradiography where only 1 or 2 rats were used for the iv or PO dosing regimens respectively.

For the duration of the study, rats were housed in metabolism cages to facilitate the collection and measurement of radioactivity in expired gases, urine and faeces. Radioactivity in the whole rat (minus GI tract), liver, kidneys, brain, muscle, skin, testes, adrenals, whole blood, erythrocytes, plasma and fat was measured by sample oxidizer treatment and liquid scintillation counting 3, 6, 24, 48, 72, 144 and 240 h after gavage administration at 1 mg/kg bw. Whole-body autoradiography was performed 5 min after iv administration or at 6 and 48 h after gavage administration. However, due to the poor quality of the autoradiograph photocopies, no meaningful information was discernible from these studies. To monitor radioactivity in excreta, urine and faeces were collected for 2 days following gavage dosing at 0.05, 1 or 5 mg/kg bw or by iv dosing at 1 mg/kg bw. Radioactivity in the expired gases was monitored for 24 h after gavage dosing at 1 mg/kg bw. Excretion of radioactivity in bile was measured for 24 h following iv or intraduodenal administration at 1 mg/kg bw.

Findings: The absorption and excretion of 1 mg/kg bw of gavage-administered benzazimide was rapid as judged by the appearance and decline of radioactivity in the whole body (minus the GI tract). In plasma, the C_{max} (1.7 μg eq/g) for radioactivity (ie for benzazimide and any of its metabolites) was achieved approximately 2 h after PO administration. At 3 h (the first sampling for whole-body radioactivity), approximately 82% of the administered dose was detected in the whole animal (minus the GI tract), whereas by 24 h this had declined to only about 1%. Radioactivity excreted in urine and measured 1, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42 and 48 h after gavage dosing showed little difference in rate or quantity (54-58%) between the lowest (0.05 mg/kg bw) and highest (5 mg/kg bw) dose administered. Similarly, radioactivity excreted in faeces over the same time interval (0-48 h) ranged between 41 and 45%. Although only 1 dose (1 mg/kg bw) of benzazimide was tested, females appeared to excrete somewhat more radioactivity in urine (66%) and slightly less in faeces (33%) relative to males, ie 57% and 42%, respectively. An iv administration of 1 mg/kg bw resulted in an almost equal ratio of radioactivity in urine (58%) and faeces (41%) in males. This result suggests almost all of the orally administered benzazimide is rapidly absorbed from the GI tract and excreted in urine and bile. This is consistent with results indicating that orally administered parent compound is almost completely absorbed and rapidly excreted. This contention was confirmed with the observation that 55% and 38% of the radioactivity of a 1 mg/kg bw dose was recovered in the bile within 24 h following iv and intraduodenal administration

respectively. However, the extent to which any bile-excreted radioactivity undergoes enterohepatic circulation was not investigated. Very little of the absorbed benzazimide was metabolised to carbon dioxide since less than 0.04% of a 1 mg/kg bw PO dose is found in air. Recovery of radioactivity from urine, faeces and expired air was able to account for 99.34% of the 1 mg/kg bw gavage administered dose.

Tissue radioactivity measurements indicated that apart from the organs involved in excretion (ie kidneys and liver), there did not appear to be any accumulation of radioactivity in tissues. The total benzazimide/metabolite concentration in all tissues (whole body minus GI tract) after 3, 6, 24 and 48 h was 0.63, 0.35, 0.014 and 0.007 µg eq/g respectively. For individual tissues the radioactivity detected after 24 h was generally low, with less than 0.05 µg eq/g being detected except for liver (0.12 µg eq/g), erythrocytes (0.11 µg eq/g) and kidneys (0.06 µg eq/g). The elimination half-life from tissues over the period from 2-10 days was approximately 4 days, whereas from plasma (or blood), calculated over 24 h, it was estimated to be between 2-3 h.

Conclusions: Following gavage administration of radiolabelled benzazimide to rats at 0.05, 1.0 and 5 mg/kg bw, the radioactivity excreted in urine at intervals between 1 and 48 h after dosing showed little difference in rate or quantity (54-58%) between the lowest and highest dose. The radioactivity excretion in faeces over 48 h ranged from 41 to 45%. Although only 1 dose (1 mg/kg bw) of benzazimide was tested, females appear to excrete somewhat more radioactivity in urine (66%) and slightly less in faeces (33%) relative to males, ie 57% and 42%, respectively. Intravenous administration at 1 mg/kg bw resulted in an almost equal ratio of radioactivity in urine (58%) and faeces (41%) in males. The results suggest that almost all of the orally administered benzazimide is rapidly absorbed from the GI tract and excreted in urine and bile. This contention was confirmed with the observation that 55% and 38% of the radioactivity of a 1 mg/kg bw dose was recovered in the bile within 24 h following iv and intraduodenal administration respectively. The extent to which any bile-excreted radioactivity undergoes enterohepatic circulation was not investigated.

2.3 ACUTE TOXICITY

2.3.1 Technical Grade Active Constituent

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

2.3.1.1 Median Lethal Dose Studies

Azinphos - Methyl

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO							
Mouse [ICR/SIM]	M	20	Corn oil	NS	20, 40, 80	15	Simmon (1978)
Rat [NS]	NS	10	Water and Cremophor	92.2	15, 17.5, 25, 35	20.7	Ciehozuis (1975)
Rat [SD]	M/F	5/sex/group	Corn oil	NS	6, 8, 10, 12, 14	12.2 (M), fasted 10.6 (F), fasted	Cooper and Terrell (1978)
Rat [SD]	M/F	4	DMSO	99	2, 4, 8, 16	5.6 (M), fasted 6.4 (F), fasted	Crawford and Anderson (1974)
Rat [SD]	F	25	Ethanol and propylene glycol	97	NS	16.4	DuBois *et al* (1955) and DuBois *et al* (1957)
Rat [NS]	M	10	Water and cremophor EL	NS	20, 25, 30, 35, 50	25.4	Flucke (1979)
Rat [Sherman]	M/F	NS	Peanut oil	NS	NS	13 (M) 11 (F)	Gaines (1969)
Rat [NS]	M	10	Water and cremophor EL	NS	5, 7.5, 8.5, 9, 10	9.1 (fasted)	Heimann (1981)
					10, 15, 25	17.25 (non-fasted)	
Rat [Wistar]	M	10 or 20/group	Water and cremophor EL	88.9	5, 6.3, 6.7, 8.0 (fasted)	6.7 (fasted)	Heimann (1982)
		10			10, 12.5, 16, 20 (non-fasted)	12.8 (non-fasted)	
Rat [NS]	M	5	Water and cremophor EL	NS	5, 6.3, 8, 8.5, 9	7.1 (fasted)	Heimann (1987a)

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Rat [SD]	F	4	CMC	99	4, 8, 16, 32	16 (fasted) 10 (non-fasted)	Lamb *et al* (1974)
Rat [Wistar]	M	15	Water and cremophor EL	91.6	1, 2.5, 3.5, 4, 5, 6, 7.5, 10	4.6 (fasted)	Mihail (1978)
	F				1, 2.5, 3.5, 5, 5.5, 6, 7.5	4.4 (fasted)	
Rat [SD]	F	4	Panasol AN-2	NS (2 samples)	7.5, 10, 15, 20	12.2 15	Nelson (1968)
Rat [CD]	M/F	2/sex	Methylene chloride + 10% arabic gum in Tween 80	> 95	NS (5 dose levels)	26 (M) 24 (F)	Pasquet *et al* (1976)
Rat [NS]	M	10	Water and cremophor EL	92.7	10 (2 groups), 15, 20, 25	15.5	Thyssen (1976a)
Rat [SD]	M/F	5	Polyehylene glycol	NS	6, 8, 10	9.0 (M) 6.7 (F)	Crown & Nyska (1987) (GLP)
Guinea Pig [NS]	M	NS	Ethanol and propylene glycol	97	NS	80	DuBois *et al* (1957) and DuBois *et al* (1955)
Dog [Beagle]	M	1 or 2/group	Water and Cremophor EL	91.6	1, 2.5, 5, 10	> 10 (0/2 deaths) fasted	Mihail (1978)
Dermal (intact)							
Rat [Sherman]	M/F	10/group	Xylene	NS	NS (72 h, non-occluded, applied material not removed)	220	Gaines (1969)
Rat [Wistar]	M	5 or 10/group	Water and cremophor EL	88.9	100, 160, 200, 250, 315, 400 (24 h, occluded, washed)	200-250	Heimann (1982)
	F				63, 100, 160, 250 (24 h, occluded, washed)	155	
Rat [Wistar]	M/F	5 or 10/sex	Water and cremophor EL	91.6	100, 500, 1000, 1500, 2500, 5000 (24 h, occluded, washed)	2500-5000	Mihail (1978)
Rat [SD]	F	4	Panasol AN-2	NS	50, 62.5, 70, 75, 100, 125, 150	72.5	Nelson (1968)
Rat [SD]	F	10	Acetone: ethanol: peanut oil (1:1:2)	95	NS	90	Pasquet *et al* (1976)
Rabbits [NZW]	M/F	2/sex/group	NS	NS	400, 750, 1000, 1500, 2500 (24 h, occluded, applied material removed)	1380	Seaman and Doyle (1978)

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Rabbits [Albino]	M/F	5	Water	NS	2000	>2000	Kenan & Nyska (1987) (GLP)
ip							
Mouse [Carworth]	M/F	NS	Ethanol and propylene glycol	97	NS	5.4 (M) 3.4 (F)	DuBois *et al* (1955) and DuBois *et al* (1957)
Rat [SD]						11.6 (M) 5.7 (F)	
Rat [Holtzman]	M	24 (weanlings)a nd 20 (adults)	Ethanol and propylene glycol	NS	NS	3.4 (weanlings) 4.9 (adults)	Brodeur and DuBois (1963)
Rat [SD]	F	4	Panasol AN-2	NS (2 samples)	4, 6, 7.5, 8, 9, 10	8.5	Nelson (1968)
Rat [Wistar]	M/F	5/sex	Cremophor EL	91	1, 5, 6.7, 8, 10 (M/F) and 9 (F)	6.9 (M), approximately 9-10 (F)	Krotlinger (1993) (GLP)
Guinea Pig [NS]	M	NS	Ethanol and propylene glycol	97	NS	40	DuBois *et al* (1955) and DuBois *et al* (1957)
						8.9	

SD = Sprague-Dawley, NZW = New Zealand White, NS = Not stated; CMC = carboxymethylcellulose

Species [strain]	Sex	Method	Group size	Vehicle	Purity (%)	Doses Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Inhalation								
Mouse [CF]	F	Whole body, 1 h	10	Xylene	86	34.0, 37.6, 38.1, 41.7, 51.0, 83.8, 99.6 (50% droplets 1.8 μm)	approximately 2300 (40)†	Doull and DuBois (1956)
Rat [NS]	M/F	Whole body, 1 h	5/sex	NS	NS	17560 (droplet size not stated)	> 17560	Bracha (1976)
Rat [Wistar]	M/F	Whole body, 4 h	5/sex	Acetone	NS	210	>210	Shapiro (1987) (GLP)
Rat [SD]	M/F	Head only, 4 h	10/sex	PEG: ethanol	88.8	80, 105, 141, 192, 250 (droplets ≤2 μm)	155 (M) 132 (F)	Shiotsuka (1987a) (GLP)
Rat [SD]	M/F	Head only, 1 h	10/sex	PEG: ethanol	88.8	248, 287, 291, 337, 414 (M/F) and 134, 583, 753 (M) (1.8-2.9 μm)	396 (M) 310 (F)	Shiotsuka (1988a) (GLP)

CF = Carworth Farms, SD = Sprague-Dawley, PEG = polyethylene glycol-400

† = value in parentheses is a corrected value calculated by the reviewing toxicologist due to an error in the original study report

2.3.1.2 Eye and Dermal Irritancy and Sensitisation Studies

A summary of findings of eye and dermal acute irritancy and sensitisation studies for the technical grade active constituent is presented in the following Table.

Eye and skin irritation and skin sensitisation studies

Route	Species	Sex	Group size	Method	Findings	Reference
Ocular	Rabbit [NZW]	NS	6	100 mg, washed	Slight irritant	WARF Institute (1976)
		M/F	8	conjunctival sac, washed	Slight irritant	Thyssen & Lorke (1981)§
		M/F	3	conjunctival sac, washed	Slight irritant	Kenan (1987) (GLP)‡
		M	6	100 µL, conjunctival sac, unwashed	Slight irritant	Zorbas (1994) (GLP)
Dermal	Rabbit [NZW]	NS	6	500 mg, intact and abraded, occlusive	Non irritant	WARF Institute (1976)
	Rabbit	M/F	8	Intact and abraded skin	Non irritant	Thyssen & Lorke (1981)§
		M/F	2 M, 4 F	500 mg, intact, occlusive	Non-irritant	Crown (1987) (GLP)‡
		M	6	500 mg, occlusive, intact	Non irritant	Zorbas (1994) (GLP)
	Human	NS	347	1% solution in petrolium ether, patch tested (conditions unspecified)	Non irritant	Lisi *et al* (1981)
Skin sensitisation	Guinea pig	M	20	Induction and challenge applications (M and K GPMT)	Sensitiser	Flucke (1986b)‡ (GLP)
			15	Induction and challenge applications (BM)	Sensitiser	Porter *et al* (1987)‡ (GLP)
			12		Sensitiser	Heimann (1987b)‡ (GLP)
			10		Non-sensitiser	Shapiro (1987) (GLP)

NS = Not stated; NZW = New Zealand White; ¶ Method modified from US FIFRA Guidelines; § US Fed Reg (1973); ‡ OECD Guideline; M and K GPMT = Magnusson and Kligman's guinea pig maximisation test; BM = Buehler Method

Eye irritation

WARF Institute Inc (1976) Report on the acute skin irritation, eye irritation and inhalation studies with Cotnion-methyl technical. Study no. not stated. Lab: WARF Institute Inc, Madison, WI, USA. Sponsor: Makhteshim, Beer-Shiva, Israel. Study duration: not stated. Report no. 6051091. Report date: 22 July 1976.

Pre GLP, non-quality assured study. (It was stated that the method adopted meets the requirements of Title 21 of the Code of Federal Regulations, 191.11).

Study and observations: An aliquot of 100 µL (about 100 mg) of Cotnion-methyl technical (azinphos-methyl; batch, purity unspecified) was instilled in one eye of each of 6 NZW rabbit (sex, bw, age and source unspecified). The untreated eye served as a control. Ocular responses (cornea, iris and the bulbar and palpebral conjunctivae) were evaluated at 24, 48 and 72 h after treatment and scored. Prior to scoring, any residual test material and

accumulated discharges in the eye were rinsed. No further information on experimental methods was provided.

Findings: Grade 1 conjunctival redness was seen in 2/6 rabbits at 24 h post treatment and not thereafter (eye irritation score of 0.67). No further ocular responses were observed.

Conclusions: Under the conditions of the study, the test substance was considered to be a slight eye irritant.

Thyssen J & Lorke D (1981) R1582 (azinphos-methyl, the active ingredient of Guthion) Study of the irritant effect on the skin and mucous membranes (eye). Study no: T 9010594 (eye). Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Federal Republic of Germany. Sponsor: Bayer AG. Study duration: July 1981. Report No. T 90 10 594. Report date: 19 October 1981.

Study performed in accordance with the guidelines recommended by the US Department of Health, Education and Welfare, Federal Register, 37 (83): 8535, 1972. No GLP statement provided.

Eight NZW male and female rabbits (source: Degenfeld, Eybach, Germany; bw range: 3-4 kg) received a single application of R1582 (azinphos-methyl; batch no. 230 105 019, purity 92.4%, dose unspecified) into the conjunctival sac of the eye of each of 5 rabbits for a contact time of 5 minutes and 3 animals for a contact time of 24 h, after which the material was washed from the eye. Animals were examined for signs of redness, swelling and ulceration at 1, 24, 48, 72 h, and at 7 days post application according to the criteria specified in the reference above. The test animals were individually housed and provided with food and water *ad libitum*.

Findings: (i) 5-minute exposure: Grade 1 erythema of the conjunctivae was observed in all animals (5/5) at 1 h post treatment. No other signs of ocular irritation were observed at the other observation times.

(ii) 24-h exposure: All animals (3/3) showed grade 2 conjunctival erythema at 1 h, and grade 1 erythema at 24 h post treatment. No further ocular responses to treatment were apparent at any other observation times.

Conclusions: Under the conditions of the study, azinphos-methyl was classified as a slight eye irritant.

Kenan G (1987) Cotnion-M: Acute eye irritation/corrosion study in rabbits. Study no: not stated. Lab: Life Science Research Israel Ltd, PO Box 139, Ness Ziona 70 451, Israel. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer Sheva, Israel. Study duration: April 6-10, 1987. Report no. MAK/118/AZM. Report date: 19 May 1987.

Quality assured GLP (US EPA & OECD) study. The method conformed to the recommendations of the OECD Guideline for Testing of Chemicals, Section 405 of 1981, and the US EPA FIFRA Guidelines (81-4) of 1982 was used.

Study & observations: A 100 mg quantity of Cotnion-M (azinphos-methyl, batch no. 50287, brownish granulated powder, purity: unspecified), was placed into the conjunctival sac of the

right eye of each of 6 albino rabbits of a local strain (3/sex, source: Loebenstein Laboratory Animals Yoqneum, bw: 1.9-2.5 kg). The left eye served as a control. The eyes were washed after 4 h, and an Elizabethan collar was used to prevent removal of residual test material from the eye and subsequent ingestion. The animals were individually housed and provided with food and water *ad libitum*. Ocular reactions (irritation of the cornea, iris and conjunctiva) to treatment were assessed at 1, 24, 48 and 73 h after treatment according to the criteria specified in the Draize method.

Findings: Grade 1-3 conjunctival redness and grade 1-2 ocular discharge were seen in all rabbits at 1 h post treatment, which resolved in 5 animals by 24 h. In I rabbit, grade-1 conjunctival redness was still evident at 24 h, but not thereafter. No other ocular reactions were observed. Based on these findings, the test compound was considered to be a slight eye irritant by the study authors.

Conclusions: Under the conditions of the study, azinphos-methyl was a slight eye irritant in rabbits.

Zorbas MA (1994) Primary eye irritation study with technical grade Guthion in rabbits. Study no: 94-335-AK. Lab: Miles Inc. Agriculture Division, Toxicology, Stilwell, KS, USA. Sponsor: Miles Inc. Agriculture Division, Kansas City, Missouri, USA. Study duration: July 5-8, 1994. Report no. 94-335-AK. Report date: 2 August 1994.

Quality assured study performed in accordance with OECD guideline 405, July 1992, and corresponding US EPA and Japanese guidelines. It was stated that the study was conducted in compliance with GLP according to FIFRA 40 CFR Part 160, TSCA 40 CFR Part 792 and OECD C (81)30 (Final) Annex 2, 1981.

Study and observations: Guthion technical (azinphos-methyl; 0.1 mL; 67 mg; batch No. 3030050/230205204; purity 92.2%), was placed in the left conjunctival sac of 6 male NZW rabbits (source: Small Stock Industries, Pea Ridge, AR, USA; about 11 weeks old, body weight unstated). The right eye served as a control. Eyes were not washed. The test animals were individually housed and provided with about 125 g of food/day and water *ad libitum*. The treated eyes were evaluated for irritation of the cornea, iris and conjunctiva at 1, 24, 48 and 72 h after treatment, according to the criteria of US-EPA-FIFRA, Pesticide Assessment Guidelines, Guideline 81-4, November 1984.

Findings: Grade 1 iridial irritation was seen in 1/6 animals at 1 h after instillation. Grade 1-2 conjunctival chemosis was observed in 6/6 rabbits at 1 h and persisted in 3/6 (grade: 1-2) animals up until 24 h. Similarly, grade 1-2 conjunctival discharge was seen in 5/6 animals at 1 post treatment and not thereafter. There was no conjunctival redness, and the cornea showed no further signs of irritation. All ocular reactions were resolved by 48 h after treatment. Based on these findings, the test compound was considered to be a mild eye irritant by the study authors.

Conclusions: Under the conditions of the study, azinphos-methyl was a slight eye irritant in rabbits.

Skin irritation

WARF Institute Inc (1976) Report on the acute skin irritation, eye irritation and inhalation studies with Cotnion–methyl technical. Study no: not stated, Lab: WARF Institute Inc, Madison, WI, USA. Sponsor: Makhteshim, Beer-Shiva, Israel. Study duration: not stated. Report no. 6051091. Report date: 22 July 1976.

Pre GLP, non-quality assured study. (The method adopted met the requirements of Title 21 of the Code of Federal Regulations, 191.11).

Study and observations: A 500 mg quantity (or 500 µL) of Cotnion-methyl (azinphos-methyl, batch, purity unspecified) was applied on the shaven, intact and abraded skin of the back and flank of each of 6 NZW rabbits (2 sites/animal, sex, body weight, source unspecified) under occlusive conditions. The test substance was held secured for 24 h, during which time the animals wore collars. After 24 h, the patches were removed and the sites were evaluated and scored for erythema and odema at that time and 48 h later. The average of the 2 scores were used to determine the primary irritation score. During the study, the animals were housed individually and provided with food and water *ad libitum*.

Findings and conclusions: No skin responses were observed in any animal at any of the observation times. Under the conditions of the study, azinphos-methyl technical was not a skin irritant in rabbits.

Thyssen J & Lorke D (1981). R 1582 (azinphos-methyl, the active ingredient of Guthion) Study of the irritant effect on the skin and mucous membranes (eye). Study nos: T 80 10 593 and T 90 10 594. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG. Study duration: July 1981. Study no. T 8010593 (skin). Report date: 19 October 1981.

Pre-GLP study. Conducted in accordance with the guidelines recommended by the US Department of Agriculture, Federal Register, 38 (187), 27019, 1973, and OECD guideline 404.

Study and observations: Each of 6 NZW rabbits, male and female (source: Degenfeld, Eybach, Germany; body weight range: 3-4 kg) received a single dermal application for 24 h of R1582 (azinphos-methyl, batch no. 230105019, purity 92.4%) in an unspecified vehicle to abraded and intact skin. Animals were examined for signs of erythema and oedema at 24 and 72 h after application. The sum of mean irritation scores recorded for each animal with intact and abraded skin at 2 observation times was divided by 4 to obtain the primary skin irritation index. The test animals were housed singly and received food and water *ad libitum*.

Findings and conclusions: No dermal responses to treatment were observed in any animal at 24 and 72 h after application, on either intact or abraded skin. Azinphos-methyl was not a skin irritant in rabbits.

Crown S (1987) Cotnion-M: Primary skin irrtation study in rabbits. Study no: not stated. Lab: Life Science Research Israel Ltd, PO Box 139, Ness Ziona 70 451, Israel. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer Sheva, Israel. Study duration: March 22-26, 1987. Report no. MAK/119/AZM. Report date: 5 May 1987.

Quality assured GLP (US EPA & OECD) study. The method conformed to the recommendations of the OECD Guideline for Testing of Chemicals, Section 405 of 1981, and the US EPA FIFRA Guidelines (81-4) of 1982 was used.

Study & observations: Each of 6 albino rabbits of a local strain (4 females and 2 males, Loebenstein Laboratory Animals, Yoqneam, bw; 1.6-2.0 kg, age unspecified) received a dermal application of azinphos-methyl technical (batch no. 50287, purity: unspecified, particulated brown powder). A quantity of 500 mg of the test substance was applied to the intact skin of the dorsal area of the trunk of each animal on a gauze pad (25 cm^2) under occlusive conditions for 4 h. The patches were removed at the end of the exposure period and the application sites were cleaned with distilled water to remove the residual test substance. The skin sites were examined for signs of erythema and oedema at 1, 24, 48 and 72 h after patch removal. The test animals were individually housed and offered food and water *ad libitum*.

Findings: No signs of dermal irritation were observed at any of the application sites following exposure to the test substance.

Conclusions: Under the conditions of the study, azinphos-methyl was not a skin irritant in rabbits.

Zorbas MA (1994) Primary dermal irritation study with technical grade Guthion in rabbits. Miles Inc. Agriculture Division, Toxicology, Stilwell, Kansas. Report No.: 94-325-AJ, dated August 2, 1994 Sponsor: Miles Inc. Agriculture Division, Kansas City, Missouri [Bayer Australia Limited (Crop Protection); Submission no. 11794, volume 1]

Study performed in accordance with OECD guideline 404, July 1992, and corresponding US EPA and Japanese guidelines, and in compliance with GLP according to FIFRA 40 CFR Part 160, TSCA 40 CFR Part 792 and OECD C(81)30 (Final) Annex 2, 1981.

Study & observations: Each of 6 male NZW rabbits (Small Stock Industries, Pea Ridge, AR, USA, about 11 weeks old, body weight: not stated) received a dermal application of guthion technical (azinphos-methyl; batch no. 3030050/230205204; purity 92.2%). A quantity of 500 mg of the test substance moistened with tap water was applied to the intact skin of the dorsal area of the trunk of each animal on a gauze pad under occlusive conditions for 4 h. After the exposure period, the patches were removed and the application sites were cleaned with paper towels moistened with water to remove the residual test substance. The skin sites were examined for signs of erythema and oedema at 0.5-1, 24, 48 and 72 h after patch removal. The test animals were individually housed and offered food (125 g/day), and water *ad libitum*.

Findings: There were no signs of dermal irritation at any of the application sites following exposure to azinphos-methyl.

Conclusions: Under the conditions of the study, azinphos-methyl was not an irritant to rabbit skin.

Skin sensitisation

Flucke W (1986b) E 1582 (c.n. azinphos-methyl) Study for skin sensitising effect on guinea pigs (Magnusson and Kligman's Maximization test). Study no: T 8021276. Lab: Bayer Institute of Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG, Study duration: November 1985. Report no. 15003. Report date: 21 August 1986.

This study was performed using the Magnusson and Kligman's guinea pig maximisation test. Conducted in compliance with the OECD Principles of GLP (Federal Law Gazette 35, 3-16, 1983).

Male guinea pigs (strain: Bor:DHPW, SPF, Winklemann, Borchen, Germany) of 275-375 g body weight were used. A group of 20 animals received 3 parallel intradermal injections on each flank at cranial, medial and caudal sites. The injections consisted of either (1) Freund's complete adjuvant (FCA) diluted 1:1 with sterile physiological saline (cranial), (2) 1% azinphos-methyl (E 1582, batch no. 230 505 073=233 596 230) in Cremophor EL 2% v/v in sterile physiological saline solution (medial) or (3) 1% azinphos-methyl in Cremophor EL 2% v/v sterile physiological saline solution diluted 1:1 with FCA (caudal). Two control groups (10/group) were similarly treated with the vehicle only (Cremophor EL 2% v/v sterile physiological saline).

After 7 days, the induction sites were shorn and irritated with 0.2 mL of 10% sodium lauryl sulphate in paraffin oil for 24 h, and covered with an occlusive dressing containing 12.5% azinphos-methyl in Cremophor EL 2% v/v in saline solution for 48 h.

The first challenge was carried out 3 weeks after the intradermal induction. The dose used was based on the results of a preliminary range-finding study using five animals (data provided). A dressing soaked in a 12.5% azinphos-methyl in Cremophor EL and physiological saline was applied on the skin of the left flank of the animals under occlusive conditions for 24 h. The animals in the control group was similarly treated with the vehicle containing no test substance. A second control group was maintained for a second challenge should it be required, but as the results of the first challenge were clear, this was not performed. The skin sites were assessed at 24 and 48 h after the first challenge. The number of control sites which showed signs of irritation were subtracted from the number of test article sites which showed signs of irritation (= corrected values). The corrected values formed the basis for a comparative assessment of the data. The test animals were acclimatised to the laboratory conditions for 8 days prior to commencement of the study. They were housed 5/cage under standard laboratory conditions and provided with food (Altromin, GmbH, Lage) and water *ad libitum*.

Findings: The corrected values after the challenge showed a sensitising effect of the test substance in 19/20 (95%) animals (see Table below). Severe redness and/or swelling (grade 3) was observed in 9/20 animals with 3/9 showing hardening of the application sites at 24 h after the challenge exposure. With the exception of one animal that showed no signs of irritation, the remaining treated animals showed either moderate redness (grade 2) or slight redness (grade 1) at 24 h. Signs of irritation in these animals persisted up until 48 h, with 11/20 animals showing eschar formation (in addition to redness and/or swelling and

hardening) in the application site. Slight erythema (grade 1) possibly caused by the dressing, was observed in 5 control animals at 24 h.

Positively reacting animals after first challenge with 12.5% azinphos-methyl

Treated group (20 animals)		Control group (10 animals)	
Test dressing	Control dressing	Test dressing	Control dressing
20	1	5	5
Corrected value: 19 (95%)		0 (0%)	

Conclusions: Under the conditions of the study, azinphos-methyl was a skin sensitiser in the Magnusson and Kligman guinea pig maximisation test.

Porter MC, Craigo RE & Hartnagel RE (1987): Dermal sensitisation evaluation of Guthion® technical in the guinea pig. Study no: not stated. Lab: Toxicology Department, Central Research Services, Miles Laboratories Inc., Elkhart, IN, USA. Sponsor: not stated, Study duration: March 23 – April 23, 1987. Report no. 884. Report date: 29 June 1987.

The study was conducted in accordance with GLP standards (40 CFR Part 160-FIFRA and 792-TSCA) and OECD Guideline 406 adopted on May 12, 1981. The Buehler method was used (azinphos-methyl; batch no. 79-R-225-42, purity 88.8%, Mobay Corporation, Stilwell, KS, USA).

Study and observations: Outbred, male Hartley albino guinea pigs (Harlan Sprague Dawley, Indianapolis, USA; 5-6 weeks old, body weight range 295-359 g) were used. A group of 15 guinea pigs received 3 topical induction applications of 25% azinphos-methyl technical in 50% ethanol/distilled water vehicle under occlusive conditions for 6 h/day, once weekly for 3 weeks (0, 7 and 14 days). In each application, an aliquot of 0.4 mL was applied under an adhesive patch, to the shaven skin of the left side of each test animal near the scapula. A group of 5 animals was treated with a 0.05% solution of 1-chloro-2, 4-dinitrobenzene (DCNB) in 50% ethanol/distilled water using the above procedure (positive controls). In addition, there were 2 vehicle control groups (5/group) which were similarly treated. The 3 induction applications were followed by a 2-week rest period. After the exposure period, the patches were removed and the application sites were scored for erythema at 24 and 48 h following patch removal. On day 28, the above test formulations and the vehicle were applied on each test animal under occlusive conditions as the challenge dose. The test material was applied on the skin near the left side of the pelvic girdle, while the vehicle was applied on the skin of the opposite side using the same procedures employed during induction. After 24 h, the patches were removed, the sites were cleaned and the skin reactions were scored for erythema at that time, and 48 h later. The skin reactions of the test substance and positive control groups were compared with those of the vehicle controls.

Body weights of the animals were measured prior to commencement, and upon completion of the study. During the study, the test animals were housed individually under standard laboratory conditions and provided with food (Purina, Ralston Purina Co.) and water *ad libitum*.

Findings: Body weight gain of the animals was unaffected by treatment. No positive erythematous skin reactions were observed following induction exposure. Challenge applications induced slight to barely perceptible erythema (grade 1) in 6/15 animals, and

clearly perceptible erythema (moderate, grade 2) in 1 animal at 24 h post patch removal. The reaction persisted in 6/7 animals up until 48 h, with 5 animals showing slight erythema (grade 1) and 1 animal exhibiting moderate erythema (grade 2).

Slight erythema (grade 1) was also observed in 2/5 controls at 24 h. DCNB treated guinea pigs (4/5) showed moderate to severe erythematous reactions at 24 h. Dermal scores recorded following challenge exposure are given in the Table below.

Dermal scores following challenge exposure

Index	azinphos-methyl		DNCB	
	Induced	Control	Induced	Control
Incidence[a]	7/15 = 0.47	2/5 = 0.40	4/5 = 0.80	0
Severity[b]	15/30 = 0.50	2/10 = 0.20	17/10 = 1.70	0

[a]Incidence = number of animals showing positive response at either 24 or 48 h divided by the number of animals tested
[b]Severity = mean of all test grades at 24 and 48 h, corrected by subtracting the reading at the vehicle site from that at the test article site

Conclusions: Azinphos-methyl is potentially skin allergenic and a skin sensitiser in guinea pigs in the Buehler test.

Heimann KG (1987b) E1582 technical (common name: azinphos-methyl) Study of skin sensitisation effect on guinea pigs (Buehler patch test). Study no: T 0022376, Lab: Institute of Toxicology/Agriculture, Fachbereich Toxikologie, Bayer AG, Wuppertal, Germany. Sponsor: Bayer AG, Study duration: March - May 1986. Report no. 16188. Report date: 5 November 1987.

GLP and quality assured study conducted according to OECD guideline 406 and corresponding US EPA and EC Guidelines. The Buehler method was used.

Study and observations: Male SPF-bred DHPW strain guinea pigs (Winkelmann, Borchen, Germany, 5-7 weeks old, body weight 309-373 g) were used in this study. A group of 12 animals received 3 topical inductions of 12.5% E1582 technical (azinphos-methyl; batch no. 230505073=233596230; purity 92.4-92.8%) in 2% Cremophor EL/saline vehicle once weekly for 3 weeks. The dose levels used for induction and challenge exposures were based on the results obtained from a preliminary range-finding study (data provided). At each application, the test material (0.5 mL) was applied to the left flank of the animals under occlusive conditions for 6 h. The third induction exposure was followed by a 2-week rest period. Two control groups (12/group) were treated likewise with the formulation vehicle only. The treated skin sites were evaluated for erythema at 48 h post application.

After the 2-week rest period, each of the treated and control group 1 animals were challenged with a hypoallergenic patch containing a 6% azinphos-methyl in 2% Cremophor EL/saline (highest non-irritant concentration). An aliquot of 0.5 mL of the appropriate test formulation was applied to the left flank of each animal under occlusive conditions. The exposure period was 6 h. A corresponding control patch containing 0.5 mL of the vehicle was applied to the right flank for comparison.

Two weeks after the first challenge, the second challenge application was made as described above with a patch containing 0.6% of the test substance in 2% Cremophor EL/saline. This

time, control group 2 was used, and the animals were treated similarly to the test group, but with the vehicle only.

The skin sites were evaluated at 48 and 72 h after patch removal for erythema and scored. The results were evaluated by subtracting the number of animals with an irritant reaction on the control side from the number of animals with an irritant reaction on the test compound side for both the treated and the control groups. Body weights of the animals were determined prior to commencement of the study and weekly thereafter. Clinical signs were checked once daily. The animals were acclimatised to the test facility for 7 days prior to commencement of the study. The test animals were housed 4/cage under standard laboratory conditions and provided with food (Altromin, GmbH, Lage) and water *ad libitum*.

Findings: Mild redness (grade 1) was observed in 3/12 animals, and moderate redness (grade 2) was seen in 3/12 at 24 h after the first challenge, compared to only 2 mildly reacting animals in the control group. Mild redness (grade 1) persisted up until 72 h in the 3 animals that had exhibited moderate redness (grade 2) at 24 h. No other skin reactions were observed at this observation time.

After the second challenge, mild redness (grade 1) was observed in 1/12 test animals and in 2 control animals (2 control animals also excluded in the evaluation as the entire depilated area were reddened) at 48 h. No further skin reactions were observed at this observation time.

Conclusions: Under the conditions of the study, azinphos-methyl was a skin sensitiser in guinea pigs. According to the study authors, the skin sensitising properties induced by azinphos-methyl were elicited only after application of relatively high concentrations (ie 6% azinphos-methyl), suggesting a threshold for the contact-allergic reaction.

Shapiro R (1987) Azinphos-methyl technical (Cotnion) EPA topical skin sensitisation test in gunea pigs (Buehler). Study no: T-6701, Lab: Product Safety Labs, 725 Cranbury Road, East Brunswick, NJ, USA. Sponsor: Makhteshim-Agan (America) Inc. Study duration: May 4 – June 10, 1987. Report no. R-4673 (PSL ref: E70427-2). Report date: 29 June 1987.

GLP, quality assured study. The Buehler method was used.

Study and observations: Male, Hartley strain young adult guinea pigs (Davidson's Mill Farm, South Brunswick, NJ, USA, bw: 319-432 g, age: unspecified) were used in this study. A group of 10 animals received topical induction applications of 100% azinphos-methyl technical (batch no: E70427-2, purity: unspecified, light brown granular) on alternate days until a total of 10 doses applications was achieved. The highest non-irritating dose levels used for induction and challenge exposures were based on the results obtained from a preliminary range-finding study (data provided). Prior to commencement of the study, the animals were acclimatised to the test facility for 5-26 days. The test animals were individually housed under standard laboratory conditions and provided with food (Purina Guinea Pig Pellets) and water *ad libitum*. At each application, the test material (500 mg or mL) moistened with the solvent (unspecified) was applied to the dorsal thoraco-lumbar region of the animals on a gauze patch (2.5 cm^2) under occlusive conditions for 6 h. The induction doses 7-10 and for the challenge dose, the dose was reduced to 250 mg due to mortality at 500 mg level. The positive control group (10 animals) received 0.05-0.08% dinitrochlorobenzene (DNCB) in 95% ethanol similarly (the induction dose concentration was raised from 0.05% to 0.08% for

the 3rd and 10th applications). The patches were removed after 6 h and the sites were cleaned with a water dampened paper towel. Twenty-four hours after each induction application, the application sites were evaluated for skin reactions (erythema and oedema) based on a 0-3 grading system and irritation scores were recorded. There was no vehicle control group in the study. Fourteen days after the 10th induction application, the challenge dose (100% azinphos-methyl) was applied similarly to a naive site on the left side of each animal. The sites were scored for sensitisation responses (erythema and oedema) at 24 and 48 h after challenge.

Findings: Four animals treated with 500 mg of the test material/induction dose died during the induction phase. One death occurred after the 4th induction dose and 4 animals died after the 7th dose. Clinical signs observed in these animals prior to death included tremors, paleness, lethargy, prostration and an apparent low body temperature. One animal in the positive control group was found dead on day 15, after the 7th induction application. This death was attributed to respiratory and circulatory distress resulting from excessive tightness of the bandage, which secured the patch. Three surviving test substance group animals exhibited very faint erythema (score of 0.5) at 24 h after the 3rd induction, with one of these 3 showing a similar skin reaction at 24 h after the 9th induction dose. None of the survivors in the test substance group exhibited any skin reactions after the challenge exposure.

Very faint to faint erythema (score of 0.5 to 1.0) was observed in all positive controls at 24 or 48 h after induction exposures. Erythema became evident in one animal at 24 h after the 1st induction dose, and in the majority of the animals after the 3rd or 4th induction application. Four of the animals showed severe erythema (score of 3) after the 10th induction exposure. Eight of the 9 surviving in this group exhibited very faint to faint erythema (score of 0.5 to 1.0) after the challenge, out of which 6 were considered to be sensitisation reactions.

Conclusions: Under the conditions of the study, azinphos-methyl technical was not a skin sensitiser in guinea pigs.

Lisi P, Caraffini S & Assalve D (1987) Irritation and sensitisation potential of pesticides. Contact Dermatitis 17: 212-218.

Study and observations: In this study, 36 pesticides including azinphos-methyl (purity, source unspecified) were patch tested in human subjects to establish the optimal test concentrations. Azinphos-methyl (1%) diluted in petroleum ether was tested on a group of 347 subjects (sex, body weight: unspecified, age: 11-84 years). In the study group, there were 71 agricultural workers and 27 other persons with the history of previous agricultural work. Work history of 249 other persons involved in the study was unspecified. According to the study authors, an unspecified number of these persons had contact dermatitis, mostly on the hands and some others were individuals admitted to different centers of Gruppo Italiano Ricerca da Contatto e Ambientali (GIRDCA) for non-allergic skin disorders. Patch tests were performed on the upper back and evaluated after 48 and 72 h. Irritant and allergic reactions were evaluated according to the method of Cronin (1980). No further details on experimental methods were provided.

Findings and conclusions: Under the conditions of the study, no irritant or allergic skin reactions were noticed with azinphos-methyl in any of the individuals. The usefulness of the study findings, however, was reduced due to lack of information on the test substance and limited details on experimental methods.

2.3.2 Isomers, Metabolites and Impurities

2.3.2.1 Median Lethal Dose Studies

A summary of findings of acute median lethal dose studies for the metabolites and impurities of technical azinphos-methyl is shown in the Table below.

Median lethal dose studies

Species	Sex	Route	Vehicle	LD50 (mg/kg bw)	Reference
Benzazimide (intermediate in production of Guthion)					
Rat [SD]	M/F	Inhalation, 4 h	NS	> 1760 (M, 0/10 deaths; F, 3/10 deaths)	Shiotsuka (1987b) (GLP)
Rabbit [NZW]	M/F	Dermal	Water	> 2000(M/F) (0/5 deaths)	Sheets (1988) (GLP)

SD = Sprague-Dawley; NZW = New Zealand White

2.3.3 Products

A summary of findings of ocular and skin irritation and skin sensitisation studies is presented in the following Table. The data on median lethal dose studies of these formulations will be presented under respective formulation headings.

Findings of ocular and skin irritation, and skin sensitisation studies conducted with azinphos-methyl formulations

Formulation	Irritation		Skin sensitisation
	Eye	Skin	
Guthion 50% WP	Moderate	Slight	ND
Guthion 35% WP	Slight	Non-irritant	Sensitiser
Azinphos 35 FL	Slight	Non-irritant	Sensitiser
E1582 32.5 WP 0111 (azinphos-methyl) & SIR 8514 3.5 (triflumuron)	Slight	Non-irritant	ND
E1582 21.3 WP 02799/0346 (azinphos-methyl) & E1586.3 (demeton)	Slight	Non-irritant	ND
Guthion 2F (20%)	Severe	Slight	ND
E1582 19.5 EC 00126/667	Severe	Moderate to Severe	Sensitiser
Guthion Fruit Tree & Garden Spray (13% azinphos-methyl)	Severe	Severe	No

ND = not determined

2.3.3.1 Guthion 62.5% WP

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [Holtzman]	M	4 or 8	15, 20, 25, 30, 40	23.6	DuBois (1970a)
		F	4, 5 or 8	10, 12, 15, 20, 25	14.8	

Route	Species	Sex	Group size	Doses Tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Dermal	Rabbit	M/F	2	1000 (24 h, intact, occluded)	> 1000 (0/2 deaths)	
Inhalation 1 h, whole body	Rat [SD]	M/F	4/sex	2000 or 5000 (dust, particle size unspecified)	≤5000 (F: 3/4 deaths) > 5000 (M: 0/4 deaths)	Crawford and Anderson (1970)

SD = Sprague-Dawley

2.3.3.2 Guthion 50% WP

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	F	8 or 16	10, 20, 25, 30	~25	Bauman & Nelson (1969)
Dermal	Rat [SD]	F	4	250, 300, 400, 500	300	Nelson (1967b)
Dermal	Rabbit [NZW]	M/F	4/sex	500, 1120, 2509 (M/F) and 223 (M) or 5620 (F) 24 h, occluded, applied material was removed)	1137 (M) 1147 (F)	Nelson (1979a)
Dermal	Rabbit [NZW]	M/F	4/sex	70, 74.1, 111.1, 162, 166.7, 250, 348, 375.1, 500, 562.6, 750, 844, 1000 (M) or 70, 162, 250, 348, 500, 750, 1000 (F) (24 h, occluded, applied material was removed)	339 (M) (F: not determined)	Hixson (1980c)
Inhalation (40 or 55 mins, Whole body)	Rat [SD]	F	5	150, 218 (dusts, particle size unspecified)	> 218 (0/5 deaths)	Nelson & Doull (1967)
Inhalation (1 h, head only)	Rat [SD]	M/F	10/sex	185, 243, 569 or 1030 (dusts, MMAD: 2.3-3.3 μm)	383 (M) 245 (F)	Shiotsuka (1986) (GLP)
Inhalation (4 h, head only)	Rat [SD]	M/F	10/sex	117, 166, 178, 203, 823 (dusts, MMAD: 2.3-3.3 μm)	198 (M) 170 (F)	
Inhalation (1 h, nose only)	Rat [SD]	M/F	6/sex	223, 503, 613, 1335, 1635 (dusts, MMAD: 3.96 μm)	916 (M) 619 (F)	Warren & Dyer 1994) (GLP)
Inhalation (4 h, nose only)	Rat [Wistar]	M/F	5/sex	114, 133, 177, 223 (dusts, MMAD: < 2.9 μm)	166.2 (M) 146.4 (F)	Pauluhn (1998) (GLP)

SD = Sprague-Dawley; NZW = New Zealand White; MMAD = Mass Median Aerodynamic Diameter

Eye and dermal irritancy studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbit [NZW] (sex unspecified)	9	100 mg, conjunctival sac, washed, unwashed	Moderate irritant	Hixson (1979)
Dermal		6	500 mg, intact and abraded, occluded	Slight irritant	

¶ = US EPA FIFRA guidelines; § = US EPA and OECD guidelines.

Eye irritation

Hixson EJ (1979) Eye and dermal irritancy of Guthion 50% WP. Study nos. 79EIL06 and 79EIL05. Lab: Mobay Chemical Co, Corporate Toxicology department, Stanley Research Centre, Stilwell, KS 66085, USA. Sponsor: Bayer AG. Study duration: September 18-28, 1979. Report no. 68336. Report date: 15 November 1979.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: A quantity of 100 mg of guthion 50% WP (batch no. 9143520, Formula no. 011011, azinphos-methyl content: 50%, solubility and stability data not provided) was applied into the left eye of each of 9 NZW rabbits (Small Stock Inc, Pea Ridge, Arkansas, USA; age, sex, body weight not stated). The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. Three rabbits had their eyes washed with 200 mL lukewarm water 45 seconds after the test compound application (group A). The eyes of the remaining 6 rabbits were not washed (group B). Evidence of ocular irritation (responses in the cornea, iris, conjunctivae) was assessed at 1, 2, 3, 4 and 7 days post treatment according to the Draize method. The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Slight conjunctival redness (grade 1) was seen in 2/3 group-A rabbits at 24 h post treatment and not thereafter. One of these 2 rabbits also had a slight discharge (grade 1). One group B rabbit exhibited corneal opacity at 24, 48 (grade 2) and 72 h (grade 1) post treatment and not thereafter. This animal also had grade 1 iritis up until 48 h post treatment together with moderate redness (grade 2) at 24 h, and slight redness (grade 1) at 48 and 72 h, and chemosis and an ocular discharge (grade 1) at 48 h post treatment. Slight conjunctival redness (grade 1) was seen in 3 rabbits at 24 h, in 1 rabbit at 48 h and in another at 72 h and not thereafter. Four of these animals showed grade 1 ocular discharge at 24 or 48 h post treatment, with 2 animals exhibiting chemosis (grade 1), which resolved by 48 h. No other information on clinical observations was provided. The mean scores for conjunctival redness in group-B animals at day 1 and 3 post treatment were 1.16 and 0.3 respectively. Based on the above findings, guthion 50% WP was considered to be a mild eye irritant by the study authors.

Conclusions: Guthion 50% WP was considered to be a moderate eye irritant.

Skin irritation

Study and Observations: A 500 mg (per site) quantity of Guthion 50% WP (batch no. 9143520, Formula no. 011011, azinphos-methyl content: 50%, solubility and stability unspecified) in physiological saline was applied to shaved, abraded and intact skin on the

back and sides of each of 6 NZW rabbits (Small Stock Inc, Pea Ridge, Arkansas, USA; age, sex, body weight, unspecified) under occlusive conditions. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. There were 4 application sites on each animal (2 intact and 2 abraded), and during the exposure periods, the animals wore plastic collars. After 24 h, the patches were removed, and the application sites were cleaned with a damp cloth. The test sites were evaluated (Draize method) for skin reactions at that time, and at 48 h later (ie at 24 and 72 h post treatment). The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: One test animal was found dead during the study (time unspecified) and autopsy revealed a creamy, purulent mass in the ventral cervical region. The study authors did not attribute this death to treatment. Consequently, another animal was included in the study and its dermal scores were used to calculate the primary irritation index. With intact skin sites, well-defined (grade 2) erythema in one rabbit and very slight erythema (grade 1) in another rabbit were seen at 24 h post treatment and not thereafter. Very slight to well-defined (grade 1 to 2) erythema together with very slight to slight odema of the skin (grade 1 to 2) were observed in all but one animal in the abraded skin group at 24 h post treatment and not thereafter. No further information on clinical observations was provided. Under the conditions of the study, guthion 50% WP was classified as a mild skin irritant (primary irritation index 0.71) by the study authors.

Conclusions: This formulation was considered to be a slight skin irritant.

2.3.3.3 Guthion 35% WP

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	M/F	5/sex	42, 80 (M/F) and 20 (M) or 52, 63 (F)	58 (M) 53 (F)	Sheets (1990a) (GLP)
Dermal	Rat [SD]	M/F	5/sex	2000 (24 h, occluded, applied material removed)	> 2000 (M/F) (0/5 deaths/sex)	Sheets (1990b) (GLP)
Inhalation, (4 h, nose only)	Rat [SD]	M/F	6/sex	248, 334, 445, 590, 780 (dusts, MMAD: 2.4-5.2 µm)	596 (M) 422 (F)	Warren (1990) (GLP)

SD = Sprague-Dawley; MMAD = Mass median aerodynamic diameter

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW] M/F	6	100 µL, conjunctival sac, unwashed	Slight irritant	Sheets (1990c)¶ (GLP)
Dermal		6	500 mg, intact, occlusive	Non irritant	
Skin sensitisation	Guinea pigs	15	Topical induction and challenge	Sensitiser	Porter *et al* (1987)¶ (GLP)

¶ US EPA FIFRA and OECD guidelines.

Eye irritation

Sheets LP (1990c) Primary eye irritation study with Guthion 35 WP in rabbits. Study no: 90-335-FY. Lab: Mobay Corporation, Health, Environment, Safety and Plant Management, Corporate Toxicology Department, 17745 South Metcalf, Stilwell, KS 66085-9104, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, Missouri 64120-0013, USA. Study duration: May 22-29, 1990. Report no. 5298. Report date: 24 August 1990.

GLP, quality assured study. Conducted according to US-EPA-FIFRA Guideline 81-4 of November, 1984, US-EPA-TSCA 40 CFR 798.4500 of July, 1988 and OECD guideline 405 Section 4 of February, 1987, and corresponding Guidelines (January, 1985) in Japan.

Study and observations: A quantity of 100 mg of guthion 35 WP (batch nos. 0-14-3651 and 0-03-0128, azinphos-methyl content: 36.3%, tan powder, formulation details provided, stability: unknown under freezer conditions) was applied into the conjunctival sac of the left eye of each of 6 NZW rabbits (Small Stock Inc, Pea Ridge, Arkansas, USA; about 15 weeks old, 4 males and 2 nulliparous, non-pregnant females, body weight unspecified). The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. Ocular irritation (responses in the cornea, iris, conjunctivae including discharge) was assessed and scored at 1, 2, 3, and 7 days post treatment. The animals were individually housed under standard laboratory conditions and provided with about 125 g of food/day and water *ad libitum*.

Findings: No corneal or iridial responses were observed in any of the treated rabbits. Grade-1, conjunctival redness was seen in all animals following treatment, and had resolved by 24 h in 1 animal and by 48 or 72 h in the remaining animals. Grade 1 chemosis was observed at 1 and 24 h post treatment in 4 and 2 rabbits, respectively and not thereafter. An ocular discharge was seen in two rabbits at 1 h post treatment (grades 1 and 3). No further clinical observations were provided. Under the conditions of the study, azinphos-methyl 35 WP was classified as a mild eye irritant by the study authors.

Conclusions: Under the conditions of the study, azinphos-methyl 35% WP was a slight eye irritant.

Skin irritation

Sheets LP (1990d) Primary dermal irritation study with Guthion 35 WP in rabbits. Study no: 90-325-FZ. Lab: Mobay Corporation, Health, Environment, Safety and Plant Management, Corporate Toxicology Department, 17745 South Metcalf, Stilwell, KS 66085-9104, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Box 4913, Hawthorn Road, Kansas City, Missouri 64120-0013, USA. Study duration: May 23 - 26, 1990. Report no. 4706. Report date: 30 August 1990.

GLP, quality assured study. Conducted according to US-EPA-FIFRA Guideline 81-5 of November, 1984, US-EPA-TSCA 40 CFR 798.4470 of July, 1988 and OECD guideline 404 Section 4 of May, 1981 and corresponding Guidelines (January, 1985) in Japan.

Study and observations: Guthion 50% WP (500 mg, batch nos: 0-14-3651 and 0-03-0128, azinphos-methyl content: 36.3%, tan powder, formulation details provided, stability:

unknown under freezer conditions) in physiological saline was applied to shaved, intact skin on the back and sides of each of NZW rabbits (3/sex, Small Stock Inc, Pea Ridge, Arkansas, USA; about 15 weeks old, body weight unspecified) under occlusive conditions. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. The test substance was applied to a 6-cm^2 skin area, for a period of 4 h. Following the exposure period, the patches were removed, and the application sites were cleaned with paper towels dampened with tap water. The test sites were evaluated for erythema and odema at 30-60 min, 24, 48 and 72 h after patch removal. Adjacent untreated skin served as the control. During the study, the animals were individually housed under standard laboratory conditions and provided with about 125 g of food/day and water *ad libitum*.

Findings: No skin responses were noted in any animal at any of the observation times nor were any other lesions or toxic signs related to treatment seen.

Conclusions: Under the conditions of the study, azinphos-methyl 35 WP was not a primary skin irritant in rabbits.

Skin sensitisation

Porter MC, Craigo RE & Hartnagel RE (1987) Dermal sensitisation evaluation of Guthion 35% WP in the guinea pig. Study no: not stated. Lab: Toxicology department, Central research Services, Miles Laboratories Inc, PO Box 40, Elkhart, IN 46515, USA. Sponsor: Agricultural Chemicals Section, Mobay Corporation. Study duration: March 23 - April 23, 1987. Report no. MTD0014 (Bayer report no. 885) Report date: 29 June 1987.

Quality assured study conducted in compliance with GLP standards 40 CFR Part 160-FIFRA and 792-TSCA. The Buehler epicutaneous patch test was used.

Study and observations: A group of 30 outbred, male Hartley albino guinea pigs (Harlan Sprague Dawley, Indianapolis, USA; 5-6 weeks old, body weight range 288-367 g) were randomly assigned to Guthion M 35% WP (G35, azinphos-methyl; 35% active ingredient; batch no: 86R0330I, Mobay Corporation, Stilwell, KS, USA, formulation details provided) test group (15 animals), test control group, DNCB positive control group, and DNCB negative control group (5/group respectively).

The test group received 3 topical induction applications of 5% azinphos-methyl 35% WP in 50% ethanol/distilled water vehicle on days 0, 7 and 14. The above dose was considered the highest non-irritant concentration, and was based on the results of a preliminary investigation (data provided). The test material (0.4 mL) was applied to the skin of the left side of each animal near the scapula under an adhesive patch. The exposure period was 6 h. Positive controls (DNCB, 0.05% solution in 50% ethanol/distilled water) and other control groups were similarly treated with the appropriate test formulation. Following the exposure period, the patches were removed and the skin sites were cleaned with warm water. The application sites were scored for erythema at 24 and 48 h after patch removal. The third induction application was followed by a 2-week rest period.

After the rest period (on day 28), the animals were topically challenged with the appropriate test formulation (azinphos-methyl 35% WP and DNCB) applied as described above near the left pelvic girdle. The vehicle (ethanol/distilled water) was similarly applied to the right side of the test and control animals. Patches were removed after 24 h and the sites were cleaned.

The application sites were observed for erythema and scored 24 and 48 h later. Body weights were determined prior to commencement and at termination of the study. The test animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Body weight gain of the animals was unaffected by treatment. Slight erythema (grade 1) was seen in one animal in the test compound group at 24 h after the third induction exposure.

At 24 h after the challenge application, 11/15 azinphos-methyl induced animals exhibited slight (grade 1) erythema. Four/11 of these animals exhibited a similar skin reaction at the vehicle control site at this observation time. At 48 h, moderate erythema (grade 2) was seen in one animal, and slight (grade 1) erythema was observed in 10 animals. No signs of irritation were observed in the control animals.

Dermal scores for induced and control groups following challenge with azinphos-methyl 35% WP and DNCB

Test group	Incidence*	Severity**
Azinphos-methyl WP 35-induced	11/15 = 0.73	18/30 = 0.60
Controls	0	0
DNCB (positive controls)	4/5 = 0.80	17/10 = 1.70
Controls	0	0

*Number of animals showing positive responses at 24 or 48 h/animals tested.
** Mean of all test grades at 24 and 48 h corrected for reading at vehicle site.

DNCB treated animals (4/5) showed moderate to severe (grade 2-3) erythema at 24 h, and slight to severe (grade 1-3) erythema at 48 h.

Conclusions: Under the conditions of the study, azinphos-methyl 35% WP was a skin sensitiser in guinea pigs.

2.3.3.4 Azinphos 35FL

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	M/F	5/sex	5, 10, 14.1, 20, 28	22 (M) 13 (F)	Cummins (1989) (GLP)
Dermal	Rat [SD]	M/F	5/sex	2470 (24 h, occluded, applied material removed)	> 2470 (M 1/5 deaths) (F 0/5 deaths)	Cummins (1988) (GLP)
Inhalation (4 h, nose only)	Rat [SD]	M/F	5/sex	1420, 1570, 2770, 3280 (group 1: 80.8% droplets < 6 μm, groups 2 and 3: 41-45% < 6 μm)	609 (M) 685 (F)	Cracknell *et al* (1989) (GLP)

SD = Sprague-Dawley

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW] M	3	100 µL in the eye, unwashed	Slight irritant	Smith & Cummins (1988a)¶ (GLP)
Dermal		3	500 mL, intact occlusive, washed	Non irritant	Smith & Cummins (1988b)¶ (GLP)
Skin sensitisation	Guinea pigs M/F	10/sex	Topical induction, challenge exposures (BM)	Sensitiser	Smith (1989)¶ (GLP)

NZW = New Zealand White; ¶ = US EPA, FIFRA and OECD guidelines; BM = Buehler Method.

Eye irritation

Smith KD & Cummins HA (1988a) Azinphos 35 FL: Acute eye irritation/corrosion test in the rabbit. Study no: MAK/078. Lab: Life Science Research Ltd, Eye, Suffolk IP23 7PX, England. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer-Sheva 84100, Israel. Study duration: August 8 - 12, 1988. Report no. 88/0692. Report date: 8 December 1988.

GLP, quality assured study. The study was designed to meet the requirements of section 4, sub-section 405 of OECD Guidelines for Testing of Chemicals (1987).

Study and observations: An aliquot of 100 µL of azinphos 35 FL (Cotnion, batch no: E5, azinphos-methyl content: 36-38%, brown solution) was instilled into the right eye of each of 3 male NZW rabbits (Rosemead Rabbits, Essex, England, body weight range: 2.9-3.1 kg, 12 weeks old). The test animals were acclimatised to the laboratory conditions for 7 days prior to commencement of the study. The untreated eye served as a control. Pain reaction of the animals to instillation of the test material was assessed by using a grading system based on; no response, blink response, animal blinks and tries to open the eye, holds eye shut, holds the eye shut and squeal or tries to escape. Ocular responses (cornea, iris, conjunctiva including discharge) were assessed and scored at 1, 48 and 72 h post treatment. The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Grade 1 conjunctival redness (erythema) and chemosis were seen in 2 animals at 1 h post treatment, while the third animal had only conjunctival redness of similar grade at this observation time. All ocular responses had resolved by 24 h post treatment. It was stated that instillation of the test substance caused slight initial pain, but no further details were provided.

Conclusions: Under the conditions of the study, azinphos 35 FL was a slight eye irritant.

Skin irritation

Smith KD & Cummins HA (1988b) Azinphos 35 FL: Acute dermal irritation/corrosion test in the rabbit. Study no: MAK/077. Lab: Life Science Research Ltd, Eye, Suffolk IP23 7PX, England. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer-Sheva 84100,

Israel. Study duration: July 12-15, 1988. Report no. 88/0595. Report date: 14 November 1988.

GLP, quality assured study. The study was designed to meet the requirements of section 4, sub-section 404 of OECD Guidelines for Testing of Chemicals of 1981.

Study and observations: An aliquot of 500 mL of azinphos 35 FL (batch no: E5 azinphos-methyl content: 36-38%, brown liquid) was applied to shaved, intact skin on the left side of the dorsum of each of NZW rabbits (3/sex, Small Stock Inc, Pea Ridge, Arkansas, USA; about 15 weeks old, body weight unspecified) under occlusive conditions. The shaven intact skin on the right side of animal was treated similarly but without the test substance and served as a control. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. The test substance was applied to a 6 cm^2 skin area for a period of 4 h. After the exposure period, the patches were removed and the application sites were cleaned with warm water. The test sites were evaluated for erythema and odema at 1, 24, 48 and 72 h after patch removal and scored. The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Slight erythema (barely perceptible, grade 1) was observed in one rabbit at 1 and 24 h after patch removal, but not thereafter.

Conclusions: Under the conditions of the study, azinphos 35 FL was not considered a primary skin irritant in rabbits.

Skin sensitisation

Smith KD (1989) Azinphos 35 FL: Delayed contact hypersensitivity study in guinea pigs. Study no: MAK/079. Lab: Life Science Research Ltd, Eye, Suffolk IP23 7PX, England. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer-Sheva 84100, Israel. Study duration: November 1 – December 2, 1988. Report no. 89/MAK079/098. Report date: 23 May 1989.

GLP, quality assured study. Conducted in compliance with the requirements of section 4, sub-section 406 of the OECD Guidelines for Testing of Chemicals of 1981. A modified version of the Buehler epicutaneous patch test was used.

Study and observations: A group of 30 Dunkin-Hartley albino guinea pigs (Olac Ltd, Bicester, Oxfordshire, England; body weight range 323-431 g, age unspecified) were used in the study. The test animals were acclimatised to the laboratory conditions for 6 days prior to commencement of the study. The induction and challenge exposure concentrations of azinphos 35 FL (batch no: E5 azinphos-methyl content: 36-38%, brown liquid) used were based on a preliminary skin irritation study (data provided). The maximum concentration of the test substance that did not cause severe skin irritation or necrosis was selected for induction, and the highest concentration considered to be sub-irritant was selected for challenge.

The test group of 20 animals (10/sex) received 3 topical induction applications of 0.25 mL azinphos 35 FL on a 4 cm^2 absorbent patch on days 0, 7 and 14. The test substance was applied to the shaven skin of the left flank of each animal under occlusive conditions for 6 h. The animals in the control group (5/sex) were not treated during the induction phase. After

the 6-h induction exposure, the patches were removed and the application sites were evaluated for skin responses (time unspecified). The third induction application was followed by a 2-week rest period.

After the rest period (on day 28), the animals were topically challenged with 0.25 mL of azinphos 35 FL, applied as described above to the shaven skin of the right flank of the animal. The exposure period was 6 h. Animals in the control group were similarly treated. The application sites were observed for erythema and scored at 24 and 48 h post patch removal following cleaning. Body weights were determined at commencement of the study and then weekly. The test animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Body weight gain of the animals was unaffected by treatment. No skin responses were seen in any animal following induction exposures. Very faint erythema (2/sex) to erythema of grade 1 (4 males and 3 females) were observed in the test group animals at 24 h after patch removal. At 48 h, 4 males and 3 females in this group showed grade 1 erythema, while 3 males and 1 female had very faint erythematous reactions. No skin responses were observed in control animals.

Conclusions: Under the conditions of the study, azinphos 35 FL was classified as a skin sensitiser in guinea pigs.

2.3.3.5 E 1582 32.5 WP 0111 (azinphos-methyl) and SIR 8514 3.5 (triflumuron)

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW]	3	100 µL into the conjunctival sac, washed	Slight irritant	Martins (1988)¶
Dermal		3	500 mg, intact, abraded, cleaned	Non irritant	

NZW = New Zealand White; ¶ = OECD Guidelines.

Eye irritation

Martins T (1988) E 1582 32.5 WP 0111 and SIR 8514 3.5 [c.n. Azinphos-methyl and Triflumuron –proposed-]. Study for irritation /corrosion potential to skin and eye (rabbit) according to OECD guidelines nos. 404 and 405. Study no: T7030040, Lab: Institute of Toxicology, Agrochemicals, Department of Toxicology, Bayer AG, Wuppertal, Friedrich-Ebert-Strasse 217-333. Sponsor: Bayer AG. Study duration: August 9-16, 1988. Report no. 17304. Report date: 25 October 1988.

Quality assured study. Performed in accordance with the OECD Guidelines 404 and 405, and in compliance with the OECD Principles of GLP of 1983.

Study and observations: An aliquot of 100 µL (equivalent to 70 mg of the test article) of E 1582 32.5 WP 0111 and SIR 8514 3.5 {azinphos-methyl (32.9%) and Triflumuron: 2-chloro-N-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzazamide (3.5%); batch no. 233813308; stability: study period, cream coloured powder; source: Bayer AG, Leverkusen, Germany)} was instilled into the conjunctival sac of one eye of each of 3 adult, male NZW rabbits

(Interfauna, UK Ltd, 3.0-3.2 kg body weight, age not stated). The untreated eye served as a control. Twenty-four hours after treatment, the treated eyes were rinsed with normal saline. Ocular responses to treatment [cornea (opacity and area affected), iris (hyperaemia and reaction to light), conjunctivae (erythema and chemosis), and discharges] were examined and scored at 1, 24, 48, 72 h and on days 7, 14 and 21 post treatment according to the guidelines used. Opacity in the aqueous humor was assessed according to the method of McDonald and Shadduck (1987). At 24 h post treatment, a drop of 1% fluorescein solution was placed on the cornea of each of the treated eyes, which were rinsed again with normal saline solution. The eyes were then examined under UV light for any damage to the corneal epithelium. Where positive effects were recorded this procedure was repeated at the later observation times. Only those effects persisting for more than 24 h were included in the evaluation. Individual Draize scores were recorded separately (for cornea, iris and erythema and swelling of the conjunctivae) at 24, 48 and 72 h post treatment and used to calculate the individual ocular "irritation grade". As only three animals were used, the interpretation was based on the individual irritation scores of the two most sensitive animals. The rabbits were individually housed under conventional laboratory conditions and provided with standard Ssniff K4 diet (100-120 g/animal once a day) and water *ad libitum*.

Findings: Grade 1 conjunctival redness was seen in all 3 rabbits, together with grade 1 conjunctival swelling and lacrimation in 1 rabbit at 1 h post treatment. The conjunctival redness persisted in 2 rabbits up until 24 h and not thereafter with no further ocular responses seen at this observation time. An irritation grade of 0.3 was calculated for these 2 animals. No further information on clinical observations was provided. Based on these findings, E 1582 32.5 WP 0111 and SIR 8514 3.5 formulation was not considered to be an eye irritant in rabbits by the study authors.

Conclusions: Under the conditions of the study, E 1582 32.5 WP 0111 and SIR 8514 3.5 formulation was considered to be a slight eye irritant in rabbits.

Skin irritation

Study and observations: A 0.5 g quantity of E 1582 32.5 WP 0111 and SIR 8514 3.5 {azinphos-methyl (32.9%) and Triflumuron: 2-chloro-N-[4-(trifluoromethoxy) phenylaminocarbonyl] benzazamide (3.5%); batch no. 233813308; stability: study period, cream coloured powder; source: Bayer AG, Leverkusen, Germany)} mixed to a paste with water and spread on a Hansamed hypoallergenic dressing, was applied on shaven intact skin (6 cm^2) of one flank of each of 3 adult, male NZW rabbits (Interfauna, UK Ltd, 3.1-3.3 kg body weight, age unspecified) under occlusive conditions. A further dressing moistened with water was applied similarly on the shaven, intact skin of the opposite flank of the animal and served as a control. Four hours after the treatment, the dressings were removed and the sites were rinsed with water. The skin response (erythema/eschar and oedema formation) were examined at 1, 24, 48, 72 h and on days 7 and 14 post patch removal and scored according to the guidelines used. The individual Draize scores were used to calculate individual "irritation grades". As only three animals were used, the interpretation was based on the individual irritation grades of the two most sensitive animals. No other information on clinical observations was provided. The rabbits were individually housed under conventional laboratory conditions and provided with Ssniff K4 diet (100-120 g/animal once a day) and water *ad libitum*.

Findings and conclusions: No skin responses were noted at any of the assessment times in any of the treated animals. Under the conditions of the study, E 1582 32.5 WP 0111 and SIR 8514 3.5 was not a primary skin irritant in rabbits.

2.3.3.6 Guthion 25% EC

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Dermal	Rat [SD]	M	4 or 8/group	200, 280, 300, 320, 360, 400, 800, 1200 (intact, unwashed)	322	DuBois & Murphy (1956)

SD = Sprague-Dawley

2.3.3.7 Guthion 25% WP

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	F	24	NS (in water)	~40	Dubois (1964)
Dermal			16	NS, in CMC (intact)	~1000	

SD = Sprague-Dawley; CMC = Carboxymethylcellulose; NS = Not stated.

2.3.3.8 Guthion 23%

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	M	24	NS	~75	DuBois & Kinoshita (1965)
Dermal			24	NS	~475	DuBois & Kinoshita (1965)

SD = Sprague-Dawley

2.3.3.9 Guthion ULV 22.5%

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Inhalation(30 min, whole body)	Mouse [Carworth]	F	10	NS (50% aerosols < 3µm)	~5700	Dubois (1967)
	Rat [Holtzman]		6	NS (50% aerosols < 3µm)	~14250	

NS = Not stated

2.3.3.10 Guthion 22.2% Liquid Concentrate

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	F	4	50, 60, 70, 80	60	Nelson & Bauman (1968)
Dermal				300, 350, 400, 450, 500, 550	350	

SD = Sprague-Dawley

2.3.3.11 Guthion 22% Spray Concentrate

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	F	4, 8 or 12/group	25, 35, 45, 50, 55, 60	35-45	Nelson & Bauman (1969)
Dermal			4	325, 350, 375, 400	350	

SD = Sprague-Dawley

2.3.3.12 Guthion 2L (22%)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	M/F	10/sex	22, 32, 47, 69, 102, 220	75 (M) 55 (F)	Nelson (1979b)

SD = Sprague-Dawley

2.3.3.13 E 1582 21.3 WP 02799/0346 (azinphos-methyl) and E 158 6.3 (demeton-S-methylsulfone)

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW]	3	100 µL into the conjunctival sac, washed	Slight irritant	Pauluhn (1986a)¶
Dermal		3	500 mg, intact, abraded, cleaned	Non irritant	

NZW = New Zealand White; ¶ = OECD Guidelines.

Pauluhn J (1986a) E 1582 21.3 WP 02799/0346 and E 158 6.3 (c.n. azinphos-methyl, demeton-S-methylsulfone): Study for irritant/corrosive effect on skin and eye (rabbit). Study no: T1021594. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld. Sponsor: Bayer AG. Study duration: January 1986. Report no. 14343. Report date: 12 February 1986.

The study methods used conformed to the OECD Guideline for Testing of Chemicals Nos. 404 and 405. No GLP or QA statements were provided.

Eye irritation

Study and observations: A quantity of 100 µL (equivalent to about 70 mg) of E 1582 21.3 WP 02799/0346 and E 158 6.3 [azinphos-methyl and demeton-S-methylsulfone (S-2-ethylsulphonylethyl-O,O-dimethylthiophosphate; Fl no: 430; formulation details, source and stability unspecified)] was instilled into the conjunctival sac of one eye of each of 3 female, NZW rabbits (Hacking and Churchill Ltd, U.K and Interfauna UK Ltd, 3.3-3.5 kg body weight, age unspecified). The untreated eye served as a control. The treated eyes were examined at 1, 24 (at which time the eyes were rinsed with physiological saline), 48, 72 h and on days 7, 14 and 21 post treatment. The Draize grades recorded separately for cornea (opacity and area affected), iris (hyperaemia and reaction to light) and conjunctivae (erythema and chemosis) were used to calculate the individual "irritation grades". Only those findings persisting for 24 h or longer were included in the assessment. At 24 h post treatment, a drop of 1% fluorescein solution was placed on the cornea of each of the treated eyes which was rinsed again with saline solution. The eyes were then examined under UV light for any damage to the corneal epithelium. Where positive effects were found this procedure was repeated at the later observation times. During the study, the animals were housed individually under conventional laboratory conditions and provided with standard food and water *ad libitum*.

Findings: Grade 1 conjunctival redness was seen in all 3 rabbits at 1 h post treatment along with grade 1 conjunctival swelling in one rabbit; conjunctival swelling and tear flow (grade 1) in another. No further ocular responses were noted, except for grade 1 conjunctival redness in one rabbit at 24 h post treatment. Based on these results, E 1582 21.3 WP 02799/0346 and E 158 6.3 was not considered to be an eye irritant in rabbits by the study authors.

Conclusions: The formulation, E 1582 21.3 WP 02799/0346 and E 158 6.3 was a slight eye irritant in rabbits.

Skin irritation

Study and observations: A quantity of 500 mg of E 1582 21.3 WP 02799/0346 and E 158 6.3 [azinphos-methyl and demeton-S-methylsulfone (S-2-ethylsulphonylethyl-O,O-dimethyl thiophosphate; Fl no: 430; formulation details, source and stability unspecified)] mixed to a paste with water was applied on shaven, intact skin (6 cm^2) on one flank of each of 3 female NZW rabbits (Hacking and Churchill Ltd, UK and Interfauna UK Ltd, 3.0-3.2 kg body weight, age unspecified) under occlusive conditions. A further patch moistened with water was placed on the shaven intact skin of the opposite flank of the animal and served as a control. Both patches were held in place by using an elastic adhesive tape during the exposure period. After 4 h of exposure, the dressing was removed and the exposed sites were washed with water. The skin reactions were evaluated and scored for erythema/eschar and oedema formation at 1, 24, 48 and 72 h and on days 7 and 14 after patch removal. The individual scores recorded separately for erythema/eschar and oedema formation at 24, 48 and 72 h were averaged to obtain the mean "irritation grade". During the study, the animals were individually housed under conventional laboratory conditions and provided with standard diet and water *ad libitum*.

Findings and conclusions: No skin reactions were observed in any of the test animals at any of the assessment times. Under the conditions of the study, E 1582 21.3 WP 02799/0346 and E 158 6.3 was not a primary skin irritant in rabbits.

2.3.3.14 Guthion 2F (20%)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	M	10	10, 19, 36.1, 68.6, 130.3	43.8	Hixson (1980a)
		F		10, 16, 25.6, 41, 65.6	29.9	
Dermal (abraded)	Rabbit [NZW]	M/F	5/sex	2000 (24 h, abraded, occluded, applied material removed)	> 2000	Hixson (1980b)
Inhalation (1 h, head only)	Rat [SD]	M	10	1360, 2150, 2190, 2200, 2256, 2340, 2640, 3550 (28% droplets <4.72 μm)	2662	Sangha (1980)
		F		1360, 1660, 2150, 2640, 3550 (28% droplets < 4.72 μm)	2467	

SD = Sprague-Dawley; NZW = New Zealand White

Eye and dermal irritancy studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW]	9	100 mg in the eye, washed, unwashed	Severe irritant	Hixson (1980d)
Dermal		6	500 mg, intact, abraded, cleaned	Slight irritant	

NZW = New Zealand White

Eye irritation

Hixson EJ (1980d) Eye and dermal irritation of Guthion 2F. Study no: 80-033-07 and 80-023-03. Lab: Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Centre, Stilwell, KS 66085, USA. Sponsor: Bayer GmbH, Leverkusen. Study duration: May 28 - June 23, 1980. Bayer Report no. 68876. Report date: 28 July 1980.

Pre GLP, non-quality assured study. Draize method was used.

Study and observations: A 100 mg quantity of Guthion 2F (azinphos-methyl; 20% ai; batch no. 0033014; Formula no. 1831; solubility and stability unspecified; Mobay Chemical Corporation, Agricultural Chemicals Division, Stilwell, KS, USA) was applied into the left eye of each of 9 NZW rabbits (6 males, 3 females, Small Stock Inc, Pea Ridge, Arkansas, USA; age, body weight not stated). The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. Three male rabbits had their eyes washed with 200 mL lukewarm water, 45 seconds after treatment (Group A). The eyes of the remaining 6 rabbits were not washed (Group B). Evidence of ocular irritation (responses in the cornea, iris, conjunctivae) was assessed at 1, 2, 3, 4, 7 and 14 days post treatment and scored. During the study, the animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Grade 1 conjunctival erythema was seen in all 3 Group-A rabbits. In one animal, this condition persisted up until 2 days, while in the remaining 2 rabbits up until 7 days, in both cases, resolving at the next observation time. Grade 1 conjunctival chemosis together with slight to moderate (grade 1-2) ocular discharge were observed in 2 rabbits at 2 days post treatment and not thereafter.

One rabbit (male) in Group-B, exhibited grade 1 conjunctival erythema, corneal opacity, chemosis and ocular discharge through day 14, post treatment. According to the study authors, "redness" in both eyes of this animal was seen at day 3, post treatment. Four remaining rabbits showed grade 1-2 conjunctival erythema and ocular discharge at 1 or 2 days post treatment and not thereafter. No ocular responses were seen in one animal in this group. No further clinical observations were provided. Based on these findings, the test substance was classified as a mild eye irritant in rabbits by the study authors.

Conclusions: Under the conditions of the study, guthion 2F formulation (containing 20% azinphos-methyl) was a severe eye irritant to rabbits.

Skin irritation

Study and observations: A 500 mg quantity of Guthion 2F (per site, batch no. 0033014, formula no. 1831, azinphos-methyl content: 20%, solubility and stability data not provided) in physiological saline was applied to shaved, intact (2 sites) and abraded (2 sites) skin on the back and sides of each of 6 male NZW rabbits (Small Stock Inc, Pea Ridge, Arkansas, USA, age, body weight range unspecified) on a 2.5 cm^2 gauze patch under occlusive conditions. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. After 24 h, the patches were removed, and the application sites were cleaned with a damp cloth. The skin sites were evaluated for irritation responses at that time, and 48 h later (ie at 24 and 72 h post treatment). During the exposure period, the test animals wore plastic collars. The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Five rabbits with intact skin showed slight erythema (grade 1) in one or both application sites with 3 of them also exhibiting slight odema (grade 1) in one application site at 24 h post treatment but not thereafter. Slight erythema and slight odema (grade 1) were observed in all 4 skin sites of three rabbits with abraded skin at 24 h post treatment. At this observation time 2 rabbits with abraded skin had grade 1 erythema (slight) of the skin in one treatment site. No skin responses were observed in any animal at 72 h post treatment. No further clinical observations were provided. This formulation was considered to be a mild skin irritant by the study authors.

Conclusions: Under the conditions of the study, guthion 2F formulation was a slight skin irritant in rabbits.

2.3.3.15 Guthion 2S

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	M/F	10/sex	15, 22, 32, 47, 60 (M/F)	37 (M)	Nelson

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
				and 10 (F)	21 (F)	(1978b)
Dermal	Rabbit [NZW]	M	4	200, 400, 800, 1600 (24 h, abraded, occluded, applied material removed)	504 (M)	Nelson (1978a)
		F		200, 400, 520, 676, 800, 879 (24 h, abraded, occluded, applied material removed)	568 (F)	
Inhalation (1h, head only)		M/F	10/sex	856, 1146, 1471 (M/F), 2343 (M) (96-99.6% droplets < 4.88 μm)	1365 (M) 1080 (F)	Nelson (1978c)

SD = Sprague-Dawley; NZW = New Zealand White

2.3.3.16 Guthion M-E 2 lb/gal Spray Concentrate

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw	LD_{50} (mg/kg bw)	Reference
Dermal	Rat [SD]	F	4	50, 150, 200, 350, 500	150	Nelson (1967a)

2.3.3.17 E 1582 19.5 EC 00126/0667

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW]	1	100 mg in the conjunctival sac, washed	Severe irritant	Krotlinger (1995a)¶ (GLP)
Dermal		4	500 mg, intact, abraded, cleaned	Moderate to severe irritant	
Skin sensitisation	Guinea pigs	40	Induction and challenge exposures (BM)	Sensitiser	Vohr (1995)¶ (GLP)

NZW = New Zealand White; ¶ = OECD Guidelines and EEC Directives; BM = Buehler Method.

Eye irritation

Krotlinger F (1995c) E 1582 19.5 EC 00126/0667 (cn. Azinphos-methyl) Study for skin and eye irritation/corrosion in rabbits. Study no: T9058321, Lab: Bayer AG, Fachbereich Toxicology, Friedrich-Ebert-Str. 217-333, 42096 Wuppertal. Sponsor: Bayer AG. Study duration: October 5-November 15, 1994. Report no. 23673. Report date: 25 January 1995.

GLP, quality assured study. Conducted in accordance with the EEC directive 92/69/EEC Part B, No: B. 4 and OECD Guidelines Nos. 404 and 405 for testing chemicals.

Study and observations: A 100 μL quantity of undiluted E 1582 19.5 EC 00126/0667 (Formulation no: 0807 based on Form. no: 00126/0667, azinphos-methyl content: 19.3%, stability: guaranteed for the study period, clear yellow-brown liquid) was applied into the conjunctival sac of one eye of a female NZW rabbit (body weight: 4.1 kg, Interfauna UK Ltd, Huntington, England, age: unspecified). Because of irritant effects seen on the skin (see

evaluation below), only one animal was used in this study. Evidence of ocular irritation (responses in the cornea, iris, conjunctivae) was assessed at 1 h and 1 (at which time the treated eye was rinsed with saline solution), 2, 3, 7, 14 and 21 days post treatment and scored. At 24 h post treatment, a drop of 1% fluorescein solution was placed on the corneal surface of the treated eye, which was rinsed again with saline solution. The eye was then examined under UV light for any damage to the corneal epithelium. Where positive effects were found, this procedure was repeated at the later observation times. The untreated eye served as a control. The test animals were acclimatised to the laboratory conditions for 2 weeks prior to the study. The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Scattered or diffuse to easily discernible areas of corneal opacity together with epithelial damage (grade 1 to 2, 75% to entire corneal surface) were observed at 21 days post treatment. Grade 1 iritis was seen up until 3 days post treatment. Similarly, grade 1-2 conjunctival erythema and swelling were seen up until 21 days post treatment. Grade 1-2 ocular discharge persisted for 3 days after treatment and not thereafter. No further clinical observations were provided.

Conclusions: Based on these results, the test formulation E 1582 19.5 EC 00126/0667 was classified as a severe eye irritant in rabbits.

Skin irritation

Study and observations: A 500 µL quantity of E 1582 19.5 EC 00126/0667 (formulation no: 0807 based on Form. no: 00126/0667, azinphos-methyl content: 19.3%, stability: guaranteed for the study period, clear yellow-brown liquid) was applied to shaven, intact skin of the dorso-lateral area of each of 4 female NZW rabbits (body weight: 2.8-4.1 kg, Interfauna UK Ltd, Huntington England, age: unspecified) on a 6 cm^2 hypoallergenic patch under occlusive conditions. A further patch moistened with deionised water was placed on the shaven skin of the opposite side of the animal. The exposure period was 4 h. After the exposure period, the patches were removed, the application sites were cleaned with water, and the skin responses were examined and scored. The untreated contra-lateral skin area served as a control. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study. During the exposure period, animals wore plastic collars. After 24 h, the patches were removed and the application sites were cleaned with a damp cloth. The skin sites were evaluated for irritation responses at 1, 24, 48, 72 h, and 7 and 14 days after patch removal. For each animal the Draize scores recorded at 24, 48 and 72 h after patch removal were added and divided by 3 to calculate the irritation index. This parameter was separately calculated for erythema/eschar formation and oedema formation. When 3 animals were used, the calculation was based on the individual scores of the two most sensitive animals. When more than 3 animals were used, the mean irritation index was calculated by averaging the total scores of all rabbits tested. If delayed reactions occurred, or where no irritation indices could be calculated, other interpretation criteria were used (unspecified). The animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: It was stated that due to the expected irritant potency of the test substance, in the first instance one animal was tested, and at a later date the study was completed by using two further animals. However, the data for 4 animals were provided in the report. According to these data, one animal died 48 h after treatment. Clinical signs observed in this animal were

emaciation, lacrimation and inappetence. No autopsy findings were provided. Grade 1-3 erythema and/or grade 1-2 oedema were seen in the 3 remaining rabbits at 3, 7 or 14 days post treatment (individual irritation indices of 2, 3 and 1.7).

Conclusions: Based on these results, the above test formulation was classified as a moderate to severe skin irritant in rabbits.

Skin sensitisation

Vohr HW (1995) E 1582 19.5 EC 00126/0667; Study for Skin Sensitising Effect in Guinea Pigs (Buehler Patch Test). Study no: T 5058327, Lab: Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Study duration: October 18 - November 25, 1994. Report no. 23726. Report date: 10 February 1995.

The study was performed according to OECD Guidelines 406 and is in compliance with the demands of Directive 92/69/EEC, Part B of July 31, 1992.

Study and observations: The Buehler epicutaneous patch test was performed with E 1582 19.5 EC 00126/0667, azinphos-methyl formulation No: 0807 based on formulation No. 00126/0667, content: 19.3%, [clear yellowish liquid]) using emulsions in sterile physiological saline with the following concentrations (based on a range-finding test and the results of the first challenge):

First to third induction:	50% (250 mg test substance/animal)
First challenge:	25% (125 mg test substance/animal) and 12% (60 mg test substance/animal)
Second challenge:	6% (30 mg test substance/animal) and 1% (5 mg test substance/animal)

The control animals were induced with sterile physiological saline solution. Three times at days 0, 7 and 14, one group of 20 (test) and 2 groups of 10 (control) female guinea pigs, strain Hsd Win:DH, previously termed Bor:DHPW (source: Winkelmann, Borchen, Germany; body weight range 282-350 g) were induced epidermal with occlusion for 6 hours (administration volume per animal 0.5 ml). The challenges were performed four and five weeks after the first induction in comparison with the control animals.

Findings: There was no difference between test substance group and control group in appearance, behaviour, and the body weight gain.

After the first challenge, 45% and 25% of the test substance animals responded with “slight localised” to “moderate confluent” skin redness to the 25% and the 12% test substance formulation, respectively [majority of the animals responding at 54 or 78 h after the challenge]. No skin reactions occurred in the control group. After the second challenge, the 6% and 1% test substance formulations led to skin redness [at 30 h after the challenge] in 10% of the test animals in both cases. There were no skin reactions in the control group.

Number of animals exhibiting skin reddening after initiation of challenges

Challenge	Test substance group (n=20)							1st and 2nd control groups (n=20)					
	Test substance patch				Control patch			Test substance patch			Control patch		
	after challenge				after challenge			after challenge			after challenge		
	30 h	54 h	78 h	total	30 h	54 h	78 h	30 h	54 h	78 h	30 h	54 h	78 h
1st													
25%	1	8	8	9	0	0	0	0	0	0	0	0	0
12%	1	3	5	5	0	0	0	0	0	0	0	0	0
2nd													
6%	2	0	0	2	0	0	0	0	0	0	0	0	0
1%	2	0	0	2	0	0	0	0	0	0	0	0	0

Conclusions: The test substance exhibited a skin-sensitising potential under the conditions of the Buehler patch test.

2.3.3.18 Guthion Spray Concentrate

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Inhalation (1 h)	Mouse (Carworth	M	10	2000 (droplet sizes unspecified)	< 2000 (10/10 deaths)	Dubois & Kleeburg (1970)
	Rat [Holtzman]		6		< 2000 (5/6 deaths)	

2.3.3.19 Guthion Liquid Concentrate

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Inhalation (1 h)	Mouse (Carworth	M	10	2000 (droplet sizes unspecified)	< 2000 (10/10 deaths)	Dubois & Kleeburg (1970)
	Rat [Holtzman]		6		< 2000 (4/6 deaths)	

2.3.3.20 Azinphos-methyl (19.3%)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [Wistar]	M/F	5/sex	18 or 25 (M/F) or 12.5 (F)	< 18 (M) (3/5 deaths) 16 (F)	Krotlinger (1995b) (GLP)
Dermal	Rat [Wistar]	M/F	5/sex	220, 800, 2000 (M/F) and 1100 (M) or 530 (F) (24 h, occluded, applied material was removed)	1021 (M) 727 (F)	Krotlinger (1995a) (GLP)

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Inhalation(4 h, head only)	Rat [Wistar]	M/F	5/sex	21, 407, 409 (77% droplets < 3 μm)	407 (M/F)	Pauluhn (1996) (GLP)

2.3.3.21 Guthion Fruit Tree and Garden Spray (13% azinphos-methyl)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	M/F	5/sex	50, 100, 150, 200	130 (M) 111 (F)	Eigenberg (1987) (GLP)
Dermal	Rabbit [NZW]	M/F	5/sex	1000, 1500, 2000 (24 h, occluded, applied material removed)	> 2000 (M/F) (1/5 deaths/sex)	Bailey (1987) (GLP)
Inhalation 4 h, head only	Rat [SD]	M/F	10/sex	326, 520, 542, 588 (aerosols of MMAD: 2.1-2.9 μm)	486 (M) 442 (F)	Shiotsuka (1987c) (GLP)
Inhalation 1 h, head only	Rat [SD]	M/F	10/sex	2277, 2758, 4266 (M/F) and 810 (F) or 3166 (M) (aerosols, of MMAD: 2.3-2.9 μm)	2830 (M) 2001 (F)	Shiotsuka (1988b) (GLP)

SD = Sprague-Dawley; NZW = New Zealand White; MMAD = Mass median aerodynamic diameter

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbits [NZW]	3/sex	100 μL, conjunctival sac	Severe irritant	Eigenberg (1987)¶ (GLP)
Dermal		3/sex	500 mL, intact, occluded	Severe irritant	
Skin sensitisation	Guinea pigs, M	40	400 μL, topical induction and challenge (BM)	Non sensitiser	Porter *et al* (1987)¶ (GLP)

NZW = New Zealand White; ¶ = US EPA Guidelines; BM = Buehler Method.

Eye irritation

Eigenberg DA (1987) Primary eye irritation of guthion fruit tree and garden spray in albino rabbits. Study no: 87-333-01, Lab: Mobay Corporation, Health, Environment and Safety, Corporate Toxicology Department, 17745 South Metcalf, Stilwell, KS 66085-9104, USA. Sponsor: Agricultural Chemicals Division, Mobay Corporation, Box 4913, Hawthorn Road, Kansas City, Missouri 64120-0013, USA. Study duration: April 13 - May 11, 1987. Report no. 874. Report date: 25 June 1987.

Quality assured, GLP study. Conducted according to the Standard Procedure No. B-6 and Standard Protocol No. 04 of the performing laboratory, which were based on the US EPA FIFRA Guidelines of November, 1984 (Guideline 81-4) and US EPA TSCA Guidelines of September, 1985 (Section 798.4500).

Study and observations: A 100 μL quantity of guthion fruit tree and garden spray (azinphos-methyl content 13%, appearance: amber liquid, batch: 87R0027S, formula no: 1475-B,

stability: indefinite) was applied into the conjunctival sac of the left eye of each NZW rabbits (3/sex, Small Stock Inc, Pea Ridge, Arkansas, USA, body weight range and age: unspecified). It was stated that because the test substance caused dermal irritation (see evaluation below), one drop of Propacaine was placed in both eyes, about 1 minute prior to the treatment with the test material. Evidence of ocular irritation (responses in the cornea, iris, conjunctivae) was assessed at 1 h and 1, 2, 3, 7, 8, 14, 21 and 28 days post treatment and scored. The right eye served as a control. The test animals were acclimatised to the laboratory conditions for at least 6 days prior to the study. The animals were individually housed under standard laboratory conditions and provided with food (Agway Prolab Rabbit Chow) and water *ad libitum*.

Findings: Grade 1-3 conjunctival redness was observed in all 6 rabbits following treatment and persisted throughout the study period in 4 animals. In 2 rabbits, erythema was resolved by 21 days post treatment. Grade 1-3 conjunctival swelling was seen up until 14 or 21 days in females, and up until 3 days in males. Corneal opacity of grade 1-2 was seen at 24 h post treatment in all rabbits, which resolved only in 2 animals by 21 or 28 days post treatment. Grade 1 iritis noticed in 1 rabbit at 24 h post treatment was resolved by 72 h post treatment. Grade 1-3 ocular discharge was seen in all rabbits for 3-14 days after treatment. No further clinical observations were provided. Based on these observations, guthion fruit tree and garden spray was classified as a primary eye irritant by the study authors.

Conclusions: Guthion fruit tree and garden spray was a severe eye irritant in rabbits.

Skin irritation

Quality assured, GLP study. Conducted according to the Standard Procedure No. B-7 and Standard Protocol No. 05 of the performing laboratory, which were based on the US EPA FIFRA Guidelines of November, 1984 (Guideline 81-5) and US EPA TSCA Guidelines of September, 1985 (Section 798.4470).

Study and observations: A 500 µL quantity of guthion fruit tree and garden spray (azinphos-methyl content 13%, appearance: amber liquid, batch: 87R0027S, formula no: 1475-B, stability: indefinite, formulation details provided) was applied to shaven, intact skin of the dorso-lateral area (6 cm^2) of each of 6 NZW rabbits (3/sex, Small Stock Inc, Pea Ridge, Arkansas, USA, body weight range, age unspecified) on a hypoallergenic patch under occlusive conditions. After about a 4-h exposure period, the patches were removed and the application sites were cleaned with paper towels dampened with tap water. The skin responses were scored for erythema and oedema at 1 h and 1, 2, 3 and 7 days after patch removal. The untreated adjacent skin area served as a control. The test animals were acclimatised to the laboratory conditions for 6 days prior to the study, and during the study, the animals wore plastic collars. The animals were individually housed under standard laboratory conditions and provided with food (Agway Prolab Rabbit Chow) and water *ad libitum*.

Findings: Well defined to moderately severe erythema (grade 1-3) and slight to severe oedema (grade 1-4) of the skin were seen in 3 rabbits at 72 h, and up until 7 days in the remaining animals (2 females and 1 male). Grade 4 oedema of the skin noticed in one male rabbit at 24 h post patch removal persisted for 2 days. Signs of skin irritation were cleared in 3 rabbits by day 7 and in all rabbits by day 14 after patch removal.

Conclusions: Based on these findings, the test formulation was a severe skin irritant in rabbits.

Skin sensitisation

Porter MC, Craigo RE & Harnagel Jr RE (1987) Dermal sensitisation evaluation of guthion fruit tree and garden spray in guinea pigs. Study no: not stated, Lab: Toxicology Department, Central Research Services, Miles Laboratories Inc, PO Box 40, Elkhart, IN 46515, USA. Sponsor: Agricultural Chemicals Division, Mobay Corporation. Study duration: March 31 – May 15, 1987. Bayer report no. 883. Report date: 29 June 1987.

Quality assured study conducted in compliance with GLP standards 40 CFR Part 160-FIFRA and 792-TSCA. The Buehler epicutaneous patch test was used.

Study and observations: A group of 40 outbred, male, Hartley albino guinea pigs (Harlan Sprague Dawley, Indianapolis, USA; 5-6 weeks old, body weight range: 309-385 g) were randomly assigned to a test group of 15 animals (guthion fruit tree and garden spray: azinphos-methyl content: 13%, amber colour liquid, batch no: 87R0027S, Mobay Corporation, Stilwell, KS, USA, formulation details provided), 2 test control groups, DNCB test group, and 2 DNCB control groups (5/group respectively). The data of a primary skin irritation study conducted to determine the highest non-irritating concentration of the test material were provided.

The test group received 3 topical induction applications of the test substance on days 0, 7 and 14. The third induction application was followed by a 2-wk rest period. The test material (0.4 mL) was applied to the shaven skin of the left side of each animal near the scapula on an adhesive patch under occlusive conditions. The exposure period was 6 h. Positive controls (DNCB in 50% ethanol/distilled water) and other control groups were similarly treated with the appropriate test formulation. After the 6-h induction exposure, the patches were removed and the application sites were cleaned with warm water. The skin sites were scored for erythema at 24 and 48 h after patch removal.

On days 28 and 42 (the first and second challenge respectively), the animals were topically challenged with the appropriate test formulation, applied as described above near the left pelvic girdle. The vehicle (ethanol/distilled water) was similarly applied to the right side of the test and control animals. Patches were removed after 24 h, the sites were cleaned with warm water and depilated. The application sites were observed for erythema and scored 24 and 48 h later. Body weights were determined at the commencement and termination of the study. The test animals were individually housed under standard laboratory conditions and provided with food and water *ad libitum*.

Findings: Body weight gain of the animals was unaffected by treatment. No skin reactions were seen in any animal in the following induction exposures to the test substance. Slight to moderate erythema was seen in 2 guinea pigs after the second and third DNCB induction exposures.

Slight erythema of the skin (grade 1) was seen in one animal in the test compound group at 48 h after the 1st challenge application. No further positive responses were observed in animals in the test substance group or naive control groups following challenge exposures.

Slight to moderate erythema, and slight to severe erythema of the skin were seen in all animals in the positive control group after the first and second challenge respectively.

Conclusions: The doubtful nature of skin response (grade 1 erythema at 48 h but not at 24 h after patch removal) observed after the first challenge, and absence of such responses after the second challenge together with negative responses in the remaining animals suggest that this test formulation is not a skin sensitiser in guinea pigs.

2.3.3.22 Guthion 12.1% Spray Concentrate with Emulsifier

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat	F	4	50, 70	70 (2/4 deaths)	DuBois (1970b)
PO	Rat	M	24	NS	96.7	DuBois & Kinoshita (1970)
Dermal			24		816.1	
Inhalation (1 h)	Rat	M	6	2000, 2500, 3000 (droplet size unspecified)	~3000	
Inhalation (1 h)	Rat	F	6	2000, 2500 (droplet sizes not stated)	~2500 (3/6 deaths)	DuBois (1970b)

2.3.3.23 Guthion 12.1 Spray Concentrate without Emulsifier

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat	M	32	NS	100.8	DuBois & Kinoshita (1970)
Dermal			24		845.3	
Inhalation (1 h)	Rat	M	6	2000, 2500, 3000 (droplet sizes not stated)	~3000	
Inhalation (1 h)	Rat	F	6	2000, 2600 (droplet sizes not stated)	~2600 (2/6 deaths)	DuBois (1970b)

2.3.3.24 Guthion Garden Spray (12.1% azinphos-methyl)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	F	24	NS (in ethanol and propylene glycol)	~70	Dubois (1963)
			20	NS (no diluent)	~70	
Dermal	Rabbit	M	24		~900	
			5		> 1500	

SD = Sprague-Dawley

2.3.3.25 Guthion Formulation 6290112E (12.1% azinphos-methyl)

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [SD]	F	20	NS (in ethanol and propylene glycol)	~85	Dubois (1963)
Dermal	Rabbit	M	6	With no diluent	> 1500	

SD = Sprague-Dawley; NS = Not stated

2.3.3.26 Guthion 2% Dust

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [SD]	F	8	50, 500	> 50 (0/8 deaths; at 500 mg/kg: 7/8 deaths)	Crawford and Nelson (1970a)
Dermal				2000 (24 h, occluded, applied material removed	> 2000 (2/8 deaths)	
Inhalation (1 h, Whole body)	Mouse [Cornet]	F	4	2000, 2500 (dust, particle size unspecified)	> 2000 (0/4 deaths)	Crawford and Nelson (1970b)
	Rat [SD]					

SD = Sprague-dawley

2.3.4 Mixtures

2.3.4.1 Azinphos-methyl (21.3% WP) and Demeton-S-Methylsulphone (6.3%)

A summary of findings of acute toxicity studies conducted with the above mixture is shown in the Table below. In all studies, doses refer to the mixture.

Median lethal dose studies

Route	Species	Sex	Group size	Doses tested (mg/kg bw) or (mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
PO	Rat [Wistar]	M/F	5/sex	2.5, 10, 25, 50, 100 (M/F) and 53, 60 (M) or 33.5, 35.5 (F)	42 (M) 30 (F)	Flucke (1986a)
Inhalation aqueous aerosols, (4 h, head only)	Rat [Wistar]	M/F	5/sex	10.1, 33.5, 199.1, 262.6	> 263 (M/F)	Pauluhn (1986b)
Inhalation dust, (4 h, head only)	Rat [Wistar]	M/F	5/sex	58, 196, 373, 780	306 (M/F)	

2.3.5 Oral Toxicity

2.3.5.1 Potentiation Studies

Dubois KP (1956) The acute toxicity of guthion and EPN given simultaneously to rats. Study no: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Sponsor and Study duration: unspecified. Report no. 1359. Report date: 16 November 1956.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: To investigate whether a combination of the two organophosphates Guthion (azinphos-methyl) and EPN (O-ethyl O-4-nitrophenyl phenylphosphonothioate) exhibit potentiation when administered simultaneously, a group of 20 female rats (Group-A, strain, body weight, age, source unspecified) were treated with one-half of the LD_{50} dose of each compound in 10% ethanol and 90% propylene glycol vehicle intraperitoneally (at 2.9 and 3.6 mg/kg body weight respectively). Another group of 20 rats (Group-B) were treated similarly with one-half of the LD_{50} of EPN plus one-half of the LD_{10} of Guthion (1.9 mg/kg bw). The mortality rate in these 2 groups was then determined. No further information on experimental methods was provided.

Findings and conclusions: The mortality rate in Group-A was 60% suggesting direct summation of the acute toxicity of the 2 compounds, whereas in Group-B, it was 25%. The acute toxicity of the latter combination was expected to be equivalent to the toxicity of 4.75 mg/kg bw dose of azinphos-methyl alone, if the acute toxic effects of the 2 compounds were additive. Percent mortality rate calculated by the study authors using log-probability plots for a dose of 4.75 mg/kg bw of azinphos-methyl was 28%, thus, indicating no potentiation of acute effects from the combined administration of these 2 compounds. This study is not adequate for regulatory purposes, due to the lack of reporting of experimental protocols.

Dubois KP (1962) Comparison of the potentiation of acute toxicity from simultaneous administration of ethyl guthion plus ethion and guthion plus ethion. Study no: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Sponsor: Bayer AG. Study duration: not stated. Report no. 10320. Report date: 5 December 1962.

[Only the data pertaining to guthion plus ethion were included in the following evaluation].

Study and observations: This study investigated the potentiation of acute toxicity of guthion (azinphos-methyl) when it was combined with either ethion or ethyl guthion. Adult, female SD rats weighing 200-250 g (6/group, age, source unspecified) were used in the study. The test animals were treated with the azinphos-methyl/ethion mixture (dissolved in 1:4 ethanol/polyethylene glycol) at 2.5/11.0 (one half of the LD_{50} value of each compound), 1.8/8.25 or 1.25/5.5 mg/kg bw (route and method unspecified). The strength of the test solutions was adjusted so that the animals always received quantities equivalent to 0.1% of their body weight. The animals were observed for a period of 14 days, although mortalities occurred during the first 2 days following treatment. Expected LD_{50} value of azinphos-methyl plus ethion was calculated (method unspecified), and the actual LD_{50} value was determined (method unspecified).

Findings and conclusions: Percent mortalities in the groups received azinphos-methyl/ethion mixture at 2.5/11.0 and 1.8/8.25 mg/kg bw were 100 and 83%, respectively. The mortality rate at 1.25/5.5 mg/kg bw was 33%. The expected LD_{50} value for an equitoxic mixture of azinphos-methyl and ethion was 13.5 mg/kg bw. It was stated that the LD_{50} value determined in the test was half of this expected value, but the actual value was not specified. The ratio between the expected LD_{50} value and observed LD_{50} value was about 2, thus indicating a potentiation effect.

Kimmerle G (1966) Guthion M (87.7%, shipment 59/64) Antidotal effect and potentiation. Study no: not stated, Lab: Institute of Toxicology, Farbenfabriken, Bayer AG. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. 20296. Report date: 7 December 1966.

Study and observations: To determine the LD_{50} value of the combination, Guthion M (azinphos-methyl, purity 87.7%), and malathion technical (purity unspecified) were mixed in the percentage ratio of their LD_{50} values (at 2.28% and 97.72% for azinphos-methyl and malathion respectively). The LD_{50} values of azinphos-methyl and malathion in non-fasting rats (strain, age, sex, body weight unspecified) were 21 and 900 mg/kg bw respectively. The anticipated LD_{50} value of the mixture was calculated by probit analysis. The LD_{50} value of the new sample in rats (strain, age, sex, body weight unspecified) was also determined.

Findings and conclusions: The ratio of the anticipated LD_{50} (460.4 mg/kg bw) to the experimentally determined LD_{50} (398 mg/kg bw) in rats was 1.16 which indicated an additive effects.

Murphy SD, Cheever KL, Chow AYK & Brewster M (1976) Organophosphate insecticide potentiation by carboxylesterase inhibitors. Proc Eur Soc Toxicol 17: 292-300.

Study and observations: In this study, potentiation of toxicity of 5 organophosphates, including Guthion (azinphos-methyl, purity > 95%, obtained from the manufacturers) in DEF (defoliant S, S, S-tributylphosphorotrithioate) pre-treated rats was examined. DEF was chosen, as this chemical is a potent inhibitor of carboxylesterases at doses which produce little or no inhibition of nervous tissue acetylcholinesterase activity. DEF was administered intraperitoneally to a group of 5 male Holtzman rats (body weight 200-250 g) at 70 mg/kg bw. Eighteen hours later, the animals were dosed with azinphos-methyl in corn oil by PO gavage or intraperitoneally (doses unspecified). Carboxylesterase activities in liver homogenates and plasma were measured using a colorimetric method. Percentage inhibition of brain AChE activity (as assayed by the method of Ellman *et al* 1961) was calculated in relation to the mean activities of a group of untreated animals. The doses required for 50% inhibition (ID_{50}s) were determined from the dose-response curve. The degree of potentiation was calculated as the ratio of the ID_{50} for brain AChE in insecticide-treated controls to the ID_{50} for brain AChE in DEF pre-treated and subsequently insecticide-dosed animals.

Findings: The data on the effect of DEF pre-treatment on tissue esterases and azinphos-methyl anticholinesterase action are presented in the following Table.

Effect of DEF pre-treatment on tissue esterases and azinphos-methyl anti-ChE activity in male rats

Degree of potentiation of:	
Azinphos-methyl (ip)	11.2-fold
Azinphos-methyl (PO)	10.2-fold
Inhibition of (%)	
Brain AChE	0
Plasma ChE	81 ± 9
Liver diethylsuccinate esterase	95 ± 2
Liver triacetin esterase	53 ± 15
Plasma diethylsuccinate esterase	74 ± 9
Plasma triacetin esterase	82 ± 17

The degree of potentiation was calculated as the ratio of the ID_{50} for brain ChE in azinphos-methyl-treated control rats to the ID_{50} for brain ChE in azinphos-methyl-treated rats that had been pre-treated with DEF.

The data presented in the above Table shows that DEF pre-treatment markedly increased the susceptibility of male rats to the anticholinesterase activity of azinphos-methyl in the absence of any inhibition of brain ChE activity. The degrees of potentiation were 10.2- and 11.2-fold for PO and ip dosing of azinphos-methyl, respectively.

The following four studies (Thyssen 1976b-1977c) were performed to investigate the combinational toxicity of azinphos-methyl and other insecticides. All the studies were performed at Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany, to the prevailing scientific standard. When the studies were performed, GLP was not compulsory.

Studies and observations: With the exceptions of animal numbers and doses used (see below), the experimental design was comparable in all four studies. To determine the LD_{50} value of the combination, the individual substances were mixed in the percentage ratio of their LD_{50} values (equitoxic quantities of both substances) to form a new sample. The anticipated LD_{50} value of this sample was calculated. The ratio of the anticipated to the determined LD_{50} resulted in a factor (F) for combination toxicity (F 0.8-1.2 = additive effect, $F < 0.8$ = sub-additive effect, $F > 1.2$ = super-additive effect). [fasted male Wistar II rats (Winkelmann) of 160-180 g body weight were used. The doses were administered in distilled water and Cremophor EL at a dosage volume of 1 mL/kg bw by PO gavage. Following treatment, the animals were observed for 7-14 days].

Statistics: LD_{50} according to Litchfield and Wilcoxon, 1949; expected LD_{50} according to Finney, 1952.

♦ Thyssen J (1976b) Studies to determine the toxic effects of the simultaneous application of azinphos-methyl or azinphos-ethyl and methamidophos. Study duration: not specified. Report no. 6354. Report date: 24 September 1976.

Chemicals used: azinphos-methyl, technical purity (batch no.: not supplied) and methamidophos, technical purity (batch no: not specified).

Number of rats: 10 or 20 males/group.
Doses of the mixed sample: 15, 17.5, 20, 22.5, 25 mg/kg bw.

♦ ***Thyssen J (1977a) Study for the combination toxicity of azinphos-methyl and propoxur. Study duration: not specified. Report no: 7174, Report date: December 14, 1977.***

Chemicals used: azinphos-methyl (batch no.: 6/05010, purity: 93.0%) and propoxur (batch no.: 75/40, purity: 99.1%).

Number of rats: 15 or 30 males/group.
Doses of the mixed sample: 25, 30, 32.5, 35 mg/kg bw.

♦ ***Thyssen J (1977b) Study for the combination toxicity of azinphos-methyl and azinphos-ethyl. Study duration: not specified. Report no: 7178 Report date: December 14, 1977.***

Chemicals used: azinphos-methyl (batch no.: 6/05010, purity: 93.0%) and azinphos-ethyl (batch no: not specified, purity: 97.5%).

Number of rats: 15 males/group.
Doses of the mixed sample: 9, 10, 11, 13, 15 mg/kg bw.

♦ ***Thyssen J (1977c) Study for combination toxicity of chlorpyrifos, cytrolane, cyolane, tamaron, gusathion-ethyl and gusathion-methyl active ingredient. Study duration: not specified. Report no: 7179. Report date: December 14, 1977.***

Chemicals used: azinphos-methyl (batch no.: 6/05010, purity: 93.0%) and 1.) chlorpyrifos (batch no. and purity: not specified, LD50: 81 mg/kg bw), 2.) cytrolane (batch no.: 372, purity: 88.5%, LD50: 5.1 mg/kg bw), 3.) cyolane (batch no.: 3380, purity: 78%, LD50: 3.2 mg/kg bw), respectively.

Number of rats: 15 males/group.
Doses of the mixed samples: 1.) 20, 25, 35, 50 mg/kg bw; 2.) 4, 5, 6.5, 7.5 mg/kg bw; 3.) 2.5, 3.5, 5 mg/kg bw.

Findings: The animals showed the typical cholinergic signs [unspecified]. The LD_{50} values are summarised in the following Table.

LD_{50} (mg/kg bw) of azinphos-methyl, the combination ingredient and the combination of both ingredients

LD_{50} Azinphos-methyl	LD_{50} Combination compound	LD_{50} Combination (factor F)	Reference
16.75	31.9 (methamidophos)	19.5 (1.2)	Thyssen 1976b
9.7	39 (propoxur)	29.3 (0.84)	Thyssen 1977a
9.7	11.8 (azinphos-ethyl)	11.1 (0.96)	Thyssen 1977b
5.3	81 (chlorpyrifos)	26 (1.7)	Thyssen 1977c
5.3	5.1 (cytrolane)	5.2 (1.0)	Thyssen 1977c
5.3	3.2 (cyolane)	3.9 (1.1)	Thyssen 1977c

Conclusions: Following acute PO administration a slight super-additive effect was observed with the combination of azinphos-methyl and chlorpyrifos. With all other compounds additive effects were evident.

♦ Doull J & Rehfus P (1957) Effects of diets containing guthion in combination with other cholinergic phosphates on dogs. Study number and duration: not specified. Lab: Department of Pharmacology, University of Chicago, IL, USA. Report no. 1489. Report date: 15 February 1957.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Adult mongrel dogs (1/sex/group, body weight: 5-12 kg, source: unspecified) were fed with diets containing Guthion technical (azinphos-methyl, purity: unstated, source: Chemagro Corporation) at 5 ppm (about 0.125 mg/kg bw/d) together with either 5 ppm parathion (diet A), 5 ppm methyl parathion (diet B), 2 ppm systox (O,O-diethyl O-2-ethylthioethyl phosphorothioate, diet C), 20 ppm EPN (O-ethyl O-4-nitrophenyl phenylphosphonothioate, diet D), or 100 ppm malathion (diet E) for 6 weeks. Test diets were prepared weekly or as needed, initially by mixing with casein and then adding required quantities to ground dog food (source: unspecified). Because 2 of the above test diets (diets C and E) caused some depression in red blood cell (RBC) ChE activity, in an attempt to understand the effects of azinphos-methyl alone, subsequently it was fed to dogs (1/sex) at 5 and 10 ppm (equivalent to approximately 0.125 and 0.25 mg/kg bw/d) from 6 weeks. All dogs were vaccinated against distemper and hepatitis and dewormed prior to treatment. Before commencement of treatment, the animals were fed with the control diet containing no azinphos-methyl for 3 weeks. Serum and RBC ChE activities were assayed before commencement of the study (using at least 5 blood samples/animal) and then weekly using a manometric method (Dubois and Magnum, 1947). The dogs were housed individually or in pairs and had constant access to prepared diets and water. No further information on experimental methods was provided.

Findings: According to the study authors, the test animals did not lose weight during the study (no actual numeric data were provided). No clinical signs related to treatment were observed. All ChE data were provided in the form of line graphs (expressed as percentages of controls). Because of lack of print clarity, the effects of the test substance on male and female ChE activities could not be distinguished from these graphs. Serum and RBC ChE activities in dogs fed with diets B and D were unaffected by treatment. About a 20% depression in serum ChE activity was seen in dogs fed with diet A during the 3rd week. About 20-25% inhibitions in both serum and RBC ChE were observed in animals fed with diet C during the study week 4. The dogs fed with diet E, exhibited about a 20% depression in RBC ChE activity during weeks 4 and 5.

In the subsequent study with azinphos-methyl alone, no biologically significant serum or RBC ChE inhibition was seen in dogs at 0.125 mg/kg bw/d at 3 weeks. No data on these 2 parameters were provided beyond this sampling time. According to the study authors, progressive inhibition in RBC ChE activity was seen in the male animal at 0.25 mg/kg bw/d, exceeding 20% depression by the end of the study.

Conclusions: Under the conditions of the study, administration of azinphos-methyl to mongrel dogs at 0.125 mg/kg bw/d (5 ppm) together with either 5 ppm parathion, 2 ppm systox or 100 ppm malathion in the diet for 6 weeks resulted in biologically significant depressions in serum or RBC ChE activities. Azinphos-methyl alone, when administered to dogs in the diet at 0.25 mg/kg bw/d for 6 weeks, resulted in RBC ChE inhibition at 6 weeks. Based on these results, a slight additive effect was apparent with the combinations of

azinphos-methyl and parathion, systox or malathion. However, due to small experimental group size and data limitations, this study was not considered adequate for any regulatory purpose.

In vitro studies

Marinovich M, Ghilardi F & Galli CL (1996) Effect of pesticide mixtures on in vitro nervous cells: Comparison with single pesticides. Toxicology 108: 201-206.

Study and observations: In this study, the toxicity of 5 pesticides including azinphos-methyl, singly and in mixtures was studied using the SH-SY5Y human neuroblastoma cell line. SH-SY5Y cells (ATCC, Rockford, MD, USA) were cultured in RPMI-1640 medium supplemented with 10% heat inactivated foetal calf serum (FCS, GIBCO), streptomycin (0.1 mg/mL) and penicillin (100 iu/mL). Initially, the cell cultures, fortified with the culture medium plus FCS containing 1 µCi/mL of [^{3}H]leucine and seeded at a density of 2 x 10^6 cells in petri dishes, were incubated at 37°C in the presence of azinphos-methyl (purity, source unspecified) in dimethylsulfoxide (DMSO) at 0.6, 6.0 and 60 µg/plate for 30 minutes, 4 or 24 h. In an attempt to assay the toxicity of the mixture of dimethoate, diazinon and azinphos-methyl, different combinations of these chemicals were incubated with the above cell culture similarly at concentrations of 1.0 + 0.6 + 0.4, 10 + 6 + 4 or 100 + 60 + 40 µg/plate respectively for 30 minutes or 4 h. The final dose volume added to the culture did not exceed 0.5%. After incubation with the test substance for 30 minutes or 4 h, the cells were resuspended in 0.1 M phosphate buffer (pH 8.0). An aliquot of this suspension was added to 5,5' dithiobis-2-nitrobenzoic acid and acetylcholine iodide for ChE assay (Ellman *et al* 1961). To evaluate any inhibitory effect on protein synthesis, the incorporation of [^{3}H]leucine into azinphos-methyl-treated cells was measured, in NaOH-solubilised and TCA-precipitated extracts, by scintillation counting. The dose that induced a 50% inhibition of protein synthesis (EC_{50}) was calculated. The protein content of cell monolayers was measured according to the method of Lowry *et al* (1951), with cytotoxicity expressed as the dose causing a 50% reduction of final cell protein content (EC_{50}). Details for treatment procedures of control cell cultures were not provided. The results were expressed as mean ± SE. As appropriate, significant differences of mean values were assessed using the ANOVA procedure, multiple range test or Student's t-test.

Findings and conclusions: The data were provided in the form of bar graphs. Concentration-related and statistically significant ($p \leq 0.05$) decreases in ChE activity (about 30-66%) compared to the controls were seen with azinphos-methyl at 6.0 and 60 µg/plate respectively at 30 minutes and 4 h. Mixtures of dimethoate, diazinon and azinphos-methyl at 10 + 6 + 4 or 100 + 60 + 40 µg/plate caused an approximately 50% significant ($p < 0.05$) depression in ChE activity at 30 min and 4 h. In comparison to the controls, azinphos-methyl caused a significant inhibition in protein synthesis (about 45%, $p \leq 0.05$) at 60 µg/plate and at 4 h only. The effect of the mixture of dimethoate, diazinon and azinphos-methyl at 100 + 60 + 40 µg/plate on protein synthesis at 4 h was about 2-fold greater than that of azinphos-methyl alone at 4 h. However, no reference to any observations or measurements of cytotoxicity was found in the report.

2.3.6 Antidote Studies

2.3.6.1 Mice

♦♦ Sterri SH, Rognerud B, Fiskum St E & Lyngaas S (1979) Effect of toxogonin and P2S on the toxicity of carbamates and organophosphorus compounds. Acta Pharmacol et Toxicol 45: 9-15.

Study and observations: Toxogonin (80 mg/kg bw) was given intraperitoneally to mice 15 min prior to the PO administration of azinphos-methyl (10, 20, 40 mg/kg bw). Twenty hours thereafter the LD_{50} was determined and the animals were taken for the analysis of acetylcholinesterase activity in erythrocytes, cerebrum and diaphragm.

Findings and conclusions: The treatment with toxogonin increased 2-fold the LD_{50} of azinphos-methyl but had no influence on the cholinesterase activities.

Edery H, Soroker D & Kuhnberg W (1970) Antidotal action of new oximes in experimental organophosphate intoxication. Israel J Med Sci 6 (2) 293-302.

Study and observations: In this study, the antidotal activity of 14 newly synthesised oximes against intoxication of 8 organophosphate compounds including gusathion (azinphos-methyl; purity, source unspecified) was examined in fasted male mice (6/group, body weight 18-22 g, source unspecified). With azinphos-methyl, antidotal activity of 4-hydroxyiminomethyl-1-(3-N, N-dimethylaminopropyl) pyridium chloride hydrochloride (Compound 30) and bis (4-hydroxyiminomethyl-pyridinium-(1)-methyl-ether dichloride (Toxogonin) was tested. Compound 30 and Toxogonin were administered to animals intraperitoneally (3 groups/compound) at 300 and 90 mg/kg bw, respectively, (as described by the study authors, "the dose levels caused no observable abnormalities") together with 25 mg/kg bw atropine sulphate in each case. Two groups of each oxime treated mice were then poisoned subcutaneously with azinphos-methyl at 18.5 mg/kg bw (2x LD_{50}), and 2 further groups at 27.75 mg/kg bw (3x LD_{50}). The 2 remaining oxime treated mice received azinphos-methyl at 46.25 mg/kg bw (5x LD_{50}) intraperitoneally. Each time, azinphos-methyl was administered 5 minutes after treatment with the oxime preparation. The animals in the control groups (6 animals/group) received either oxime plus atropine sulphate or azinphos-methyl alone.

Findings: The survival rate in groups treated with Compound 30 plus atropine sulphate and Toxogonin plus atropine sulphate, and subsequently dosed with azinphos-methyl at 18.5 mg/kg bw was 100%. A similar survival rate was seen in the group treated with Toxogonin plus atropine sulphate and then dosed with azinphos-methyl at 27.75 mg/kg bw. The survival rate in the group that received Toxogonin plus atropine sulphate and later dosed with azinphos-methyl at 46.25 mg/kg bw intraperitoneally was 66%, whilst 0% survival rate was recorded for the remaining groups.

Conclusions: Treatment with Compound 30 and Toxogonin, each jointly with atropine sulphate, appear to provide protection against azinphos-methyl acute toxicity in mice.

2.3.6.2 Rats

Dubois KP (1960) Effect of pyridine-2-aldoxime methiodide (2-PAM) and related compounds on the acute toxicity of guthion and dipterex. Study No. not stated. Lab:

Department of Pharmacology, University of Chicago, Chicago 37, IL USA. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 5724. Report date: 6 September 1960.

Pre GLP, non quality assured study. No test guidelines were cited.

Study and observations: This short report presents the data on a study conducted to investigate the antidotal effects of pyridine-2-aldoxime (2-PAM), diacetyl monoxime (DAM) and 1, 1-trimethylene-bis(4-formyl-pyridinium bromide) dioxime (TMB-4) in adult, female SD rats (5 rats/group; body weight and source not stated). The test animals were poisoned with ip administration of azinphos-methyl (source, batch not stated) of unspecified purity in a mixture of ethanol and propylene glycol (1:4) at 5.0 (LD_{50})/kg bw, immediately after which they were dosed with either 2-PAM, DAM (100 mg/kg bw) or TMB-4 (75 mg/kg bw) in 0.1 M phosphate buffer similarly.

Findings and Conclusions: The groups that received TMB-4 and DAM exhibited about 50% and 75% mortality, respectively, thus, showing no antidotal effects against azinphos-methyl poisoning. No mortalities were seen in rats treated with 2-PAM. However, 2-PAM did not show any protective action in rats receiving azinphos-methyl at 10 mg/kg bw, in which group 100% mortality was observed. In a separate experiment, the effect of atropine sulfate (100 mg/kg bw) plus 2-PAM (100 mg/kg body weight) combination was tested in a group of rats treated with azinphos-methyl at 10 mg/kg bw. The mortality rate in this group was 60%. Thus, 2-PAM did not demonstrate any significant antidotal action against acute azinphos-methyl poisoning and the effect of 2-PAM was only slightly augmented when it was combined with atropine sulfate.

Kimmerle G (1966) Guthion M (87.7%, shipment 59/64) Antidotal effect and potentiation. Study no: not stated, Lab: Institute of Toxicology, Farbenfabriken, Bayer AG. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. 20296. Report date: 7 December 1966.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and Observations: This short report presented the data on a study conducted to investigate the antidotal effects of atropine sulfate, Pralidoxime (PAM), and Obidoxime chloride (BH6) following PO administration of azinphos-methyl technical as an aqueous emulsion (purity: 87.7%) to fasted rats (15/group, strain, age, sex, body weight and source unspecified). Azinphos-methyl was administered to the test animals at 5, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 70 and 80 mg/kg bw. The LD_{50} values were determined following treatment with either atropine sulfate at 50 mg/kg bw, PAM 50 mg /kg bw, BH6 at 20 mg/kg bw, atropine sulfate plus PAM (each at 50 mg/kg bw), atropine sulfate (50 mg/kg bw) plus BH6 (20 mg/kg bw), or without any of these chemicals. Antidotal chemicals were administered to the animals intraperitoneally.

Findings and Conclusions: The LD_{50} value of azinphos-methyl in fasted rats without antidotes was 14 mg/kg bw. Treatment with atropine sulfate alone increased this value by about 2-fold. The increases noted with PAM or BH6 alone were about 1.3-fold. The combined effect of atropine sulfate plus PAM or atropine sulfate plus BH6 was greater than atropine sulfate alone, the increases in LD_{50} being about 3.4- and 4-fold respectively. Thus, treatment with atropine sulfate plus PAM or BH6 appears to be more effective as an antidote

against azinphos-methyl toxicity compared to treatment with atropine sulfate, PAM or BH6 alone.

♦ ***Sanderson DM (1961) Treatment of poisoning by anticholinesterase insecticides in the rat. J Pharm Pharmacol 13: 435 - 442, 1961.***

Study and observations: Azinphos-methyl (12 mg/kg bw) as commercial liquid formulation was administered undiluted once to Wistar rats (150-50 g), followed immediately by ip injection of atropine or pyridine-2-aldoxime (2-PAM) alone or in combination.

Findings and conclusion: Atropine and 2-PAM alone were effective against azinphos-methyl poisoning in rats. The combination was less effective.

♦ ***Lorke D & Kimmerle G (1969) Effect of reactivators on organophosphate poisoning. Reprint from Naunyn-Schmiedeberg's Arch Exp Path 263: 237 (abstract).***

Study and observations: Rats were poisoned with azinphos-methyl and treated by reactivators [pyridine-2-aldoxime (2-PAM), toxogonin] alone or in combination with atropine after initial signs of poisoning had appeared.

Findings and conclusions: The therapeutic effect was good (LD_{50} increased by 50-200%), when the reactivator was given without atropine. In combination with atropine, an enhanced effect was observed.

♦ ***Crawford CR & Doull J (1970) Antagonism of the lethal effects of Dipterex and Guthion with atropine and related drugs. Fed. Proc. 29: 349 (abstract no. 589).***

Study and observations: Azinphos-methyl in polypropylene glycol was administered once, orally, at unspecified dose levels to non-fasted adult male and female CF1 mice and Charles river rats. When the signs were well established, the following antidotes were given parenterally to antagonise poisoning: atropine, scopalamine, methantheline or propantheline, alone or in combination with pyridine-2-aldoxime (2-PAM).

Findings and conclusions: Atropine was effective against azinphos-methyl poisoning in rats and mice but the best results were obtained with scopalamine and propantheline in rats. 2-PAM alone did not protect against azinphos-methyl and its effect in combination was dependent on both the species and antidote used.

♦ ***Edery H, Soroker D & Kuhnberg W (1970) Antidotal action of new oximes in experimental organophosphate intoxication. Israel J Med Sci 6: 209 - 218, 1970.***

Study and observations: Rats and mice were treated intraperitoneally with toxogonine or 4-hydroxyiminomethyl-1-(3-N,N-dimethylaminopropyl)pyridinium chloride hydrochloride (a synthetized oxime in this laboratory) each together with atropine. Five minutes later azinphos-methyl was administered by subcutaneous (sc) injection (multiples of LD_{50}).
Findings and conclusions: The treatment with the oxime/atropine combination was successful in reducing the toxic action of azinphos-methyl.

2.4 SHORT-TERM, REPEAT-DOSE TOXICITY

Summary of doses (mg/kg bw/d or mg/m^3) at which no inhibition of ChE activity was seen following azinphos-methyl administration.

Species	Duration	Route	Plasma (P)	Erythrocyte (E)	Brain
Rat	28 days	PO	0.35/0.46 M/F	1.3/0.46 M/F	> 3.37/1.54 M/F
	29 days	PO	> 3.2/0.8 M/F	0.2/0.8 M/F	0.8/0.8 M/F
	16 weeks	PO	0.25	0.25	> 1.0
	16 weeks	PO	< 0.25	< 0.25	< 0.25
	60 days	ip	< 0.5	ND	< 0.5
	10 days	Inhalation	25.0	ND	25.0
	12 weeks	Inhalation	1.24	1.24	ND
Rabbit	3 weeks	Dermal	2.0	2.0	ND
Dog	12 weeks	PO	0.5	0.125	ND

ND = not determined.

2.4.1 Oral

2.4.1.1 Rats

Hecht (1955) Toxicology of R 1582 = E 17 147. Study no: not stated. Lab: Toxicological and Industrial Hygiene Laboratory, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. 24. Report date: 4 March 1955.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Azinphos-methyl (R1582=E17147) (purity, batch no. and source unspecified) in water and emulsifier 233 (0.02%) was administered by PO gavage to 5 male rats (150-180 g body weight, strain age, source not stated) at 2 mg/kg bw/d for 2 weeks (12 doses). From the limited information provided it appears that only the clinical signs and weight gain of the test animals were examined. There was no control group in this study and no further details for experimental methods were provided.

Findings: The study authors stated that none of the animals exhibited any signs of poisoning during treatment, and in particular no fibrillary twitching or sanguineous tears were observed. It was stated that the test animals gained about 14 g in body weight during the treatment period but no supporting data were provided.

Conclusions: When azinphos-methyl was administered by PO gavage to male rats at 2 mg/kg bw/d for 12 days over a 2-week period, no clinical signs were observed in any of the treated animals. However, this study is inadequate for regulatory purposes due to lack of a control group, data limitations and the fact that only one dose level was used.

Harper KH & Brown JM (1965) Effect of gusathion (100 ppm) upon cholinesterase activity in weanling rats. Study no: not stated. Lab: Huntingdon Research Centre, England. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. 1303/65/221. Report date: 19 August 1965.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Groups of 10 female weanling Wistar CD rats (initial body weight: 99-120 g, source, age not stated) were fed with Gusathion (azinphos-methyl; purity, batch and source unspecified) in the diet at 0 (control) and 100 ppm (estimated to be equivalent to 0 and 10 mg/kg bw/d) for 3 weeks. Prior to commencement of the study, the test animals were kept under observation for 1 week. The details for procedures on diet preparation and analytical confirmation of the test substance in the diet were not provided. The animals were observed daily for signs of toxicity. Body weight and food consumption were recorded weekly. After the 3-wk feeding period, blood samples were collected from the animals by cardiac puncture for plasma and red blood cell (RBC) ChE assay using the Δ pH method. The brain was removed and weighed following exsanguination by severing the aortic arch, and its ChE activity was determined. Significance of inter-group differences was tested using the ANOVA procedure ($p \leq 0.001$). No further details on experimental methods were provided.

Findings: No information on any clinical observations was provided. Compared with controls, group mean body weight in rats fed azinphos-methyl at 10 mg/kg bw/d was reduced time-relatedly showing a deficit of about 7% at 3 weeks. At this observation time, group mean food consumption in this group was reduced by about 11% compared to the controls. The depressions seen in both the above parameters at 3 weeks were considered to be treatment-related. Plasma, RBC and brain ChE activity data at termination are presented in the Table overleaf. According to the data presented in this Table, treatment-related, statistically significant and ≥ 20% inhibition in plasma, RBC and brain ChE activity was seen in rats at 10 mg/kg bw/d at 3 weeks. Brain and RBC ChE activity inhibition appeared more pronounced than plasma ChE inhibition.

Plasma, RBC and brain ChE activities in rats fed azinphos-methyl for 3 weeks.

Dose (mg/kg bw/d)	ChE activity (group mean)		
	Plasma	RBC	Brain
0	1.11	0.40	0.73
10	0.74* (33%)	0.16* (60%)	0.28* (62%)

*Significantly different from the controls ($p \leq 0.001$); values in parenthesis represent % inhibition compared to the control.

Conclusions: Administration of azinphos-methyl in the diet to female weanling rats at 10 mg/kg bw/d for 3 weeks resulted in treatment-related depression in group mean body weight and food consumption (by about 7% and 11%, respectively), and statistically significant inhibition in plasma, RBC and brain ChE activity at termination. As this was a single dose study, a NOEL could not be established.

Löser E (1966) Effect of gusathion in the diet on the cholinesterase activity of rats. Study no: not stated. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. RefR1582270466. Report date: 27 April 1966.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Groups of male rats (10/dose, average body weight: 165 g, age, source unspecified) were fed with gusathion (azinphos-methyl, purity, source, batch unspecified) in the diet at 0 (control), 5, 10, 20, 50 and 100 ppm (estimated to be equivalent to 0, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg bw/d) for 9 weeks. The details for procedures on animal acclimatisation and diet preparation were not provided. In addition, the concentrations of the test substance in diets were not analytically confirmed. After the 9-week feeding period, the animals were sacrificed using an unspecified method. The animals were individually housed in Makrolon cages and had constant access to prepared diets and water throughout the study. Body weights and food consumption were recorded weekly. Whole blood ChE was determined prior to, and at 3 days after the commencement of the study, then at weeks 1, 2, 3, 4, 5, 7 and 9 using the method of Pilz and Kimmerle (1962). No further information on experimental methods was provided.

Findings: It was reported that one animal at 2.5 mg/kg bw/d died of pneumonia and multiple abscesses after 59 days, apparently unrelated to treatment. A line graph provided showed that average body weight in animals receiving azinphos-methyl at 2.5 and 5.0 mg/kg bw/d was depressed at termination by about 11% and 15% respectively compared to the controls. According to the graph, the animals at 5.0 mg/kg bw/d did not gain any weight during the first 2 weeks on study, and at 2 weeks their average body was reduced by about 44% compared with controls. Further, it was stated that at termination, the inter-group differences were "significant", but no supporting data were provided. A bar graph on average food consumption in animals revealed that the food intake in rats at 2.5 and 5.0 mg/kg bw/d was about 17% lower than that of the controls. Whole blood ChE data presented as percentages of corresponding pre-treatment value (considered as 100 for all groups) are given in the following Table. Apparent dose-related depressions in whole blood ChE activity were seen at all sampling times, exceeding generally 20% at 1.0, 2.5 and 5.0 mg/kg bw/d.

Whole blood ChE activity (% of pre-treatment) in rats fed azinphos-methyl in the diet for 9 weeks

Dose (mg/kg bw/d)	Sampling time							
	at 3 days	wk 1	wk 2	wk 3	wk 4	wk 5	wk 7	wk 9
0	97.8	100	100	91.2	96.7	96.7	95.6	100
0.25	93.5	95.7	89.2	91.4	84.9	82.8	84.9	93.5
0.5	95.6	91.1	81.1	84.4	81.1	82.2	84.4	94.4
1.0	84.4	68.8	62.5	58.3	59.4	55.2	61.5	66.7
2.5	43.5	32.6	28.3	25.0	26.1	29.3	31.5	33.7
5.0	20.2	18.1	13.8	11.7	12.8	13.8	11.7	13.8

wk = week; no statistical analysis was performed on this data

Conclusions: Administration of azinphos-methyl in the diet to male rats at 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg bw/d for 9 weeks resulted in depression of terminal average body weight at 2.5 and 5.0 mg/kg bw/d by about 11% and 15%, respectively. Dose-related reductions in whole blood ChE activity were seen at 1.0, 2.5 and 5.0 mg/kg bw/d, compared to corresponding pre-treatment values. The regulatory value of the findings, however, is reduced due to data limitations and lack of clinical observations. No treatment-related effects were seen at 0.5 mg/kg bw/d.

♦ ***Eiben R, Schmidt W & Loeser E (1983) R1582 (common name: azinphos-methyl, the active ingredient of ®Guthion). Toxicity study on rats with particular attention to ChE activity (28-day feeding study as a range-finding test for a 2-year study. Study no: T 7011708, Lab: Institute of Toxicology Bayer AG, Wuppertal, Germany. Sponsor: Bayer AG, Germany. Study duration: June 1982 to July 1982. Report no. 11813. Report date: 18 May 1983.***

GLP (40 CFR part 160), non-quality assured study. No test guidelines were cited.

Main deviations from current OECD guidelines: Haematological and clinical biochemistry determinations (with the exception of ChE), and histopathological examinations were not performed.

Study and observations: Azinphos-methyl (batch no. 230105056; purity 93.3%) at concentrations of 0, 5, 20 and 50 ppm in pulverized rat diet containing 1% peanut oil was fed *ad libitum* to groups of 5 male and 5 female SPF BOR:WISW (SPF/Cpb) strain rats (source: Winkelmann, Borchen, Germany; mean body weight: 233 g for males and 158 g for females) for a period of 28 days. Observations: health status and clinical signs once or twice per day; food consumption and body weights weekly; ChE activity in RBC and plasma determined on days 1, 4, 14 and 28 by a modified Ellmann *et al* (1961) method in retro-orbital blood; brain ChE activity at necropsy; gross pathology on all rats sacrificed at the end of treatment. Heart, lungs, liver, spleen, kidneys, adrenals, gonads were weighed and preserved but not examined histopathologically.

Statistics: Significance of inter-group differences was tested using Mann-Whitney and Wilcoxon U-Test.

Findings: The average doses ingested at 5, 20 and 50 ppm were 0.35, 1.30 and 3.37 mg/kg bw/d for males and 0.46, 1.54 and 3.96 mg/kg bw/d for females, respectively.

There were no effects on appearance, behaviour and mortality up to and including 50 ppm, no detrimental effect on body weight gain and food consumption, and gross pathological examination gave no indications of test compound-related organ damage up to and including 50 ppm. There was a dose- and time-dependent inhibition of plasma and erythrocyte ChE activity at dose levels of 20 ppm and above. [A statistically significant depression (26%) in plasma ChE activity was seen at 20 ppm in males, only at day 28. A statistically insignificant depression (20%) in plasma ChE activity was seen in males at this same dose on day 4 and 14, however no plasma ChE inhibition was observed in females. A statistically significant decrease in plasma ChE activity of 44-66%, relative to the control group, was seen in females at 50 ppm. At this dose, inhibition of plasma ChE activity in males was 25-34%, and this was consistent across the entire sampling period. RBC ChE activity in males was unaffected by at 20 ppm, but in females inhibition of RBC ChE activity was statistically significant (17-22%). At 50 ppm, RBC ChE inhibition was 14-22% in males and 34-35% in females and statistically significant on days 14 and 28.] Brain ChE activity was inhibited [$p \leq 0.01$] in the female group receiving 50 ppm in the diet.

Effect of dietary-administered azinphos-methyl on ChE activity (u/mL) in rats

Dose level Sex	0 ppm M/F	5 ppm M/F	20 ppm M/F	50 ppm M/F
Plasma				
1 d	0.51/1.26	0.52/1.21	0.47[8%]/1.39	0.46/1.07
4 d	0.49/1.25	0.50/1.27	0.39[21%]/1.28	0.37/0.70* [25%]/[44%]
14 d	0.48/1.34	0.48/1.41	0.38[21%]/1.42	0.32*/0.63** [34%]/[53%]
28 d	0.50/1.66	0.50/1.57	0.37[26%]*/1.70	0.37*/0.65** [26%]/[61%]
RBC				
1 d	2.81/2.89	3.26/3.01	3.81/3.11	3.13/3.11
4 d	2.58/2.84	2.61/3.03	2.74/3.28[+15%]**	2.37/2.61
14 d	2.71/3.04	2.80/3.10	2.45/2.53[17%]*	2.13**/2.02** [22%]/[34%]
28 d	2.66/3.23	2.73/2.87	2.57/2.53[22%]**	2.30**/2.11** [14%]/[35%]
Brain				
28 d	1.40/1.39	1.68/1.32	1.56/1.14[18%]	1.28/0.65** [9%]/[53%]

Mean values from 5 animals/sex/group; * $p < 0.05$; ** $p < 0.01$, d = day; Values in square brackets represent % inhibition compared to corresponding controls.

Conclusions: The NOEL for dietary administration of azinphos-methyl to rats over 28 days was 5 ppm, equivalent to 0.35 mg/kg bw/d in males and 0.46 mg/kg bw/d in females. A dose-related inhibition of plasma and erythrocyte ChE activity was observed at dietary concentrations of 20 and 50 ppm, whereas brain ChE activity was inhibited at 50 ppm in females only. [From this preliminary investigation, the dose levels of 5, 15 and 45 ppm were chosen for the chronic toxicity study].

Broadmeadow A, Lee P, Ashby R, Brown PM & Flower JSL (1986) Cotnion technical: Preliminary toxicity study by oral (gavage) administration to CD rats for four weeks. Study no: not stated. Lab: Life Science Research Ltd, Eye, Suffolk IP23 7PX, England. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer-Sheva 84100, Israel. Study duration: October 9 - November 7, 1985. Report no. 85/MAK056/779. Report date: 19 February 1986.

The study authors stated that the study was conducted according to the internationally recognised GLP standards existed at that time, the procedures and the data were subjected to specific examination, and the final report was reviewed by the QA unit. However, no supporting GLP or QA statements were provided. No test guidelines were cited.

Study: In this dose range-finding study, groups of CD rats (5/sex/group, Charles River, UK, 3-5 weeks old, body weight: 50-69 g) were treated with Cotnion technical (azinphos-methyl, purity 93%, batch: 0013, Makhteshim Chemical Works, Israel) in corn oil by PO gavage at 0, 0.2, 0.8 and 3.2 mg/kg bw/d for 29 days. The test doses were prepared fresh daily by serial dilution prior to administration. The dosage volume was 15 mL. The animals were acclimatised to the laboratory conditions for 1 week before commencement of treatment. They were housed (5/group) under conventional laboratory conditions and offered food (Labsure, K andK Greeff Chemicals, UK) and water *ad libitum*, except overnight and during sampling.

Observations: Clinical signs were checked 3 times/day. Mortality, morbidity and evidence of systemic toxicity or ill health were recorded daily. Detailed examinations including palpation, food consumption assessment (including food conversion ratio) were conducted weekly. Body weights were recorded on the day that treatment commenced, then twice weekly and at termination. Water consumption was not measured. Ophthalmoscopic examinations were conducted prior to commencement and then at 21 days. The following haematological and clinical chemistry examinations were conducted at 21 days using retro-orbital blood samples: PCV, Hb, RBC, WBC, platelet count, MCH, MCHC, and ALT, AST, urea, creatinine, glucose, total bilirubin, total protein, electrophoretic protein fractions, sodium, potassium, chloride, calcium and phosphorous concentrations. Plasma (using both acetyl and butyryl choline as substrates) and RBC ChE activity in retro-orbital blood samples (Ellman *et al* 1961), was measured at day 26 on study. Urinanalysis was carried out at 27 days for appearance, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilin, nitrite and cells. After completion of scheduled treatment, the animals were sacrificed by CO_2 inhalation and necropsied. The left half of the brain was sampled for ChE assay (Ellman *et al* 1961). The following organs were dissected and weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus and uterus. Samples of the above and a range of other tissues, including bone marrow smears were processed for histopathology. Where appropriate, the statistical significance of inter-group differences was tested using the Student's t-test or Dunnet's test.

Findings: There were no premature mortalities. No treatment-related clinical signs were observed in any of the treated animals. Body weight, food consumption, food conversion ratio and haematological parameters were unaffected by treatment. Ophthalmoscopy did not reveal any treatment-related occular lesions at any dose. The data on plasma, RBC and brain ChE activities are presented in the following Table.

Plasma, RBC and brain ChE activities in rats treated with azinphos-methyl orally for 26 days.

Dose (mg/kg bw/d)	ChE activity (iu/L, mean ± SD)			
	Plasma		RBC	Brain
	Butyryl	Acetyl	Acetyl	Acetyl
Males				
Control	1142 ± 133	727 ± 117	2121 ± 309	12800 ± 200
0.2	1245 ± 200	811 ± 127	1871 ± 134	13000 ± 600
0.8	1194 ± 58	783 ± 60	1705 ± 144* (20%)	12900 ± 700
3.2	1001 ± 155	607 ± 116	855 ± 78** (60%)	7000 ± 1600** (45%)
Females				
Control	3169 ± 811	1927 ± 603	1933 ± 140	12300 ± 600
0.2	2714 ± 530	1672 ± 308	1840 ± 213	13600 ± 400
0.8	2929 ± 419	1876 ± 312	1902 ± 313	12200 ± 2600
3.2	1863 ± 325* (41%)	1111 ± 234* (42%)	717 ± 80** (63%)	6300 ± 900** (49%)

* Biologically significant (≥ 20%) compared to the corresponding controls. Values in parentheses represent % inhibition compared to the corresponding control.

At 3.2 mg/kg bw/d, plasma butyryl and acetyl ChE activities in males appeared slightly depressed (by about 12% and 16% respectively) and significant reductions ($p \leq 0.01$) in plasma butyryl and acetyl ChEs were seen in females. Treatment-related inhibition of RBC ChE activity was seen reaching statistical significance ($p \leq 0.001$ or 0.01) at 0.8 and 3.2 mg/kg bw/d in males compared to the corresponding controls, and at 3.2 mg/kg bw/d in females. At termination, the brain ChE activity in animals at 3.2 mg/kg bw/d was

significantly reduced ($p \leq 0.001$) compared to the concurrent controls. Necropsy did not reveal any treatment-related effects. No inter-group differences were seen in organ weights and histopathology.

Conclusions: Administration of azinphos-methyl by PO gavage to rats at 0.2, 0.8 and 3.2 mg/kg bw/d for 29 days resulted in significant depressions in plasma butyryl and acetyl ChE activities in females at 3.2 mg/kg bw/d, and in RBC ChE activity in males at 0.8 and 3.2 mg/kg bw/d, and in females at 3.2 mg/kg bw/d. Brain ChE activity in rats at 3.2 mg/kg bw/d was significantly reduced compared to the concurrent controls. Based on RBC ChE inhibition in males at 0.8 mg/kg bw/d, the NOEL for this study was 0.2 mg/kg bw/d. From this preliminary investigation, the study authors concluded that the dose levels of 0.2, 0.8 and 3.2 mg/kg bw/d would be suitable for a 13-week study.

2.4.1.2 Cattle

Anderson CA (1963) The effect of feeding guthion on the blood cholinesterase activity and milk production of dairy cows. Study no: not stated. Lab: Research Department, Chemagro Corporation. Sponsor: Bayer AG, Germany. Study duration: not stated. Report no. 16950. Report date: 8 January 1963.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Groups of dairy cows (3/dose, breed, age, body weight, source not specified) were fed with guthion (azinphos-methyl; purity, source, batch unspecified) in grain supplement at 0 (control), 16.8 and 33.6 ppm (equivalent to approximately 0, 0.25 and 0.5 mg/kg bw/d) for 28 days. The control group consisted of 4 animals. Half of the daily dose was given in the morning and the remainder in the evening, in each case mixed in about 225 g of the supplement. After the 28-day feeding period, the animals were kept on the untreated control diet for one week. Milk production was measured daily. Whole blood ChE was determined on days 0 (commencement of feeding), 1, 3, 7, 10, 14, 21, 28, and at day 7 post-treatment using the Δ pH method. No further details on experimental methods were provided.

Findings: It was stated that shortly after commencement of treatment, one animal at the low-dose group entered the feed room and consumed an unspecified amount of concentrated feed causing "protein poisoning". Therefore, this animal was removed from the experiment. Average daily milk production was reduced in cows at 0.25 mg/kg bw/d by about 37% compared to the controls during both the treatment and recovery periods. A similar decrease in milk production was seen in cows at 0.5 mg/kg bw/d during the first 2 treatment weeks, which decreased progressively during the remaining study period. At day 7 post-treatment, the average daily milk production at 0.5 mg/kg bw/d was about 25% of that of the controls. The cows fed at 0.25 mg/kg bw/d showed about 20-25% depressions in whole blood ChE activity, starting from day 15 with no signs of recovery seen during the 7-day post-treatment recovery period. About 30-50% depressions in whole blood ChE activity were seen in cows at 0.5 mg/kg bw/d starting from day 7, showing a slight trend of recovery during the recovery period. The decrease in milk production and whole blood ChE inhibition seen at both dose levels were considered to be treatment-related and biologically significant. The study authors stated that except for the drop in milk production, the treated animals did not exhibit any "gross toxic symptoms". No further information was provided.

Conclusions: Under the condition of the study, feeding of azinphos-methyl at 0.25 and 0.5 mg/kg bw/d in grain supplement to dairy cows for 28 days resulted in treatment-related and biologically significant reduction in average daily milk production and whole blood ChE inhibition.

White RG, Nelson DL & Allen AD (1968) The toxicity of guthion to cattle. Study ref: 64-69 AH-68A-621. Lab: Research and Development Department, Chemagro Corporation. Sponsor: Bayer AG, Study duration: not stated. Report no. 2306. Report date: 26 August, 1968.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Guthion (azinphos-methyl; purity, source unspecified. batch: 670 and 679) was administered to 12 heifers (average body weight: 334.8 kg, source, age unspecified) of a mixed breed at 3.6 mg/kg bw/d for 6 days. One half of the dose was given in the morning and the remainder in the evening, in each case in a gelatin capsule. Two similar heifers served as controls. The animals were fed with their normal ration through out the study. Whole blood ChE was measured on 3 consecutive days prior to commencement of treatment, on days 2 and 5, and at 2 and 7 days of the 7-day post treatment recovery period. The animals were observed intermittently for clinical signs during the course of the study. When the acute signs of poisoning were evident the animals were treated with iv injections of (0.25-1.0 mg/kg bw) atropine sulfate plus 2-PAM (10-15 mg/kg bw). No further details on experimental methods were provided.

Findings: Five of the 12 treated animals died on days 1, 2 and 4 on study (1, 2 and 2 animals respectively). Paralysis was seen in one heifer on treatment day 1 and in 2 heifers on treatment day 2. Clinical signs observed in animals were diarrhoea, profuse salivation, muscle fasciculation and colic (described by the study authors as "moderate cholinergic symptoms"). Further, it was stated that the clinical signs in the surviving animals persisted until day 5 post-treatment and they made an "uneventful recovery" during the last 2 days of the 7-day recovery period. A line graph showed that whole blood ChE activity in the treated animals was depressed by about 80% compared to the controls at 2 days. At 5 days, the ChE depression was about 60% compared to the controls. A slight recovery of ChE activity (about 20%) was noticeable during last 5 days of the post treatment recovery period. Necropsy examination of 3/5 animals that died revealed haemorrhages from mechanical trauma to the gastro-intestinal tract.

Conclusions: Under the conditions of the study, administration of azinphos-methyl orally at 3.6 mg/kg bw/d to heifers for 6 days caused mortalities in 5/12 animals and acute cholinergic signs such as paralysis, diarrhoea, profuse salivation, muscle fasculation and colic. No NOEL could be established, as this was a single dose study.

Crawford CR & Anderson RH (1973). The effect of daily oral administration of guthion to cattle at doses of 5 and 15 ppm for 30 days. Study ref: 70-187. Lab: Research and Development, Chemagro Division of Baychem Corporation, Sponsor: Bayer AG. Study duration: not stated. Report no. 35408. Report date: 8 January 1973.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Groups of Holstein bull calves weighing approximately 124 kg (3/group, age, source unspecified) were treated with guthion (azinphos-methyl; 54.1% WP, batch: 2090269, source unspecified) orally at 0.15 and 0.45 mg/kg bw/d, once daily for 30 days. The doses were administered in gelatine capsules. The control animals received empty gelatine capsules. Individual blood samples were collected (method unspecified) for whole blood ChE determination one week prior to commencement of treatment (3 samples), on treatment day 1 (time unspecified), and weekly thereafter. Individual body weights and feed consumption were recorded before commencement of treatment, on treatment day 1 and weekly thereafter. The ChE activity in all blood samples was determined at the end of the 30-day study. No further details on experimental methods were provided.

Findings: No treatment-related depressions in whole blood ChE activity were seen in any of the treated animals at any of the sampling times. Body weight gain and feed consumption were unaffected by treatment.

Conclusions: Administration of azinphos-methyl orally to bull calves at 0.15 and 0.45 mg/kg bw/d, once daily for 30 days, had no effects on body weight gain, feed consumption or whole blood ChE activity.

2.4.1.3 Horses

Giri SN, Peoples SA, Llaguno GV & Mull RL (1974) Oral toxicity of azinphosmethyl in horses. Am J Vet Res 35(8): 1031-1035.

Study and observations: Adult riding horses (3/group, age: 4-20 years, body weight range: 400-496 kg, sex: unspecified, crossbreeds of Thoroughbred and Saddlebred) were used in this study. Azinphos-methyl (Guthion 50% WP, source: Chemagro, Division of Baychem Co., Kansas City, MO, USA) was fed to the animals in daily grain ration at 0, 5, 15 ppm for 30 days or at 25, 50, 75 and 100 ppm for 7 days (equivalent to approximately 0, 0.1, 0.3, 0.5, 1, 1.5 and 2 mg/kg bw/d, respectively). The animals at 0.5, 1, 1.5 and 2 mg/kg bw/d were observed for 10 additional days after cessation of treatment. The doses were prepared fresh daily in acetone, then mixed with the grain ration and fed to the animals from a bucket to avoid any losses. The controls were fed similarly with the same amounts of grain ration to which only acetone (a similar volume) was mixed. During the study, the animals were individually housed and were offered feed and water *ad libitum*. Plasma and erythrocyte (RBC) ChE activities in jugular vein blood were determined on 3 consecutive days prior to commencement of treatment, at 3, 7, 14, 21 and 30 days (at 0, 0.1 and 0.3 mg/kg bw/d), at 2, 5 and 7 days, and at 2, 7 and 10 days post-treatment (at 0.5, 1.0, 1.5 and 2.0 mg/kg bw/d).

Findings: No treatment-related effects on plasma or RBC ChE were observed in horses at 0.1, 0.3 and 0.5 mg/kg bw/d. According to the line graphs provided, dose- and time-related decreases in plasma and RBC ChE activities were seen in animals at 1.0 mg/kg bw/d and above. At 1, 1.5 and 2 mg/kg bw/d, plasma ChE activity was inhibited by 20%, 40% and 70%, and RBC ChE activity was depressed by 40%, 50% and 50%, respectively. Except for RBC ChE activity at 2.0 mg/kg bw/d, plasma and RBC ChE activities in animals at 1, 1.5 and 2 mg/kg bw/d recovered to baseline values by the end of the post-treatment recovery period. RBC ChE activity at 2.0 mg/kg bw/d was slower to recover and showed only about 30% recovery by the end of the recovery period. The study authors stated that none of the treated animals exhibited any clinical signs of ChE toxicity following treatment.

Conclusions: Following administration of azinphos-methyl orally to horses in grain rations at 0, 0.1 and 0.3 mg/kg bw/d for 30 days and 0.5, 1, 1.5 and 2 mg/kg bw/d for 7 days, dose- and time-related, depressions in plasma and RBC ChE activities were observed at 1 mg/kg bw/d and above. Except for RBC ChE activity at 2 mg/kg bw/d, plasma and RBC ChE activities at 1, 1.5 and 2 mg/kg bw/d recovered to baseline values by the end of the 10-day recovery period.

2.4.2 Dermal

2.4.2.1 Rats

Dubois KP, DiGiacomo R, Meskauskas J & Root M (1966) Subacute toxicity of a guthion formulation to rats. Study number, laboratory, sponsor, and study duration: not stated. Report no. 19011. Report date: October 1966.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: This study investigated the effects of guthion (azinphos-methyl) in rats following repeated dermal skin application. Young SD rats (5/sex, 240-254 g body weight, source, age not stated) were treated dermally with azinphos-methyl SC (200 g/L, source, batch unspecified) 5 days/week for 3 weeks at 50 mg/kg bw/d for males and at 120 mg/kg bw/d for females. The doses of the test substance diluted in water (volume unspecified) were applied to an abraded skin area, covering about 10% of the animal's body surface. No details were provided for procedures on animal acclimatisation, housing, feeding and method of test chemical application and treatment of controls. Body weight was measured weekly. After the final application, the following haematological parameters (blood collection method unspecified) were evaluated: one-stage prothrombin time, Hb, Hct and WBC. After these tests, the animals were sacrificed (method unspecified) and the following organs were removed and weighed: brain, liver, kidneys, spleen, heart, lungs, testes, thymus and adrenal glands. In addition, sternal marrow, mesentric lymph node, stomach, duodenum-pancreas, ileum, colon, urinary bladder and ovaries were processed for histopathology. Parts of the brain of each of 3 animals/treated group was used to assay for ChE activity at termination using a manometric method (Dubois and Magnum, 1947). No further information on experimental methods was provided.

Findings: At termination, the average body weight in males was depressed by about 23% compared to the corresponding controls. This may be due to the administration of the test substance. No inter-group differences in average body weights were seen in females at this observation time. Haematological parameters were unaffected by treatment. Compared to the controls, marked inhibition of brain ChE activity was seen at termination (approximately 24% and 30% for males and females respectively) in treated animals.

Consistent with the decrease in average body weight, about 20-30% depressions in absolute liver, kidney, spleen, heart, lung, testis and thymus weights were seen in treated males compared to the corresponding controls. Mild consolidative lung changes characteristic of a chronic pneumonic type seen in the control and treated females (2/group) at necropsy were considered incidental. Moderate cytoplasmic vacuolisation of the zona fasciculata of the adrenal gland was observed in treated females during histopathological examination. This finding was described by the study authors as a secondary change to exhaustion in animals, resulting from lack of food (inanition) due to treatment.

Conclusions: Dermal application of azinphos-methyl to rats at 50 mg/kg bw/d for males and 120 mg/kg bw/d for females 5 days/week for 3 weeks resulted in biologically significant depression in average body weight in males and marked inhibition in brain ChE activity in both sexes. The regulatory value of the study findings, however, is reduced due to data limitations and lack of clinical observations. No NOEL could be established as only one dose level was used.

2.4.2.2 Rabbits

Fluke W & Schilde B (1980) Gusathion-M active ingredient (R 1582) Subacute cutaneous study of toxicity to rabbits. Study no: Gusathion/003=R 1582/004, Lab: Institute of Toxicology, Bayer AG. Sponsor: Bayer AG, Germany. Study duration: July 26 - August 15, 1979. Report no. 8959. Report date: 20 February 1980.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study: Technical grade gusathion (azinphos-methyl, purity: 94.1%, batch: 30.3.79 I, source: not stated) was applied on the shaven, dorso-lateral intact (3 animals/sex/group) or abraded (3 animals/sex/group) skin of NZW rabbits (source: Hacking and Churchill, Huntington, UK, body weight range: 2.2-3.0 kg, age: not stated) at 0 (control), 2.0 and 20 mg/kg bw/d for 3 weeks. Test compound was formulated in Cremophor EL and demineralised water prior to each treatment and applied on a 63 cm^2 skin site for 6 h/d, 5 d/wk over 3 weeks under non-occluded conditions. The dosage volume was 0.5 mL. The application sites were cleaned with soap and water after each exposure. The animals in the control group were treated with the Cremophor/water formulation similarly. During the treatment and exposure periods, the animals were restrained to prevent any accidental PO ingestion of the test chemical. The rabbits were individually housed under conventional laboratory conditions and provided with food (Z222 rabbit grain, Hoeing Co., Verden/Aller) and water *ad libitum*. About 24-48 h after the last application, the animals were anaesthetised using hexabarbitone sodium, sacrificed by exsanguination and autopsied.

Observations: General appearance and behaviour of the animals were checked daily. The application sites were examined for signs of skin reactions (redness and swelling) and scored before application of the test substance and after the 6 h exposure period according to the guidelines specified in the US Federal Register (38: 187, 27109 of 1973) or Draize method (1959). Body weights were recorded before commencement of the study and then weekly. Haematology (RBC, WBC, Hb, Hct, thrombocyte count, differential blood count), clinical chemistry (ALT, AST, blood sugar, and plasma and RBC ChE using an unspecified method) and urinalysis (pH, protein, sugar, haemoglobin, urobilinogen and sediment) were performed prior to commencement, and at the end of the study. Blood samples required for analyses were collected from the ear vein. Urine samples collected from each rabbit over a 16-h period overnight were used in urinalysis. Additionally, plasma and RBC ChE activities were assayed after the 10^{th} or in the morning prior to the 11^{th} application. Brain ChE was determined at termination. The weight of the following organs was determined: heart, lung, liver, spleen, kidneys, adrenals, testicles or ovaries and thyroid. Samples of these organs and of epididymis, uterus and treated and untreated dorsal skin samples from each animal were processed for histopathology.

Findings: There were no treatment-related mortalities. Appearance and behaviour of the animals during treatment were normal. Excretion of soft unpelleted faeces was seen in both

the control and treated groups alike. Therefore this finding was considered to be unrelated to treatment. At termination, the females at 20 mg/kg bw/d were lighter (about 70% and 36% for intact and abraded skin groups respectively) than the corresponding controls. No further inter-group differences in group mean body weights were noted. There were no abnormal skin reactions attributable to treatment. Similarly, skin fold measurements did not reveal any indications of skin thickening.

Plasma and RBC ChE data of the treated animals are presented in the following Table. In males inhibition of plasma ChE activity was noted at 20 mg/kg bw/d in both groups (intact and abraded skin) of animals on application day 10, and only in rabbits with abraded skin sites on application day 15. Depressions in plasma ChE activity of comparable magnitude were also observed in females at 20 mg/kg bw/d at both these sampling times but this effect was limited to the group with abraded skin. In addition, a similar effect was seen on plasma ChE activity in females with abraded skin at 2.0 mg/kg bw/d on application day 15. RBC ChE activity in both sexes was inhibited at 20 mg/kg bw/d at both sampling times irrespective of whether the skin was intact or abraded. Brain ChE activity was unaffected by treatment.

Plasma and RBC ChE activities (u/mL) in rabbits treated with azinphos-methyl sc for 15 days.

Dose (mg/kg bw/d		Plasma			RBC		
		Sampling day					
		0	10	15	0	10	15
Males							
0	intact	0.65	0.63	0.47	1.74	1.87	1.80
	abraded	0.69	0.57	0.65	2.00	1.94	2.19
2	intact	0.70	0.57	0.64	1.98	1.61	1.83
	abraded	0.56	0.49	0.57	2.17	1.88	2.05
20	intact	0.68	0.41 (35%)	0.51	1.64	1.23 (34%)	1.38 (23%)
	abraded	0.56	0.41 (35%)	0.51 (22%)	1.74	1.30 (33%)	1.36 (38%)
Females							
0	intact	0.68	0.56	0.69	1.65	1.74	1.83
	abraded	0.69	0.65	0.75	1.57	1.67	1.70
2	intact	0.64	0.55	0.64	1.86	1.74	1.64
	abraded	0.53	0.46	0.54 (28%)	1.92	1.73	1.69
20	intact	0.76	0.50	0.61	1.78	1.29 (26%)	1.24 (32%)
	abraded	0.66	0.41 (37%)	0.51 (32%)	2.16	1.19 (29%)	1.26 (26%)

Values in parentheses represent % inhibition compared to corresponding controls.

Urinalysis, autopsy examinations, absolute and relative organ weights and histopathology did not reveal any inter-group differences.

Conclusions: Based on reductions in terminal body weights in females, inhibition of RBC ChE in males and females, and plasma ChE inhibition in males at 20 mg/kg bw/d, the NOEL for the animals with intact skin was 2 mg/kg bw/d. In animals with abraded skin, inhibition in plasma ChE was seen at 2 mg/kg bw/d and above.

2.4.3 Intraperitoneal

Dubois KP & Murphy SD (1956) The subacute toxicity of gusathion (Bayer 17147) to rats. Study no: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago

37, IL, USA. Sponsor: Bayer AG. Study duration: not stated. Report no. 1055. Report date: 17 January 1956.

Pre GLP, non-quality assured study.

Study and Observations: Groups of 5 young adult female SD rats (initial body weight 212-246 g, source not stated) were intra-peritoneally dosed with gusathion (Bayer 17147, azinphos-methyl, purity, source and batch not specified) in absolute alcohol and polyethylene glycol mixture at 0.5, 1, 2 and 3 mg/kg bw/d for 60 days. Initially, the test substance was dissolved in warm absolute alcohol, and then diluted with polypropylene glycol. Absolute alcohol and propylene glycol ratio in the final mixture was 1:4. The strength of the solutions was adjusted so that each group received a volume of the dosing solution equivalent to 0.1% of body weight. No details were provided for procedures on animal acclimatisation, treatment of control animals, housing and feeding. Mortality among the treated animals was recorded at 5-day intervals during the first ten days and then at 20- and 30-day intervals during the remaining study period. Body weights were measured at 10, 20, 30 and 60 days. Three animals at each 0.5 and 1 mg/kg bw/d were sacrificed at 24 h after the last dose (method unspecified), and ChE activity in brain, serum and submaxillary gland was determined using an unspecified method. No further details on experimental methods were provided.

Findings: All animals at 3 mg/kg bw/d died during the first 5 days on study. In rats receiving the test substance at 2 mg/kg bw/d, the mortality rate was about 80%, with most animals dying during the second month. No further mortalities were recorded. Percent increases in body weight in animals at 0.5 and 1 mg/kg bw/d were about 25.5 and 20.7, respectively. The study authors stated that "marked inhibition in body weight gain" was observed in animals at 2 mg/kg bw/d during their survival times but no individual data were provided. Because there were no control data, a meaningful group comparison of weight gain could not be made. It was stated that the animals at 2 mg/kg bw/d exhibited "marked symptoms of acute poisoning" each day throughout the treatment period, and recovery always occurred within 24 h before the administration of the next dose. However, no details on onset, types and duration of signs observed were provided.

The data on ChE activity in brain, submaxillary gland and serum at termination are presented in the following Table.

Inhibition of ChE activity in rats treated with azinphos-methyl ip for 60 days.

Dose (mg/kg bw/d)	Total dose (mg/kg bw)	Percent inhibition of ChE activity*		
		Brain	Submaxillary gland	Serum
0.5	30	27	19	24
1.0	60	54	31	38

*Calculated from averages of at least 3 animals/group.

ChE activity was inhibited by 19–27% at 0.5 mg/kg bw/d in brain, submaxillary gland and serum, and by 31–54% at 1 mg/kg bw/d.

Conclusions: Administration of azinphos-methyl ip at 0.5, 1, 2 and 3 mg/kg bw/d for 60 days resulted in 80% and 100% mortality at 2.0 and 3.0 mg/kg bw/d and reduced weight gain at 0.5, 1 and 2 mg/kg bw/d respectively. Depressions in brain, submaxillary gland and serum ChE activity were seen at both tested dose levels. The study was not adequate for regulatory purposes due to lack of a control group, statistical analysis, and clinical observations.

2.4.4 Inhalational

DuBois KP & Flynn M (1969) Effects of repeated inhalation exposure of rats to Guthion. Study no: not stated. Lab: Toxicity Laboratory, University of Chicago, Chicago, Illinois 60637, USA. Sponsor: Bayer AG. Study duration: not stated. Report no. 26254. Report date: 2 December 1969.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Groups of 6 adult female rats (source, strain, age and body weight not specified) were exposed by inhalation to guthion (azinphos-methyl, batch no. 9050026, purity, source unspecified) at nominal concentrations of 10, 25 or 50 µg/L air as an aerosol in an unspecified volume of ethanol for 1 h/day for 5 or 10 days. Treatment procedures of the control group were not specified. Three animals/group were sacrificed using an unspecified method after 5 days of exposure. The remaining animals were killed at the end of the 10-day exposure period. Cholinesterase (ChE) activity in brain, submaxillary gland and serum was determined using a manometric method (DuBois and Magnum, 1947). No further details on exposure conditions were provided.

Findings: No inhibition of ChE activity was seen in the animals at 10 and 25 µg/L (nominal) for one hour each day for 5 and 10 days.

ChE activity in female rats exposed to azinphos-methyl aerosols 1 h/day for 5 or 10 days

Dose (µg/L air)	ChE activity (µL CO_2/10 min/50 mg tissue)*			
	Time (days)	Brain	Submaxillary gland	Serum
Control	-	93.8 (92.3-94.6)	25.4 (23.6-27.8)	12.0 (11.2-12.6)
10	5	98.4 (96.1-102.4)	24.0 (23.5-24.4)	11.4 (1.9-11.7)
	10	91.3 (84.2-98.6)	23.9 (22.9-25.6)	12.8 (12.3-13.6)
25	5	85.6 (76.7-92.9)	21.5 (18.1-23.5)	13.8 (10.9-16.7)
	10	76.7 (72.8-81.9)	20.5 19.3-21.4)	12.9 (12.4-13.5)
50	5	85.8 (82.9-90.7)	21.8 20.7-23.4)	13.2 (12.0-15.3)
	10	48.2 (44.1-56.0) [48%]	17.5 (15.7-20.2) [31%]	8.9 (8.2-9.7) [26%]

*Values (mean and range) from 3 animals/group in the exposed groups; Values in square brackets represent percent inhibition compared to the controls.

However, depressions in ChE activity (by about 26%, 31% and 48% for serum, RBC and brain respectively) were noted at 50 µg/L after 10 days of exposure.

Conclusions: Under the conditions of the study, inhalation of azinphos-methyl at 10, 25 or 50 µg/L, 1 h/day for 5 or 10 days resulted in biologically significant reduction in plasma, submaxillary gland and brain ChE activity at 50 µg/L after 10 days of exposure. The NOEL for this study was established at 25 µg/L. However, the value of the study findings is reduced due to data limitations, small experimental group size, lack of a vehicle control group, haematological, clinical biochemistry, gross necropsy and histopathological data, and the fact

that only female animals were used in the study. In addition, exposure duration in this study was short (ie 1 h/day) compared to the exposure period of 6 h/day specified in current OECD guidelines.

♦ ***Kimmerle, 1976: Subchronic inhalation toxicity of azinphos-methyl in rats. Arch Toxicol 35:83-89, 1976.***

Study: Groups of SPF Wistar rats (10/sex/dose, source: Winkelmann, Borchen, Germany; body weight range: 150-170 g and 130-150 g for males and females respectively, age unspecified) were exposed in dynamic inhalation chambers for 6 hours/day, 5 days/week for 12 weeks to azinphos-methyl (technical grade; batch no, purity not specified) as a liquid aerosol in polyethylene glycol 400/ethanol (1:1) vehicle at mean aerosol concentrations of 0, 0.195, 1.24 and 4.72 mg/m^3 air. The control group was exposed to vehicle only. Effective concentrations were monitored using a GC method. The droplet size was measured with a cascade impactor: 97% of droplets had a diameter of 1 ± 0.5 μm. [The test animals were individually housed under conventional laboratory conditions and had constant access to food (Altromin standard food) and water].

Observations: Animals were inspected daily and body weights recorded weekly. ChE activity in plasma and RBCs were determined fortnightly by the method of Pilz and Eben (1967). Laboratory examinations (haematology, ALT, AST, AP, urea, creatinine and bilirubin) were performed after the 12-week exposure period [method of sacrifice unspecified] followed by necropsy, gross examination, organ weights and microscopic evaluation of principle organs [thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals and gonads]. Brain ChE activity was determined using the method of Ammon (1933). Statistical methods: not specified.

Findings: The exposure of rats to azinphos-methyl aerosol at concentrations of up to 4.72 mg/m^3 did not cause any significant changes in appearance and behaviour. Only the male rats exposed to the highest concentration showed a significant lower body weight gain. [At termination, the males at 4.72 mg/ m^3 were about 20% lighter than the corresponding controls and this change was considered to be test substance-related].

The hematological, the clinical biochemistry (except ChE activities) and the urinalysis parameters were not altered by treatment. Plasma and erythrocyte ChE activities were inhibited by about 30-40% at the highest concentration whereas brain ChE activity was not significantly changed (values not reported). [Plasma ChE activity was inhibited by up to 19% in males at the high dose, but RBC ChE activity was inhibited by 25-48% compared with controls from week 2 onwards. In the high-dose females, plasma ChE activity was inhibited by 28% at 4 weeks, but at other sampling times plasma ChE was generally unaffected by treatment. RBC ChE activity was inhibited in the high-dose females by 11-38% at all sampling intervals].

None of the organ weights showed significant differences between groups. No morphological change or variation from normal was seen in any of the tissues examined that was considered to be associated with the treatment. [Plasma and RBC ChE activities in treated rats at different sampling times are presented in the following Tables].

ChE activity (μ equivalents of acetylcholine) in male rats (daily exposure: 6 h)

Dose	0 mg/m^3		0.195 mg/m^3		1.24 mg/m^3		4.72 mg/m^3	
Time	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
0 wk	2.36	3.80	2.26	3.81	2.30	3.32	2.39	3.52
2 wk	2.20	3.77	2.27	3.98	2.39	3.51	1.93* [13%]	2.83 [25%]
4 wk	3.26	3.64	3.12	3.90	3.09	3.36	2.96	1.90* [48%]
6 wk	2.32	3.73	2.38	3.74	2.38	3.14	2.26	2.39* [36%]
8 wk	2.37	3.73	2.31	3.66	2.49	3.26	1.92* [19%]	2.54* [32%]
10 wk	2.34	4.08	2.32	3.91	2.28	3.49	1.96* [16%]	2.89* [30%]
12 wk	2.46	4.04	2.41	3.99	2.44	3.36	2.07 [16%]	2.27* 44%]

Mean values of 5 animals/group; Inhibition of ChE more than 20%; Values in square brackets represent percent % compared to controls.

ChE activity (μ equivalents of acetylcholine) in female rats (daily exposure: 6 h)

Dose	0 mg/m^3		0.195 mg/m^3		1.24 mg/m^3		4.72 mg/m^3	
Time	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
0 wk	3.30	3.50	3.32	3.40	3.28	3.27	3.35	3.34
2 wk	3.32	3.44	3.35	3.65	3.31	3.55	3.09*[7%]	2.83 [18%]
4 wk	4.24	3.06	4.20	3.21	4.14	3.06	3.08*[28%]	1.90* [38%]
6 wk	3.96	3.93	3.96	3.79	3.86	3.83	3.30[17%]	2.39* [40%]
8 wk	3.60	3.63	3.65	3.85	3.58	3.62	3.93*	2.54* [30%]
10 wk	4.00	3.88	4.04	4.05	3.98	4.02	3.58[11%]	2.89* [26%]
12 wk	4.05	3.55	4.02	3.56	4.00	3.97	3.45[15%]	2.27* [36%]

Mean values of 5 animals/group; Inhibition of ChE more than 20%; Values in square brackets represent % inhibition compared to controls.

Conclusions: The NOEL for inhalation administration of azinphos-methyl to rats over 12 weeks (exposure 6 h/day) was 1.24 mg/m^3 (1.24 μg/L). At concentrations of 4.72 mg/m^3 air (4.72 μg/L), decreased body weight gain in males and inhibition of ChE activity in plasma and RBC were observed. [The inhibition of plasma ChE activity was on occasion, about 20% compared with controls, but this effect was not considered to be biologically significant.]

2.5 SUBCHRONIC TOXICITY

2.5.1 Rats

♦ ***Doull J & Rehfuss PA (1956) The effect of diets containing Guthion (Bayer 17147) on rats (final report). Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Sponsor; Bayer AG. Study number & duration unspecified. Report no. 1077. Report date: 3 May 1956.***

Pre GLP, non-quality assured study. No test guidelines were cited.

Main deviations from current OECD guidelines: Haematological and clinical biochemistry determinations (with the exception of ChE) were not performed. Histopathology was carried out on 2 male and 2 female rats per dose level only. There is no mention of analysing diet to confirm dose levels. The 12-page report is not very detailed (missing raw data).

Study and observations: Groups of 13 SD rats/sex (source, age: not specified, body weight range 72-84 g) were fed diets containing 0, 2, 5 and 20 ppm Guthion (azinphos-methyl, 25% WP, batch no:, purity not specified, source: Chemagro Corporation, equivalent to

approximately 0, 0.1, 0.25 and 1.0 mg/kg bw/d) for 16 weeks (in the report also designated as 120 days). [Five kg batches of the diets were prepared as needed by adding required amounts of the test substance to ground food (Purina Rat Diet) during the study. The test animals were individually housed and had constant access to food and water during the study]. At the end of the exposure period, 3-5 animals/group were used for ChE determinations in serum, RBC, submaxillary gland and brain], using the method of DuBois and Magnum (1947). The remaining animals were killed [by cervical dislocation] at various times after cessation of exposure to plot ChE activity recovery. General appearance and cholinergic signs were recorded daily, food consumption and body weight at regular intervals. [It was stated that food consumption and body weights were recorded daily during the first 10 days and at 2-day intervals thereafter. Since the food consumption in both sexes remained relatively constant during the first month on study, measurements were not taken during the remaining part of the study. However, the line graphs on food consumption provided indicate that the measurements have been made during the first 2 months]. Autopsy, with gravimetry of principal organs, and histopathology of 17 organs and tissues (including femoral nerve and spinal cord) were performed on 2 males and 2 females/group at the end of the treatment period. Statistical examinations: not applied to the data.

Findings: No mortalities occurred and food consumption, growth and general appearance were not affected by treatment [The data were presented in the form of line graphs]. At 20 ppm, ChE activity was inhibited in the brain by about 10% and in RBC and serum by about 30%. [By about 30% and 40% for serum and RBC ChE respectively]. When treatment at 20 ppm was discontinued, inhibition of ChE activity persisted for less than 4 days in serum, for about 10 days in brain, and for more than 20 days in RBC [The data were provided in the form of line graphs]. There were no appreciable changes in the gross or microscopic appearance of the tissues, including central and peripheral nervous tissue. [Furthermore, the study authors stated that none of the animals exhibited any clinical signs, which could be attributed to the test chemical].

ChE activity (μL CO_2/10 min/50 mg wet tissue) in rats following dietary-administration of azinphos-methyl for 16-weeks.

Dose level (ppm)	0	2	5	20
Sex	**M/F**	**M/F**	**M/F**	**M/F**
Serum	6.9/24.4	6.4/22.4	6.5/22.7	4.4/18.5* [36%/24%]
RBC	10.1/10.3	9.4/10.7	9.3/9.4	6.1/6.4* [40%/38%]
Brain	99.3/98.2	99.7/96.7	95.0/97.9	90.2/84.5 [10%/14%]
Submaxillary gland	22.6/24.1	23.2/23.3	22.8/23.8	23.1/24.1

Mean values from at least 3 animals/group.

Conclusions: The NOEL for dietary administration of azinphos-methyl to rats over 16 weeks was 5 ppm, equivalent to about 0.25 mg/kg bw/d (calculated by means of a conversion factor of 0.05). At dietary concentrations of 20 ppm, inhibition of ChE activity in serum, RBCs, and brain was observed.

Doull J & Anido P (1957a) Effect of high dietary levels of guthion on rats. Study no: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Sponsor: Bayer AG. Study duration: not stated. Report no. 1762. Report date: 5 June 1957.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study: Groups of at least 18 male, weanling SD rats (body weight range: 55-69 g, source: not stated) were fed with diets containing guthion (azinphos-methyl) at 0, 50 and 100 ppm (equivalent to approximately 0, 2.5 and 5 mg/kg/day) for 16 weeks. The experimental diets were prepared by mixing required quantities of azinphos-methyl 25% WP formulation (source: Chemagro Corporation, formulation details and batch not specified) with ground rat food (Rockland Rat Diet). Male rats and the above dose levels were chosen for this study because a previous 16-week feeding study in rats (Doull & Rehfuss, 1956) had shown atrophic changes in the testis of 2/12 rats at 20 ppm (equivalent to approximately 1 mg/kg bw/d), in the absence of any gonadal damage in female rats at 1 mg/kg bw/d. The experimental diets were prepared weekly in batches of 5 kg.

Observations: The animals were observed daily for mortality and cholinergic signs, and weighed weekly. After 8 weeks of exposure, 3 rats/group were sacrificed (method unspecified), necropsied, and the following tissues were weighed and processed for histopathology: brain, lung, heart, spleen, kidney, liver, adrenal gland, submaxillary gland and testes. ChE activity in brain, submaxillary gland, serum and RBC of these animals was determined using a manometric method (Dubois & Magnum 1947). Samples from the stomach, ileum, colon and skeletal muscle were also collected for histopathological examination. Similar procedures were carried out using at least 3 rats after completion of the 16-week feeding period. At this time, 3 and 2 additional rats at 2.5 and 5 mg/kg bw/d respectively were returned to the control diet for a 3-week recovery period. At the end of the recovery period they were sacrificed (method unspecified) and brain, serum, RBC and submaxillary gland ChE activities were determined. During the study, the animals were housed in an air-conditioned room, and had constant access to the prepared diets and water. No details were provided for animal acclimatisation or any analyses to confirm the dose levels.

Findings: Both dietary levels of azinphos-methyl caused dose-related mortalities. According to the line graph provided, the survival rates at 2.5 and 5 mg/kg bw/d at 8 weeks were about 89% and 78%, and at 16 weeks about 55% and 44% respectively. There were no mortalities in the control group. At termination, the animals at 2.5 and 5 mg/kg bw/d were about 10% and 18% lighter than the controls. However, no individual numerical data on this parameter were provided. Cholinergic signs such as salivation, lacrimation, diarrhoea and occasional episodes of tremors were observed during the first 4 weeks in all treated animals, including fibrillation and convulsions in one rat. The details on onset and duration of the signs were not provided. It was stated that some of these signs subsided during the remaining study period, but the treated animals continued to exhibit hyperexcitability, tremors and some mortality. ChE activities in different tissues at intercurrent sacrifice and termination are given in the following Table.

ChE activity in male rats treated with azinphos-methyl in the diet for 16 weeks.

Dose (mg/kg bw/d)	ChE activity (µL CO_2 produced/10 min/50 mg wet tissue)			
	Brain	Submaxillary gland	Serum	RBC
At 8 weeks				
Control	92.6	19.7	5.6	9.2
2.5	37.9 (59%)	14.3 (27%)	3.3 (40%)	2.1 (77%)

5.0	21.4 (76%)	10.9 (45%)	2.6 (53%)	1.4 (85%)
At 16 weeks				
Control	98.0	21.7	5.4	10.0
2.5	51.4 (47%)	17.9 (17%)	3.3 (39%)	2.9 (71%)
5.0	24.4 (74%)	12.8 (41%)	2.0 (63%)	2.7 (73%)

Average of duplicate determinations from at least 3 animals/group; Values in parentheses represent % inhibition compared to corresponding controls.

Treatment-related inhibition in ChE activity in all tissues was seen at both sampling times. The study authors stated that at the end of the 3-week recovery period, the animals at 2.5 mg/kg bw/d showed only RBC ChE inhibition, whereas the rats at 5 mg/kg bw/d exhibited about 25% and 52% inhibition in brain and RBC ChE, respectively (no numeric data were provided).

No inter-group differences were observed in organ weights nor were any treatment-related histopathological changes in any of the tissues seen. There was no evidence of testicular atrophy at any dose level.

Conclusions: Under the conditions of the study, feeding azinphos-methyl to male SD rats in the diet at 2.5 and 5 mg/kg bw/d for 16 weeks, resulted in treatment-related mortalities, reduced weight gain and depressions in brain, RBC, submaxillary gland and serum ChE activity. No NOEL was established (≤ 2.5 mg/kg bw/d) because of ChE inhibition observed at both dose levels. The regulatory value of the findings of this study is limited due to lack of statistical analyses, data limitations (haematology, clinical biochemistry), and only male rats and two dose levels were used.

Broadmeadow A (1987) Cotnion technical: Toxicity study by oral (gavage) administration to CD rats for 13 weeks. Report no. 86/MAK057/342. Lab: Life Sciences Research Ltd., Eye, Suffolk, England. Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel. Study duration: 19 February 1986 – 22 May 1986. Report date: January 1987.

Guidelines and GLP: A formal statement was provided which indicated that the study complied with current OECD, US EPA and Japanese GLP standards on agricultural chemicals. The study and report were quality assured.

Aim: To determine the toxicity of Cotnion technical (azinphos-methyl) to CD rats over 13 weeks, and to determine the dose selection for a future combined toxicity and oncogenicity study.

Materials and Methods

Cotnion technical (azinphos-methyl) (93% purity; batch no. 0013; Makhteshim Chemical Works Ltd., Beer-Sheva, Israel) was administered in corn oil (unspecified concentration) by PO gavage to 10 CD rats/sex/group (56-84 g, unspecified starting age; Charles River UK Ltd) at 0, 0.2, 0.8 and 3.2 mg/kg bw/d (equivalent to 0, 0.215, 0.860 and 3.44 pure Cotnion mg/kg bw/d respectively) for 13 weeks. The dose volume was 5 mL/kg bw/d. All dosing formulations were prepared daily and their stability and homogeneity determined on day 1 and during week 13 by an unspecified means. The purity of Cotnion was confirmed at the end of the study by the sponsor.

Rats were acclimatised for 7 days then randomly allocated to each group. Room temperature and humidity were kept at 18-25°C and 40-70% respectively. A 12 h light/dark cycle was maintained throughout the study and air was ventilated at 15 changes/h. Rats were housed 5/sex/group/cage in high density polypropylene cages (Type RC1; North Kent Plastics Ltd., Dartford, Kent, England). Tap water (controlled by the East Anglia Water Company, Lowestoft, Suffolk, England) and Laboratory Animal Diet No.1 (Labsure, Manea, Cambridgeshire, England) were available *ad libitum* except overnight prior to, or during collection of blood or urine samples.

All rats were examined at least three times daily for deaths, morbidity, or clinical signs. A detailed weekly examination including palpation for growths was performed. A complete necropsy was performed on any rats dying during the study. Food consumption and body weights were recorded weekly, and water consumption was recorded daily. Food conversion ratios were calculated as the amount of food consumed per unit body weight gained. Ophthalmoscopy was performed using a Fision Binocular Indirect Ophthalmoscope after instillation of a mydriatic (0.5% tropicamide, Mydriacyl, Alcon; unspecified location) on all rats prior to the study, and on rats in the control and 3.2 mg/kg bw/d groups after 12 weeks of treatment.

After 12 weeks of treatment, all rats were anaesthetised with ether and fasted blood samples (unspecified volume) taken from the retro-orbital sinus prior to dosing. The following haematology parameters were measured: PCV, Hb, RBC, total WBC, differential WBC, platelet count, MCH, MCV and MCHC. The following clinical chemistry parameters were measured: ALT, AST, RBC and plasma ChE, urea, creatinine, glucose, total bilirubin, total protein, electrophoretic protein fractions, Na, Cl, Ca and P. After 11 weeks of treatment, fasted overnight urine samples were collected from each rat that had been placed in metabolism cages. The following urinary parameters were measured: appearance, volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bilirubin, urobilin, nitrite, blood and microscopy of spun deposits.

Following treatment, all surviving rats were sacrificed by carbon dioxide inhalation then subjected to a complete necropsy, including a detailed examination of the external features and orifices, the neck and associated tissues, the thoracic, abdominal and pelvic cavities. All organs and tissues were examined as considered appropriate by the study author. Brain ChE activity was measured. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs (with bronchi), ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and uterus (with cervix). The previous organs along with the following were histopathologically examined in all rats from the control and 3.44 mg/kg bw/d groups: aorta (thoracic arch), caecum, colon, duodenum, ileum, jejunum, lymph nodes (cervical and mesenteric), oesophagus, pancreas, rectum, salivary gland, sciatic nerve (left), sternal bone (with marrow), stomach, thymus, trachea, urinary bladder and any lesions. The kidneys, liver and lungs of all rats were examined.

Statistical analysis: Intergroup differences in body weight change, haematology, clinical chemistry, urinalysis and brain ChE activity were determined by a Students t-test using a pooled error variance. Intergroup differences in organ weights were determined using a Dunnett's test. Pathology data were analysed using the Fisher exact probability test.

Results

Stability and homogeneity analysis: Homogeneity analysis of azinphos-methyl in the vehicle (corn oil) revealed that the analytical concentrations of 0.041 ± 0.001 (low dose) and 0.593 ± 0.010 mg/mL (high dose) complied with the nominal concentrations of 0.04 and 0.64 mg/mL respectively. Azinphos-methyl was found to be within 4 % of the nominal concentrations of 0.04 and 0.64 mg/mL in corn oil after 2 days incubation at an unspecified temperature.

Mortalities and clinical signs: One female in the 0.860 mg/kg bw/d group died during routine blood sampling, however the study authors reported that histopathological examination revealed no evidence that the death was caused by azinphos-methyl. Salivation was observed in males at and above 0.860 mg/kg bw/d from approximately week 3, with the majority of rats affected from week 8 at both 0.860 (up to 8/10) and 3.44 mg/kg bw/d (up to 10/10). The study author reported that salivation was observed shortly after dosing in the majority of males, but occasionally it was seen prior to dosing or up to an hour after dosing in some animals. Salivation was observed infrequently in females with the exception of 2/20 mid-dose animals only during week 7, and 4/10 high-dose animals only during week 9.

Body weight: There was no treatment-related effect on body weight in males. Females treated with 0.215 and 0.86 mg/kg bw/d azinphos-methyl showed a statistically lower body weight gain to week 7 ($p < 0.05$ and 0.01 respectively) with the body weight gain to week 13 of the 0.86 mg/kg bw/d group also statistically lower than the control ($p < 0.05$). However, in the absence of a dose-response effect or any effect at the highest dose (3.44 mg/kg bw/d) these observations were not considered to be treatment-related.

Food and water consumption: There was no treatment-related effect on food or water consumption. There was a slight increase in food conversion ratios in females during weeks 8-13 (13, 15 and 29% greater than the control at 0.215, 0.86 and 3.44 mg/kg bw/d respectively), but in the absence of statistical significance, this effect was not considered to be treatment-related.

Ophthalmoscopy: There was no treated-related ocular lesions observed.

Haematology: The mean (± SD) platelet count of males in the 3.44 mg/kg bw/d group (621 ± 67 $10^3/cm^3$) was significantly lower than the control group (677 ± 50 $10^3/cm^3$; $p < 0.05$). In the absence of pre-treatment haematology values, and the fact that all groups, including the control fell below the historical control range (700-1200 $10^3/cm^3$; Derelanko 2000) it was unlikely that this was a treatment-related effect. Females treated with 0.215 mg/kg bw/d azinphos-methyl showed an incidentally higher number of neutrophils ($p < 0.01$) relative to the control, however in the absence of any effect at the 2 highest doses this result was not considered to be treatment-related. Similarly, the decreased RBC ($p < 0.05$) and increased MCV ($p < 0.05$) in females at 0.860 mg/kg bw/d was not considered to be treatment related due to the absence of an effect at the highest dose. Total WBCs and lymphocytes were significantly elevated in females at 3.44 mg/kg bw/d (13.7 ± 3.9 $10^3/cm^3$ and 12.3 ± 3.7 $10^3/cm^3$ respectively; $p < 0.05$) relative to the control (10.4 ± 2.4 $10^3/cm^3$ and 9.3 ± 2.5 $10^3/cm^3$ respectively), however, in the absence of pre-treatment values and the fact that data fell within the historical control range (Deerlanko 2000) this was not considered to be a treatment-related effect.

Clinical Chemistry: There was a clear treatment-related effect on the inhibition of plasma butyryl, plasma acetyl, RBC and brain ChE activities in both males and females (see Table below). At the highest dose (3.44 mg/kg bw/d), there was a statistically significant ($p < 0.001$) inhibition of plasma butyryl (30-47%), plasma acetyl (42-49%), RBC (77-78%) and brain (64-67%) ChE activities in both males and females. At the mid-dose (0.86 mg/kg bw/d), there was a statistically significant inhibition of plasma butyryl (16%, $p < 0.01$), plasma acetyl (14%, $p < 0.05$), RBC (17%, $p < 0.001$) and brain (9%, $p < 0.05$) ChE activities in males, while in females only RBC ChE activity was significantly inhibited (29%, $p < 0.001$). At the lowest dose (0.215 mg/kg bw/d), plasma butyryl (18%, $p< 0.001$), plasma acetyl (18%, $p < 0.01$) and RBC activities (8%, $p < 0.05$) were inhibited in males while only RBC ChE activity was inhibited in females (9%, $p < 0.001$). There was no effect on brain ChE activity in either males or females at the lowest dose. Although the inhibition of plasma ChE activity in males at 0.215 and 0.86 mg/kg bw/d was statistically significant, it was not considered to be toxicologically relevant as no dose-response relationship was observed and the level of inhibition was below 20% of the control. Similarly, the statistically significant inhibition of RBC ChE activity in both males and females at 0.215 mg/kg bw/d, and in brain ChE activity in males at 0.86 mg/kg bw/d were not considered to be toxicologically relevant as they were also below 20 % of the control.

Effect of 13 weeks of azinphos-methyl administration (PO gavage) on ChE activity in CD rats (% inhibition of control).

DOSE	0 mg/kg bw/d		0.215 mg/kg bw/d		0.86 mg/kg bw/d		3.44 mg/kg bw/d	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma butyryl ChE	0	0	18***	3	16**	10	30***	47***
Plasma acetyl ChE	0	0	18**	-3	14*	7	42***	49***
RBC ChE	0	0	8*	9***	17***	29***	77***	78***
Brain ChE	0	0	3	4	9*	3	67***	64***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

There were some statistically significant variations in AST, Na, Cl and Ca in males, and glucose, total protein, electrophoretic protein fractions, Na, Ca and P in females. However, as the effects followed no dose-response pattern, fell within historical control ranges, and that no pre-treatment clinical chemistry samples were measured, they were not considered to be treatment-related. The study author reported that the statistically significant depression in A/G ratio in females at every dose ($p < 0.001$-0.01) was possibly treatment-related, but in the absence of a similar effect in males (which appeared to be more sensitive to azinphos-methyl than females as shown by the inhibition of ChE activity and presence of clinical signs) this was considered unlikely.

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

Gross pathology: There was no treatment-related effect on absolute organ weights, while incidental variations in relative organ weights such as increased relative liver weight ($p < 0.05$) in high-dose females and mid-dose males, reduced relative heart weight ($p < 0.05$) in low and high-dose females, were not considered to be treatment-related as they were marginally different to the control and no dose-response effect was evident. Four high-dose males had pale gelatinous material in their duodenum, ileum or jejunum while a single mid-dose female had a yellow viscous fluid in the jejunum. There were no other treatment-related macroscopic abnormalities detected.

Histopathology: There was no treatment-related effect on the occurrence of histopathological abnormalities.

Conclusions: The NOEL in CD rats following daily PO gavage of azinphos-methyl for 13 weeks was 0.215 mg/kg bw/d, based on a statistically significant ($p < 0.01 – 0.001$) inhibition of RBC ChE activity (males and females) and observations of salivation (males) at and above 0.86 mg/kg bw/d. At 3.44 mg/kg bw/d, inhibition of brain and plasma ChE activities (males and females), and the presence of a viscous yellow fluid (males) in the small intestine, were observed.

Comments: The main deficiencies noted in the study were the absence of pre-treatment haematology, clinical chemistry or urinary parameters measurements, and that no historical control data was provided.

2.5.2 Dogs

Doull J & Anido P (1957b) Determination of safe dietary level of guthion for dogs. Study no: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago, IL USA. Sponsor: Bayer AG. Study duration: not stated. Report no. 1759. Report date: 1 June 1957.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Adult mongrel dogs (body weight: 6-17 kg, 1 dog/sex/group, source: not stated), were fed with guthion (azinphos-methyl 25% WP, source: Chemagro Corporation, batch not specified) in the diet at 5, 10, 20 and 50 ppm (estimated to be equivalent to 0.125, 0.25, 0.5 and 1.25 mg/kg bw/d) for 12 weeks. The dogs were dewormed and vaccinated against distemper and hepatitis prior to commencement of the study. Prior to commenced of treatment, dogs were fed with the control diet containing no azinphos-methyl for 3 weeks. During this time biweekly determinations of serum and red blood cell (RBC) ChE activities were made to establish baseline values. The test diets were prepared weekly by adding required quantities of azinphos-methyl to ground dog food (Arcady Dog Chow) to provide the desired dietary levels. The dogs were housed individually and provided with exercise runs, and had constant access to prepared diets and water throughout the study. The animals were observed daily for clinical signs. Body weights were recorded weekly. Serum and RBC ChE activities were measured weekly using a manometric method, (μL of CO_2 produced/50 mg tissue/10 min) (Dubois and Magnum, 1947). After completion of the feeding period, the animals were returned to the control diet for a 3-week recovery period during which time their serum and RBC ChE activities were assayed. There was no control group in this study. No further details for experimental methods were provided

Findings: General appearance and weight gain of the animals were normal. No "toxic symptoms" that could be attributed to azinphos-methyl were observed in any of the treated animals. However, no individual data for any these parameters were provided. Food and water consumption, haematology and clinical chemistry parameters were not examined. According to 2 line graphs provided (for plasma and RBC ChE activity), serum ChE activity in dogs at 1.25 mg/kg bw/d declined gradually with time reaching about 20% inhibition at 10 weeks and about a 25% inhibition at 12 weeks. RBC ChE activity in dogs at 0.25, 0.5 and 1.25 mg/kg bw/d was decreased in a dose- and time-related manner. The enzyme activity was inhibited at 0.25 and 0.5 mg/kg bw/d by about 20% at 9 weeks. RBC ChE inhibition at 1.25

mg/kg bw/d, however, was more pronounced and reached about 40% and a maximum of 50% at 9 and 10 weeks, respectively. Although the RBC ChE activity in dogs at 0.25 and 0.5 mg/kg bw/d showed a trend towards recovery starting from week 10, the enzyme activity at 0.5 and 1.25 mg/kg bw/d remained depressed during the remaining study period.

The study authors stated that serum and RBC ChE activities in animals on 1.25 mg/kg bw/d had returned essentially to normal levels during the first week and by the end of the 3-week recovery period respectively, but no supporting data were provided.

Conclusions: Under the conditions of the study, administration of azinphos-methyl in the diet to mongrel dogs at 0.125, 0.25, 0.5 and 1.25 mg/kg bw/d) for 12 weeks resulted in depression in serum ChE activity at 1.25 mg/kg bw/d, and RBC ChE inhibition at 0.25, 0.5 and 1.25 mg/kg bw/d compared with pre-dose activity. Based on RBC ChE inhibition at 0.25 mg/kg bw/d and above, the NOEL for this study was 0.125 mg/kg bw/d. However, the validity of the study findings is reduced due to lack of a control group, small experimental group size, data limitations (body weights, food consumption, haematology and clinical chemistry, clinical observations), and large variation in starting body weights of experimental animals. Thus the study was not considered adequate for regulatory purposes.

♦ ***Löser E & Lorke D (1967) Cholinesterase activity in dogs following administration of Gusathion in food. Study no: not stated. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: not specified. Report no. 292. Report date: 13 April 1967.***

Pre GLP, non-quality assured study. No test guidelines were cited.

Main deviations from current OECD guidelines: Only one animal/sex/dose group was used. Hematological and clinical biochemistry determinations (with the exception of ChE) as well as gross necropsy and histopathological examinations were not performed. Body weight and food consumption data were not reported. There is no mention of analysing the diets to confirm dose levels.

Study and observations: Groups of Beagle dogs (1 animal/sex/dose, source: Appleton, England; age: about 10 months) were fed diets containing chemically pure Gusathion active ingredient (batch number and purity: not specified) at dietary concentrations of 0, 20, 50, 100, 200 and 400 ppm [equivalent to approximately 0, 0.5, 1.25, 2.5, 5.0 and 10 mg/kg bw/d] for 19 weeks. ChE activity was determined in whole blood [obtained from the saphenous vein] before treatment, after 2 days, and then weekly, according to the method of Pilz *et al* (1965).

Findings: General condition was impaired at 50 ppm and above, and levels of 100 ppm and above induced signs of cholinergic stimulation (occasional muscular spasms and tremors at 100 ppm, and uncoordinated movements and intense muscular spasms at 200 and 400 ppm). [The study authors stated that the dogs at doses up to and including 200 ppm always consumed their daily food ration]. Doses of 50 ppm and above caused weight loss and animals at 400 ppm frequently refused to eat. The female animal dosed at 400 ppm died after the study week 9. Dose-related blood ChE inhibition was observed in all dose groups; significant inhibition (> 20%) occurred already at the lowest dose of 20 ppm. [No necropsy data were provided].

Whole blood ChE activity (% of pre-treatment value) in male and female dogs (mean values from 1 animal/sex/group)

Dose level	0 ppm	20 ppm	50 ppm	100 ppm	200 ppm*	400 ppm*
Pre-dose	100	100	100	100	100	100
2 days	89.0	100	98.5	84.0	88.0	50.5
1st week	100	100	90.0	85.6	49.1	35.4
2nd week	97.5	100	87.3	68.0	76.0	25.7
3rd week	100	92.5	84.2	61.6	60.0	31.0
4th week	91.6	58.4	73.0	37.6	62.0	24.8
5th week	98.4	69.1	57.1	48.0	47.2	20.4
6th week	93.2	85.8	73.7	50.4	57.4	27.4
7th week	94.9	76.7	76.0	52.0	63.0	29.2
8th week	94.9	78.3	64.7	49.6	63.8	20.4
9th week	100	90.8	70.7	54.4	47.1	18.6
10th week	100	93.4	68.4	53.7	45.4	18.6
11th week	100	84.3	71.4	49.6	50.0	35.4
12th week	100	79.2	61.7	45.6	38.9	24.8
13th week	96.8	90.0	73.7	40.0	38.9	18.6
14th week	100	71.8	63.2	56.1	62.0	40.4
15th week	100	84.1	74.4	69.6	77.8	44.2
16th week	95.0	81.8	66.2	60.8	61.1	26.6
17th week	100	86.8	75.2	73.6	57.4	31.8
18th week	100	95.0	74.5	60.8	-	-
19th week	100	93.5	82.0	50.5	-	-

* Animals given this concentration were started 2 weeks later but were given compound for the same total length of time.

Conclusions: The NOEL for dietary administration of azinphos-methyl to dogs over 19 weeks was < 20 ppm [about 0.5 mg/kg bw/d]. Inhibition of ChE occurred at dose levels of 20 ppm and above, clinical signs at 50 ppm and above, and mortality at 400 ppm.

2.6 CHRONIC TOXICITY

2.6.1 Mice

♦ ***National Cancer Institute (NCI) (1978) Bioassay of azinphosmethyl for possible carcinogenicity. NCI Carcinogenesis Technical Report Series No. 69. Lab & Sponsor: NCI, National Institute of Health (NIH), Bethesda, Maryland, USA. Study duration: unspecified. Report date: 1978.***

Guidelines and GLP: The method employed was that of the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: Only two dose levels were used. The concurrent control group consisted of 10 male and 10 female mice only.

The study is considered supplementary.

Material and Methods: Groups of 50 male and 50 female 35 days old B6C3F1 mice (source: Charles River Breeding Laboratories Inc., Wilmington, MA, USA; body weight range not specified) [quarantined for 14 days and then assigned to control or dose groups] received

azinphos-methyl (Guthion®, Mobay Chemical Corp., Kansas City, MO. USA, batch no. not specified, purity 90%) for 80 weeks by admixture in the diet [Wayne® Lab Blox animal mean (Allied Mills Inc., Chicago, IL, USA)] (with 2% corn oil) at concentrations of 0, 31.2 and 62.5 ppm [estimated to be equivalent to 0, 4.7 and 9.4 mg/kg bw/d respectively] active ingredient (males) and 0, 62.5 and 125.0 ppm [estimated to be equivalent to 0, 9.4 and 18.75 mg/kg bw/d respectively] active ingredient (females). [Dietary analysis was performed using gas-liquid chromatography to ensure compliance with the theoretical azinphos-methyl content] The dose levels were selected on the basis of a previous subchronic feeding study conducted to estimate maximum tolerated doses of azinphos-methyl.

A group of 10 male and 10 female mice served as matched controls; the pooled controls for statistical tests consisted of the matched controls combined with 130 male and 120 female untreated mice from similar bioassays of 11 other test chemicals. [Mice were housed in plastic cages, 5 per cage for females and 2-3 per cage for males. Temperature and humidity were maintained at 20-24°C and 40-70% respectively. There were 10-12 air changes/h with a 10 h light/14 h dark cycle maintained throughout the study. Food and water were available *ad libitum*]

Following 80 weeks of exposure, the animals were retained untreated but under observation for a further 12 or 13 weeks. All animals were observed twice daily for signs of toxicity, weighed at regular intervals [unspecified], and palpated for masses at each weighing. All animals found dead, killed in a moribund condition or killed [by unspecified means] at scheduled sacrifice [92 or 93 weeks] were subjected to a gross and microscopic examination of major tissues, major organs, and all gross lesions [skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gall bladder, pancreas, stomach, small and large intestine, kidney, urinary bladder, pituitary gland, adrenals, thyroid gland, mammary gland, prostate or uterus, testis or ovary and brain].

Statistical methods: Survival analysis, Kaplan and Meier (1958), Cox (1972), and Tarone's (1975) extension of Cox's methods. Incidences of lesions, one-tailed Fisher exact test at significance level of 0.05, with Bonferroni inequality correction, and Cochran-Armitage test for linear trend in proportions, as well as time-adjusted analysis when appropriate. Statistical comparisons were made against the matched and pooled control groups.

Findings: [Dietary analysis: Analysis of the azinphos-methyl content of the diet indicated that the measured levels of 31.2, 61.2 and 126.0 ppm complied with the target levels of 31.2, 62.5 and 125.0 ppm].

General examinations: Clinical signs, including hyperactivity and convulsions, were observed in treated animals but also in control animals. [At week 49 all dosed females appeared to be hyperactive. Other clinical signs observed in the second year of the study included rough hair coats, alopecia, abdominal distension and hyperactivity (incidences unspecified). Rough hair coats were observed in low and high-dose males from week 74 and 60 respectively. (incidences unspecified). Convulsions were periodically observed in 1 high-dose female, 1 high dose male and 1 control male during the second year of the study.] The body weights of male mice and low-dose female mice were comparable to the controls throughout the study [see Table below]. The mean body weight of the high-dose female group was up to approximately 17% lower than the control group throughout the treatment period, but was comparable to the controls after week 80 [no statistical analysis was performed on the body weight data]. There were no treatment-related differences in the

incidences of mortality in any of the experimental groups [although there was a slight increase in moribund sacrifice at the highest dose (See Table below)].

Effect of dietary administration of azinphos-methyl over 80 weeks on mortality and body weight in mice

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
No. of animals initially in study	10/10	50/50	50/50
Natural death	1/0	1/3	1/2
Moribund sacrifice	1/3	4/3	7/6
Terminal sacrifice	8/7	45/44	42/42
Survival rate (%)	80/70	90/88	84/84
Body weight [g] (week 0) @	21/18	22/18	21/18
Body weight [g] (week 52) @	35/31	35/31	36/28

@ Approximate mean body weight, derived from graphically presented data

Pathological examinations: No treatment-related gross pathological changes were reported and all the tumour types observed during the study had been encountered as a spontaneous lesion in the mouse strain employed. [There was no difference in non-neoplastic lesions between azinphos-methyl and control groups (see relevant Table below). There was a trend of increased hepatocellular carcinoma in azinphos-methyl-treated male mice (see relevant Table below). A statistically significant linear trend ($p = 0.006$) was detected in the incidence of hepatocellular carcinomas when the matched control group was used in a Cochran-Armitage test. However, no statistical significance was established when a Fisher exact test was employed using either the matched or pooled control. Hepatocellular carcinomas are known to be a common age-related neoplasm in untreated male B6C3F1 mice (historical control range of 6-29%; Haseman *et al* 1998) and consequently the occurence in this study was not considered to be treatment-related. Additionally, the low genotoxic potential of azinphos-methyl (see Section 9) suggests that this finding has no relevance to human populations.] There was no statistical evidence of an association of tumours with the administration of azinphos-methyl at either dose level in either sex of mouse.

Summary of the incidence of neoplasms in mice following treatment with azinphos-methyl in the diet for 80 weeks

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
No. of animals examined microscopically	10/10	49/49	50/49
No. of animals with primary tumours	4/5	23/10	23/17
No. of animals with benign (b) tumours	5/1	16/5	10/6
No. of animals with malignant (m) tumours	1/5	8/7	15/11
No. of animals with tumours uncertain benign or malignant	0/0	0/0	0/1
No. of animals with secondary tumours	0/3	1/2	0/4

Incidence of neoplasms in mice following treatment with azinphos-methyl in the diet for 80 weeks

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
Skin (@)	10/10	50/50	50/50
- Fibrosarcoma	0/0	0/0	1/0
- Leiomyosarcoma	0/0	1/0	0/0

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
Subcutaneous tissue (@)	10/10	50/50	50/50
- Leiomyosarcoma	0/0	0/0	1/0
- Fibrosarcoma	0/0	0/0	1/0
- Malig. lymphoma, lymphocytic type	0/0	0/0	0/1
Lung (#)	10/10	49/50	50/50
- Alveolar/bronchiolar adenoma	1/0	6/1	4/3
- Alveolar/bronchiolar carcinoma	1/0	2/0	0/0
- Papillary cystadenocarcinoma, MET	0/1	0/0	0/0
- Lymphoma, metastatic	0/1	0/0	0/1
- Granulocytic leukaemia	0/0	0/1	0/0
Bone marrow (#)	10/10	49/47	50/50
- Lymphoma, metastatic	0/1	0/0	0/0
- Hemangioma	0/0	1/0	0/0
- Hemangiosarcoma, metastatic	0/0	1/0	0/0
Spleen (#)	8/9	46/49	46/50
- Malig. lymphoma, lymphocytic type	0/0	1/0	0/3
- Hemangioma	0/0	1/0	0/0
- Hemangiosarcoma	0/0	1/1	0/1
- Malig. lymphoma, NOS	0/1	0/0	0/0
- Malig. lymphoma, histiocytic type	0/0	0/2	0/0
Lymph node (#)	9/9	46/40	46/45
- Hemangioma	1/0	0/0	0/0
- Malig. lymphoma, NOS	0/0	0/1	1/2
- Malig. lymphoma, histiocytic type	0/0	0/1	1/0
- Lymphoma, metastatic	0/1	0/0	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Salivary gland capsule (#)	10/10	49/49	50/49
- Hemangioma	0/0	0/1	0/0
Liver (#)	8/10	49/49	50/50
- Hepatocellular adenoma	2/0	8/0	7/1
- Hepatocellular carcinoma	0/1	3/0	12/0
- Hemangiosarcoma	0/0	2/0	0/0
- Lymphoma, metastatic	0/1	0/0	0/1
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Small intestine (#)	10/10	49/48	50/50
- Malig. lymphoma, lymphocytic type	0/1	0/0	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
Kidney (#)	10/10	49/49	50/50
- Malig. lymphoma, lymphocytic type	0/0	0/0	0/1
- Malig. lymphoma, histio-type metas	0/0	0/1	0/0
- Cortex: lymphoma, metastatic	0/1	0/0	0/0
Pituitary (#)	10/7	49/39	50/40
- Chromophobe adenoma	0/0	0/1	0/0
Adrenal (#)	10/10	49/47	50/49
- Lymphoma, metastatic	0/1	0/0	0/0
Thyroid (#)	10/9	49/42	50/46
- Cystadenoma, NOS	0/1	0/0	0/0
- Papillary cystadenoma, NOS	0/0	0/0	0/1
- Papillary cystadenocarcinoma, NOS	0/1	0/0	0/0
Mammary gland (@)	10/10	50/50	50/50
- Papillary cystadenocarcinoma, NOS	0/0	0/0	0/1
- Fibroadenoma	0/0	0/0	0/1
- Lymphoma, metastatic	0/0	0/0	0/1
Uterus (#)	7	48	48
- Endometrial stromal polyp	0	2	0
- Leiomyosarcoma	0	0	1
Cervix uteri (#)	7	48	48

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
- Leiomyosarcoma	0	1	0
Ovary (#)	9	47	41
- Granulosa-cell tumour	0	0	1
Pelvic cavity (@)	10/10	50/50	50/50
- Liposarcoma	0/0	0/0	0/1
Eye/lacrimal gland (@)	10/10	50/50	50/50
- Papillary cystadenoma, NOS	1/0	0/0	0/0
Multiple organs (@)	10/10	50/50	50/50
- Malig. lymphoma, NOS	0/1	0/0	0/0
- Malig. lymphoma, lymphocytic type	0/0	0/1	0/0
- Lymphoma, lymphocytic, metastatic	0/0	0/0	0/1
- Malig. lymphoma, histio-type metas.	0/0	0/1	0/0
- Granulocytic leukaemia	0/0	0/0	0/1

No. of animals examined microscopically; @ No. of animals necropsied; NOS = not otherwise specified

Conclusion: The NOEL for dietary administration of azinphos-methyl to B6C3F1 mice over 80 weeks (then observed for 12-13 weeks) was 62.5 ppm [estimated to be equivalent to 9.4 mg/kg bw/d]. Clinical signs and decreased body weight gain were observed at higher dose levels [in females at 125 ppm (estimated to be equivalent to 18.75 mg/kg bw/d)]. Azinphos-methyl was not shown to be carcinogenic in B6C3F1 mice of either sex.

[*Comments:* The study design and level of reporting had a number of deficiencies. Individual animal data were not provided. The sample size (n = 10) of matched controls was low compared to the treatment groups (n = 50). Only 2 dose levels per sex were investigated with different doses given to each sex (ie 31.2 and 62.5 ppm for males, 62.5 and 125 ppm for females). The incidences of clinical signs were inadequately reported. Some data (eg body weight) was not statistically analysed. Food consumption was not recorded. Haematology, clinical chemistry or urinalysis were not investigated.]

♦ ***Hayes RH (1985) Oncogenicity study of azinphos-methyl (®Guthion) in mice. Report No. 612. Study No. 80-271-02. Lab: Mobay Chemical Corporation, Environmental Health Research, Corporate Toxicology Department, Stilwell, Kansas, USA. Sponsor: Mobay Chemical Corporation, Agricultural & Chemicals Division, Kansas City, MO, USA. Study duration: 17 March 1980 - 19 March 1982. Report date: 10 April 1985.***

Guidelines and GLP: The study was performed according to OECD guideline 451, with additional haematological and clinical chemistry determinations. The study is GLP compliant [Federal Register, 45: 26373-26385, 1980. Study was quality assured].

The study is considered acceptable.

Material and Methods: Groups of 50 male and 50 female 38 day old CD1 outbred strain mice (source: Charles River Breeding Laboratories, Wilmington, MA, USA) [Mice were quarantined for 3 days prior to commencement of the study] received azinphos-methyl (batch no. 79-R-225-42, purity 88.6%) [Mobay Chemical Corporation, Agricultural and Chemicals Division, Kansas City, MO, USA] in the diet (with 1% corn oil) for 104 weeks at nominal concentrations of 0, 5, 20 and 40 ppm [estimated to be equivalent to 0, 0.75, 3.0 and 6 mg/kg bw/d]. [Diet (Ralston Purina Rodent Chow 5001-4, unspecified source) containing azinphos-methyl was prepared weekly. Each batch of diet was analysed for azinphos-methyl content,

stability and impurities, using either gas, liquid or thin-layer chromatography, low resolution mass spectrometry, or titration.]

The study was initially started with 80 ppm [estimated to be equivalent to 12 mg/kg bw/d] as the high dietary level, but this was reduced to 40 ppm after one week, due to severe reaction to treatment, including mortality, at 80 ppm. [Mice were 6 weeks old at initiation of the study. Five animals were housed per suspended, stainless steel cage. Room temperature and humidity were maintained at 20-23°C and 35-55%. A 12 h light/12 h dark cycle was maintained throughout the study. Food and potable water (supplied by Rural Water District No. 2, unspecified location) were available *ad libitum.*]

[Animals were observed twice daily for clinical signs, moribundity and mortality. Weekly observations for abnormalities and masses by palpation were made. Body weights and food consumption was recorded weekly]. Haematology values [Hct, Hb, RBC, WBC, MCV, MCHC, platelet and differential leukocyte count] and ChE activities were determined in 10 animals/sex/group at 6, 12 and 24 months. All animals found dead, killed [by CO_2 asphyxiation] in a moribund condition or killed at scheduled sacrifice [unspecified time] were subjected to a gross pathological examination [all orifices, cranial cavity and their viscera, cervical tissue and organs] and tissues preserved for histopathology. [The following organs were weighed: adrenals, brain and brainstem, gonads, heart, kidneys, liver, lungs and spleen. The following tissues and organs were examined histopathologically: adrenals, bone and marrow, brain, cervical lymph nodes, cervix, dorsal aorta, oesophagus, eyes, gall bladder, harderian glands, heart, kidney, large intestine, larynx, liver, lungs, mammary gland, mesenteric lymph modes, skeletal muscle, optic nerves, ovaries, pancreas, parathyroids, pituitary, prostate gland, ribs-costochondrial junction, sciatic nerve, seminal vesicles, skin, skull, small intestine, spinal cord, spleen, sternum, stomach, salivary gland, testicles, thymus, thyroids, trachea, urinary bladder, uterus and all gross lesions.]

Statistical methods: Body weight, feed consumption, haematological parameters, and organ weights were subjected to an analysis of variance followed by Duncan's new multiple range test. All significant differences were reported at the 95% confidence level.

Findings: [*Dietary analysis:* Analysis of the azinphos-methyl content of the diet indicated that the measured levels of 4.14, 17.3 and 34.2 ppm complied with the target levels of 5, 20 and 40 ppm. The half-life of azinphos-methyl in rodent chow at room temperature was determined to be 19 days.]

General examinations: The average doses ingested at 5, 20 and 40 ppm were 0.79, 3.49 and 11.33 mg/kg bw/d for males and 0.98, 4.12 and 14.30 mg/kg bw/d for females, respectively.

At the initial high dietary level of 80 ppm, 4 females were found dead after 4 days, and a decrease in body weights of 10 to 13% was noted in males and females after one week [no statistical analysis was performed]. Following the reduction in the high dietary level from 80 to 40 ppm, there were no clinical signs of reaction to treatment and mortality [incidence] remained unaffected by treatment. Body weight gain and feed intake of both sexes were unaffected by treatment throughout the study at dose levels up to and including 40 ppm [see Table below]. [There was no difference in the incidence or types of abnormalities/masses detected by palpation, or clinical signs, between azinphos-methyl-treated and control mice. A transient bout of Sendai virus infection was detected in all groups from week 68-71]

Effect of dietary administration of azinphos-methyl over 104 weeks on mortality and body weights in mice

Dose level (ppm) Sex	0 M/F	5 M/F	20 M/F	40 M/F
No. of animals	50/50	50/50	50/50	50/50
Mortality, 104 wk (%)	44/60	34/40	48/68	44/46
Body weight, 104 wk (g)	38.6/35.4	39.0/34.4	38.8/36.5	38.9/35.3

Clinical laboratory examinations: There were no biologically significant changes in the haematological profile at any of the dose levels of either male or female animals at 6, 12 and 24 months. A toxicologically relevant inhibition (up to 80%) of ChE activity occurred in plasma, RBC and brain at 20 and 40 ppm [Tables following]. At 5 ppm there was no biologically significant inhibition of ChE activity, except for female RBC at 2 years which was 22% lower than the control value. At a subsequent analysis one week later, erythrocyte ChE activities in different females from the control and 5 ppm were almost identical in both groups (0.84 and 0.83 µmol/ml/min, respectively). [At 20 ppm, inhibition of plasma ChE ranged from 17-31% in males and 22-53% in females, while at 40 ppm it ranged from 48-56% and 67-77% in males and females respectively (see relevant Table). At 20 ppm, inhibition of RBC ChE ranged from 34-57% in males and 42-58% in females while at 40 ppm it ranged from 50-81% and 46-77% in males and females respectively (see relevant Table overleaf). At 20 ppm, the level of inhibition of brain ChE was 16% in males and 26% in females, while at 40 ppm the level of inhibition was 63 and 67% for males and females respectively (see relevant Table). No statistical analysis was performed on the ChE data.]

Effect of dietary administration of azinphos-methyl over 104 weeks on ChE activities (plasma, RBC: µmol/ml/min; brain: µmol/g/min) in mice

Dose level (ppm) Sex	0 M/F	5 M/F	20 M/F	40 M/F
Plasma, 6 mo	3.11/5.76	3.33/5.45	2.57/3.08	1.62/1.48
Plasma, 1 yr	3.88/6.51	4.81/5.44	2.63/3.27	1.32/1.50
Plasma, 2 yr	4.33/4.98	3.95/4.93	2.97/3.86	1.89/1.65
RBC, 6 mo	1.33/1.16	1.11/1.03	0.88/0.67	0.67/0.63
RBC, 1 yr	1.04/0.87	0.99/0.81	0.45/0.39	0.20/0.20
RBC, 2 yr	0.95/0.79	0.80/0.62	0.54/0.40	0.35/0.32
Brain, 2 yr	14.7/14.4	12.9/13.6	12.3/10.6	5.4/4.7

Result of statistical analysis not given in the report.

[Effect of dietary administration of azinphos-methyl over 104 weeks on ChE activities (expressed as% inhibition compared to the control group, as calculated by reviewing toxicologist)

Dose level (ppm) Sex	5 M/F	20 M/F	40 M/F
Plasma, 6 mo	-7/5	17/53	48/74
Plasma, 1 yr	-24/16	32/50	66/77
Plasma, 2 yr	9/1	31/22	56/67
RBC, 6 mo	17/11	34/42	50/46
RBC, 1 yr	5/7	57/55	81/77
RBC, 2 yr	16/22	43/58	63/59
Brain, 2 yr	12/6	16/26	63/67

Result of statistical analysis not given in the report.]

Pathological examinations: No treatment-related effects were revealed on organ weights and on gross or microscopic examinations. The statistically significant increased absolute heart weight in females at 20 ppm is considered not biologically significant since the effect was not seen at 40 ppm.

There was increased frequency of adenomatous hyperplasia in the lungs with increased dosage [see following Table]. Although the incidence in male mice at 40 ppm was statistically

Incidence of adenomatous hyperplasia and alveolar/bronchiolar adenoma in the lungs of mice administered azinphos-methyl in their diet for 104 weeks

Dose level (ppm) **Sex**	**0** **M/F**	**5** **M/F**	**20** **M/F**	**40** **M/F**
Number of animals	50/50	50/50	50/50	50/50
Adenomatous hyperplasia: incidence severity	7/6 2.6/2.2	10/16 1.7/1.9	14/7 2.0/1.4	15*/11 2.2/2.5
Alveolar/bronchiolar adenoma	14/7	8/6	4/6	8/9

* $p < 0.05$; severity values are the average of histopathological severity codes 1-5 (1 = minimal, 2 = mild; 3 = moderate; 4 = marked; 5 = severe).

significantly increased, the severity of hyperplasia was less than in controls. In contrast to the increased frequency of adenomatous hyperplasia the incidence of alveolar/bronchiolar adenoma in the lungs of the 40 ppm males was lower than in controls. Thus, there was no dose-related increase in the combined incidence of adenomatous hyperplasia and alveolar/bronchiolar adenoma in the lungs of male mice. [The study authors concluded that the presence of Sendai virus infection may have contributed to the observed inflammatory and pulmonary hyperplastic changes. Overall there was no difference in the incidence and types of non-neoplastic observations between azinphos-methyl-treated and control mice.]

Neoplasms were similar in type, localisation, time of occurrence and incidence in control mice and mice receiving azinphos-methyl up to and including 40 ppm in the diet [see following Tables]. Although there was no dose-related increase in the number of animals with tumours at the 40 ppm level, there was an increased frequency of total tumours found and of total malignant tumours in males which was statistically significantly different from the controls ($p < 0.05$). However, the tabulation of malignant tumours is based on the number of malignant designations per tissue per animal and, thus, haematopoietic neoplasms are counted in each tissue rather than once per animal. In males fed 40 ppm, the slight increase in malignant lymphoma bearers markedly increases the total tumour and total malignant tumour categories due to multiple site involvement.

Summary of the incidence of neoplasms in mice administered azinphos-methyl in their diet for 104 weeks

Dose level (ppm) **Sex**	**0** **M/F**	**5** **M/F**	**20** **M/F**	**40** **M/F**
Total no. of animals	50/50	50/50	50/50	50/50
Animals with tumours	32/28	32/28	23/31	35/30
Animals with only benign tumours	19/12	17/10	12/9	16/13
Animals with only malignant tumours	7/11	8/13	7/13	14/12
[All animals with malignant tumours]	[13/16]	[15/18]	[11/22]	[19/17]

Total no. of tumours	116/224	86/172	117/177	170*/179
Total no. of malignant tumours	86/204	56/154	99/153	143*/157

* $p<0.05$

Incidence of neoplasms in mice administered azinphos-methyl in their diet for 104 weeks

Dose level (ppm)	0		5		20		40	
Sex	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Total no. of animals	50	50	50	50	50	50	50	50
Skin (#)	50	50	50	50	49	50	49	50
- Lymphoma (m) all types	1	4	3		3	1	1	1
- Adenocarcinoma NOS(m)				1				
- Fibrosarcoma (m)				1				
- Metastasis NOS (m)						1		
- Sarcoma NOS (m)						1		
Mammary gland (#)	30	50	20	50	23	50	19	50
- Lymphoma (m) all types	1	7	2	1	2	4	1	2
- Fibrosarcoma (m)		1		1				
- Adenoacanthoma (m)		1						
- Sarcoma NOS(m)		1						
- Adenocarcinoma NOS(m)				3		2		
Salivary gland, submax.(#)	50	50	50	50	50	50	50	49
- Lymphoma (m) all types	3	4	2	4	3	2	2	4
Lymph node, cervical (#)	45	48	47	47	42	49	44	47
- Lymphoma (m) all types	3	6	3	7	4	6	5	6
- Metastasis NOS (m)						1		
Lymph node, mesenteric (#)	50	46	50	48	48	49	50	49
- Lymphoma (m) all types	3	7	3	8	4	8	7	7
- Metastasis NOS (m)		1				2		1
Trachea (#)	50	48	50	50	50	48	50	49
- Lymphoma (m) all types		1			1		1	2
Larynx (#)	50	49	50	50	50	49	50	49
- Lymphoma (m) all types		3		1	1		2	2
- Myelo. neoplasia NOS (m)				1				
Oesophagus (#)	50	47	50	50	50	48	50	50
- Lymphoma (m) all types		1					1	1
Thyroid (#)	48	48	50	47	50	48	49	48
- Lymphoma (m) all types		1		3			2	2
- Adenoma NOS (b)				1				
- Adenocarcinoma NOS(m)								1
Parathyroids (#)	20	22	21	24	19	29	24	27
- Lymphoma (m) all types							1	
Sternum (#)	49	49	47	50	50	50	49	49
- Lymphoma (m) all types	3	6			3		3	3
- Hemangiosarcoma (m)			1					
Ribs costochondral jct.(#)	50	50	49	48	50	50	49	50
- Lymphoma (m) all types	2	6	2		3		4	3
- Alveol/bronchiol. ca.(m)		1				2	1	
- Sarcoma NOS (m)				1				
Thymus (#)	46	46	44	44	43	46	47	48
- Lymphoma (m) all types	3	7	1	6	4	7	5	5
- Alveol/bronchiol. ca.(m)							1	
- Metastasis NOS (m)						1		
Heart (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types		4	3	3	3		3	3
- Alveol/bronchiol. ca.(m)		1					1	
- Metastasis NOS (m)						1		

Dose level (ppm)	0		5		20		40	
Sex	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Aorta (#)	48	45	49	47	49	48	50	47
- Lymphoma (m) all types	2	3		1	2		4	3
- Alveol/bronchiol. ca.(m)		1					1	
Lung (#)	50	50	50	50	50	50	50	50
- Alveol/bronchiol.ca.(m)	2	2	3	2	5	3	6	2
- Alveol/bronchiol. ad.(b)	14	7	8	6	4	6	8	9
- Lymphoma (m) all types	3	7	2	5	4	8	5	6
- Leukaemia NOS (m)				1				1
- Metastasis NOS (m)						2		2
Liver (#)	50	50	50	50	50	50	50	50
- Hepatocellular ca. (m)	5		6		2		4	
- Hepatocellular ad. (b)	8	2	10	1	8	3	8	2
- Hemangiosarcoma (m)	3	1				2		
- Hemangioma (b)		1		2	1	1	1	
- Lymphoma (m) all types	3	6	2	6	3	5	7	6
- Sarcoma NOS (m)		1				1		1
- Myeloprol. neoplasia (m)				1				
- Metastasis NOS (m)						2		2
Gall bladder (#)	48	43	45	45	41	40	46	40
- Lymphoma (m) all types	2	3		3	2		1	2
Spleen (#)	49	50	50	50	50	50	50	50
- Lymphoma (m) all types	3	6	2	7	4	8	6	6
- Hemangiosarcoma (m)			1			1		
- Hemangioma (b)			1	1		2		
- Myelo. neoplasia NOS (m)				1				
- Metastasis NOS (m)		1						
Pancreas (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	3	6		6	3	5	4	7
- Hemangiosarcoma (m)	1							
- Metastasis NOS (m)						2		
Stomach (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	2	6	1	4	1	1	1	4
Small intestine (#)	50	50	49	50	50	50	50	50
- Adenocarcinoma (m)			2				1	
- Lymphoma (m) all types	2	1		4	1	1	3	2
Caecum (#)	49	49	48	47	50	50	49	50
- Lymphoma (m) all types	1	4		2		1	1	3
Large intestine (#)	50	48	50	49	50	48	50	50
- Lymphoma (m) all types	1	4		4	2	1		1
Adrenals (#)	50	50	50	50	50	50	50	49
- Cortical adenoma NOS(b)	2		1				1	1
- Pheochromocytoma (b)						1		
- Lymphoma (m) all types	3	5	2	6	2	3	3	4
- Metastasis NOS (m)						2		
Kidneys (#)	50	50	50	50	50	50	50	50
- Adenoma NOS (b)	1							
- Lymphoma (m) all types	3	6	1	8	4	6	7	6
- Endometr. strom.sarc.(m)		2				2		1
- Metastasis NOS (m)						1		
Testes (#)	50		50		50		50	
- Interstit. c. tumour (b)			1				2	
- Hemangioma					1			
- Lymphoma (m) all types							1	
Urinary bladder (#)	49	48	50	50	50	49	50	50
- Carcinoma (m)				1				
- Lymphoma (m) all types	2	6	1	4	3	4	3	5
- Metastasis NOS (m)						1		

Dose level (ppm)	**0**		**5**		**20**		**40**	
Sex	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Seminal vesicles (#)	50		50		49		50	
- Lymphoma (m) all types	3		1		3		5	
Prostate (#)	50		50		50		50	
- Lymphoma (m) all types	3		1		4		2	
Muscle, gastrocnemius (#)	50	50	49	50	49	50	50	50
- Lymphoma (m) all types	1	1	1		1		2	3
Sciatic nerve (#)	49	49	48	48	50	50	50	50
- Lymphoma (m) all types			1		1		1	
Bone (#)	50	49	49	48	49	49	49	50
- Osteosarcoma (m)							1	
- Lymphoma (m) all types	2	1			1		1	1
Bone marrow (#)	50	50	50	50	50	50	50	50
- Lymphoma (m) all types	1	5	1	1	3	5	5	3
- Myelo. neoplasia NOS (m)				1				
- Metastasis NOS (m)								1
Spinal cord (#)	49	48	50	48	50	50	50	50
- Neurofibroma (b)		1						
- Lymphoma (m) all types	2	3		1	1	1	3	1
Eyes (#)	50	50	50	50	50	50	49	50
- Lymphoma (m) all types	1	2	1		2	2	1	1
- Metastasis NOS (m)						1		
Optic nerve (#)	44	44	38	42	44	33	42	47
- Lymphoma (m) all types	1	1		1	1		3	1
Harderian glands (#)	50	48	50	50	50	50	50	50
- Adenom (b)	4	2	8		3	3	7	2
- Adenocarcinoma (m)								1
- Lymphoma (m) all types	2	3	1	1	3	3	3	2
Pituitary (#)	49	50	50	48	50	50	49	49
- Adenoma (b)	1	1		2		3		
- Carcinoma NOS (m)						1		
- Lymphoma (m) all types	1	4			1	1	1	2
Skull (#)	50	50	50	49	50	50	50	50
- Osteoma NOS (b)			1					
- Lymphoma (m) all types	3	3	1		1	2	3	2
- Metastasis NOS (m)						1		
Brain (#)	50	49	50	50	50	50	50	50
- Glioma NOS (b)				1		1		
- Lymphoma (m) all types	1	2		1	1			1
- Metastasis NOS (m)						1		
Ovaries (#)		49		49		48		50
- Granulosa c. tumour (b)				4		1		3
- Hemangioma (b)		1						1
- Hemangiosarcoma (m)		1						
- Adenoma NOS (b)								1
- Lymphoma (m) all types		7		6		3		3
- Metastasis NOS (m)						1		2
Uterus (#)		48		50		50		49
- Endom. stromal polyp (b)		1						2
- Endom. stromal sarc. (m)		5		3		6		4
- Leiomyoma (b)		1		1				
- Hemangioma (b)		2		1		3		2
- Hemangiosarcoma (m)				1				1
- Adenocarcin. NOS (m)						1		1
- Carcinoma NOS (m)				2		1		2
- Sarcoma NOS (m)						1		
- Lymphoma (m) all types		4		4		2		3
- Metastasis NOS (m)				1				

Dose level (ppm)	0		5		20		40	
Sex	male	female	male	female	male	female	male	female
Cervix (#)		38		49		50		46
- Endom. stromal sarc. (m)		3				1		3
- Sarcoma NOS (m)		1						
- Leiomyoma (b)		1						1
- Lymphoma (m) all types		3		2		3		3

Total no. of organs examined; NOS - not otherwise specified; (b) benign; (m) malignant

Conclusion: The NOEL for dietary administration of azinphos-methyl to mice over 2 years was 5 ppm, equivalent to 0.79 mg/kg bw/d in males and 0.98 mg/kg bw/d in females [based on a biologically significant decrease in plasma, RBC and brain ChE activity at 20 ppm (equivalent to 3.49 and 4.12 mg/kg bw/d for males and females respectively]. A dose-related depression of ChE activity was observed at 20 ppm and above, and mortality and a decrease in body weight occurred at 80 ppm. There was no evidence that azinphos-methyl [up to and including 40 ppm (estimated to be equivalent to 6 mg/kg bw/d)] is tumorigenic in mice.

[*Comments:* Standard deviations or errors were not included with the mean data.]

2.6.2 Rats

Harper KH, Bensen HG, Mawdesley-Thomas LE & Street AE (1966) Toxicity of Gusathion during repeated administration to rats for two years. Report No. 1616/66/144. Lab:. Huntington Research Centre, Huntington, England. Sponsor: Bayer, Germany. Study duration: March 1964 – July 1966. Report date: 2 June 1966.

Aim: The aim of this study was to assess the prolonged PO toxicity of Gusathion (azinphos-methyl) to rats over 2 years.

Materials and Methods: A pre-mix of Gusathion (azinphos-methyl) (purity, batch no. and source unspecified) containing 2000 ppm was prepared every two weeks throughout the study. Details pertaining to the production of this pre-mix were unspecified. Target dietary levels of 0, 2.5, 5, 20, 50 and 100 ppm Gusathion (estimated to be equivalent to 0, 0.125, 0.25, 1, 2.5 and 5 mg/kg bw/d) were obtained by direct dilution into Spiller's Laboratory Small Animals Diet (batch and source unspecified). Again, no details were given with regard to the manufacture of the diet.

Four-hundred and sixty male and female rats of a Wistar strain (Manor Farm Breeding Laboratories, Staatsburg, USA) were quarantined and acclimatised for 5 days prior to the commencement of the study. Initial ages were unspecified while starting weights were approximately 93 g for males and 84 g for females. Thirty male and thirty female rats were randomly placed into the following groups: Group 1 (50-100 ppm), Group 2 (20 ppm), Group 3 (5 ppm), Group 4 (control), Group 5 (2.5 ppm) and Group 6 (control). Satellite groups consisting of 10 males and 10 females were randomly assigned to the same groups and used for laboratory tests (eg urinalysis), with animals from the main groups conscripted toward the end of the study as mortalities increased.

The dose level received by Group 1 animals was increased from 50 to 100 ppm after week 47. Dosing of Groups 1-4 commenced on the 17th March 1964 and was completed in Januray 1966, while dosing of Groups 5 and 6 commenced on the 24th August 1964 and ended in July 1966. Thus Groups 5 and 6 were treated for an extra month. Five rats were housed per

suspended, wire-bottomed cage (ie there were 6 randomly distributed replicates/group). Animals were supplied with diet and tap water *ad libitum* during the experiment. Details of laboratory conditions were unspecified (ie room temperature and humidity). Any rats that died or exhibited gross signs of disease during the first 2 weeks were replaced with 'group spares' that had been treated with an identical level of Gusathion for the same time.

Animals were observed for any signs of reaction to Gusathion (unspecified time and frequency). Body weights were recorded initially and then weekly throughout the study. Food consumption per cage was recorded weekly. Water consumption was assessed by visual inspection (unspecified time and frequency). Rats found dead during the study were subjected to a detailed macroscopic examination and any affected organs identified.

Pathology: At the end of the study all remaining rats were killed by inhalation of carbon dioxide. Macroscopic examination of tissues was performed, with the following organs weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes and thyroid. Half of the brain of up to 6 males and 6 females from each group was taken for ChE estimation with the other half of the brain used for histopathological examination.

Laboratory analysis: At 2, 10, 26, 39, 52, 65, 78, 91 and 97 weeks, samples of blood (unspecified volume) were withdrawn from the lateral veins of up to 10 male and 10 females from each group for measurement of plasma and RBC ChE levels. At 2, 52 and 96 weeks urinalysis and haematological analysis were performed on 5 male and 5 female rats from Groups 1 (50-100 ppm) and 4 (0 ppm) with analysis extended to Groups 5 (2.5 ppm) and 6 (0 ppm) at 96 weeks. Details regarding the collection of urine were unspecified. Urinary parameters measured included: pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigments, bile salts and urobilinogen. Samples of blood (unspecified volume) were withdrawn from the lateral caudal vein and analysed for the following parameters: Hct, Hb, RBC, WBC, differential white cell count, MCH and MCV.

Histopathological examination was performed on an unspecified number of animals from Groups 1 and 4 with only representative animals from the intermediate groups examined due to the high percentage of sporadic deaths. The following organs/tissues were examined: adrenals, aorta, bone marrow, brain, colon, duodenum, eye, heart, ileum, kidneys, liver, lungs, lymph node, optic nerve, ovaries, pancreas, pituitary, prostate, sciatic nerve, skeletal muscle, spinal cord, spleen, stomach, testes, thyroid, urinary bladder and uterus.

Any suspected differences between controls and treatment groups were statistically analysed by ANOVA. It was assumed that Groups 1-3 were compared to Group 4, and Group 5 was compared to Group 6.

Results

Mortalities and clinical observations: There were numerous deaths across all groups throughout the study (see following Table) but in the absence of a relationship with dose, mortality was not obviously associated with treatment. Most dead animals showed signs typical of ageing such as enlarged and haemorrhagic pituitaries, enlarged hearts, cystic nephritis and peri-arteritis. Microscopic examination of a selection of rats indicated that the majority had a history of weight loss and a nasal discharge. Collectively these observations suggest that the study animals were of poor health unrelated to Gusathion administration.

Following the increased dosage of Group 1 animals from 50 to 100 ppm, convulsions were observed in 5 females after 7 to 32 weeks. All of these individuals were sacrificed between 24 h to 28 weeks after convulsions were initially observed.

There was no evidence of a difference in food consumption, food conversion ratios and body weights between Gusathion-treated and control rats.

Urinalysis and Haematology: There was no difference in urinary and haematological parameters between Gusathion-treated and control rats.

Effects of dietary-administered Gusathion on rats over 97 weeks (n = 30 unless indicated)

Group	**1** **50 –100 ppm**	**2** **20 ppm**	**3** **5 ppm**	**4** **control**	**5** **2.5 ppm**	**6** **control**
Deaths						
♂	28/40	29/40	30/40	33/40	29/40	22/30
♀	34/40	27/40	33/40	33/40	22/40	23/30
Group Mean Plasma ChE Δ pH+						
♂						
wk 39	0.61*** (13%)	0.62*** (13%)	0.71* (0%)	0.71	0.63	0.59
wk 65	0.57**** (21%)	0.67	0.68	0.72	0.78	0.66
wk 97	0.64*** (30%)	0.75* (18%)	0.85	0.92	1.06	0.85
♀						
wk 39	0.87**** (49%)	1.35**** (21%)	1.44*** (15%)	1.70	1.58	1.48
wk 65	0.72**** (45%)	1.38	1.27	1.31	1.46	1.35
wk 97	0.85* (24%)	1.01	0.98	1.12	1.31	1.45
Group Mean RBC ChE Δ pH+						
♂						
wk 39	0.39*** (25%)	0.48	0.55	0.52	0.50	0.51
wk 65	0.26**** (54%)	0.47**** (18%)	0.56	0.57	0.51	0.49
wk 97	0.38*** (33%)	0.56	0.51	0.57	0.62	0.49
♀						
wk 39	0.28**** (45%)	0.39**** (24%)	0.53	0.51	0.51	0.49
wk 65	0.23**** (60%)	0.43**** (26%)	0.58	0.58	0.46	0.46
wk 97	0.26* (33%)	0.46	0.41	0.39	0.53	0.51
Group Mean Brain ChE Δ pH+						
♂	0.39** (19%)	0.47	0.51	0.48	0.58	0.46
♀	0.27**** (49%)	0.51	0.50	0.53	0.52	0.64
Tumour Incidence (%)						
Adrenals	5	0	0	4	-	-
Pituitary	41	50	33	51	-	-
Mammary tissue	3	19	14	15	-	-
Thyroid	5	6	5	6	-	-
Spinal cord	0	0	5	0	-	-

Skin	0	0	10	0	-	-
Connective tissue	0	0	0	13	-	-
Parathyroid	0	0	0	2	-	-

+ values in parentheses are % inhibition compared to the control (Group 4) calculated by the reviewing toxicologist; * statistically different to the control at p = 0.05; ** statistically different to the control at p = 0.02; *** statistically different to the control at p = 0.01; **** statistically different to the control at p = 0.001

Clinical Chemistry: There was a clear Gusathion-related effect on plasma, RBC and brain ChE activity (see Table above). Group 1 had significantly lower plasma and RBC ChE activities throughout the study compared to the control group (p = 0.001-0.05; up to 30 and 49% inhibition of plasma ChE in males and females respectively; up to 33 and 60% inhibition of RBC ChE in males and females respectively). Brain ChE in Group 1 was also significantly inhibited compared to the control group with the effect in females more significant (p = 0.001, 49% inhibition) than in males (p = 0.02, 19% inhibition). During the first 39 weeks, Groups 2 and 3 males had significantly lower plasma ChE activity than the control (p = 0.01 and 0.05 respectively) but the inhibition in activity in Group 3 (5 ppm) was not biologically significantly. A similar pattern was observed in Group 2 (20 ppm) and 3 (5 ppm) females, however the result was statistically more significant than that occurring in males (p = 0.001 and 0.02; 21 and 15% inhibition respectively). Plasma ChE activity of Groups 2 (20 ppm) and 3 5 ppm) generally recovered to control levels for the remainder of the study. Group 2 (20 ppm) males still had slightly depressed plasma ChE activity at 97 weeks. Group 2 males showed a transiently depressed (p < 0.001, 18% inhibition) RBC ChE activity at week 65 while Group 2 females had a depressed RBC ChE until week 65 (p < 0.001, 24-26% inhibition) after which they recovered to control levels.

Gross pathology at necropsy: Animals that survived the duration of the study showed no intergroup differences in gross pathology. There were no effects on organ weights that could be attributed to Gusathion. Tumour incidences were not related to Gusathion administration (see Table above).

Histopathology: There were no histopathalogical abnormalities observed in rats that died during the study and in rats that were sacrificed at the end of the study. The majority of animals across all groups exhibited signs of heart degeneration, chronic pneumonitis and kidney nephropathy.

Conclusions: This study indicated that Wistar rats could tolerate the incorporation of up to 100 ppm Gusathion (azinphos-methyl) (estimated to be equivalent to 5 mg/kg bw/d) in their diet over 97 weeks. Toxicological effects included depressed plasma, RBC and brain ChE activity, and convulsions in some rats when the dietary intake of Gusathion was increased from 50 to 100 ppm. This study also indicated that Gusathion is not carcinogenic as no Gusathion-related effect on tumour incidence was observed. Females appeared to be more sensitive to Gusathion than males as shown by the statistically more significant effect on plasma, RBC and brain ChE activity. The NOEL was 5 ppm (estimated to be equivalent to 0.25 mg/kg bw/d) based on depressed plasma and RBC ChE activities at 20 ppm (estimated to be equivalent to 1 mg/kg bw/d).

Comments: The experimental design and level of reporting are commensurate with a summary study of this age, however a number of deficiencies were apparent. The report date of the 2nd June 1966 is earlier than the duration of the study which was until July 1966. Not all of the groups were started on their diet simultaneously with Groups 1-4 started on the 17th

March 1964 and finishing in January 1966, and Groups 5 and 6 started on the 24th August 1964 and ending in July 1966. This difference in start date implies that Group 5 and 6 rats were 5 months older than Group 1-4 rats when commenced on their diet. Consequently it is less than favourable to link results from Groups 1- 4 with Groups 5 and 6 with regard to setting a NOEL due to this apparent age difference. Details of the preparation of the diet were not given. The Gusathion content of the diet was not analysed. No indication of the variability of data was provided such as standard deviations or errors. It was unclear which control Groups were used for statistical comparisons (ie Group 4 or 6). Urinalysis and haematology were not performed on all groups. Two methods for determining ChE levels were utilised, with the method of Michel (1949) used until week 39 and then changed without reason to the method of Williams and Frawley (1957). Consequently ChE data up to week 39 was numerically lower than data from week 39. Statistical analyses were inadequately described. The increased dosing of the high-dose group from 50 to 100 ppm did not take into consideration any delayed or cumulative effects of the test compound.

♦ ***Worden AN, Wheldon GH, Noel PRB & Mawdesley-Thomas LE (1973) Toxicity of Gusathion for the rat and dog. Lab: Huntingdon Research Centre, Huntingdon PE18 6ES, England. Dates of experimental work: not specified in detail (start of acclimatisation: February 1964). Published in: Tox Appl Pharmacol 24:405-412.***

This published paper is a summary of data from Harper *et al* 1966 (see above).

Guidelines and GLP: The test method employed was the internal standard [of the Huntingdon Research Centre] at the time the study was performed and is in general compliance with OECD guideline 452. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: The test procedures are not described in detail. The 8-page report (publication) is not very detailed (missing data for food consumption and body weight, for clinical, laboratory and pathological examinations; missing raw data).

The study is considered supplementary.

Material and methods: Groups of 40 male and 40 female Wistar rats (source: Manor Farm Breeding Laboratories, Staatsburg, NY) were administered Gusathion (source and purity not specified) in the diet over a period of two years [initial age and weight of animals unspecified]. The initial concentrations were 0, 5, 20 or 50 ppm [estimated to be equivalent to 0, 0.25, 1 and 2.5 mg/kg bw/d]. A supplementary group receiving 2.5 ppm [estimated to be equivalent to 0.125 mg/kg bw/d] and an additional control were introduced into the study at week 23. In the highest dose group, the concentration in the diet was increased from 50 to 100 ppm [estimated to be equivalent to 5 mg/kg bw/d] after 47 weeks. Blood and urine samples were taken at regular intervals [2, 52 and 96 weeks] and examined almost according to OECD guideline 452. [Plasma and RBC ChE levels were measured at 10, 26, 39, 52, 65, 78 and 97 weeks] The pathological examinations were not described.

Findings: Several females [5] exhibited signs of toxicity (convulsions) at the 100 ppm dose. There was a significant inhibition of plasma, erythrocyte and brain ChE activity at the highest dose group, more marked in females than in males. An initial inhibition of plasma and erythrocyte ChE activity was observed at 20 ppm. There was no evidence that the test substance had any effect on tumour incidence.

Conclusion: The NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm, equivalent to about 0.25 mg/kg bw/d (calculated by means of a conversion factor of 0.05). A depression of plasma and erythrocyte ChE activity was observed at 20 ppm and above, and clinical signs occurred at 100 ppm.

♦ ***National Cancer Institute (NCI) (1978) Bioassay of azinphosmethyl for possible carcinogenicity. NCI Carcinogenesis Technical Report Series No. 69. Lab & Sponsor: NCI, National Institute of Health (NIH), Bethesda, Maryland, USA. Study duration: unspecified. Report date: 1978.***

Kimmerle (1980) Comments on the bioassay of azinphosmethyl for possible carcinogenicity (NCI Carcinogenesis Technical Report Series No. 69, 1978). Mobay Chemical Corporation, Stanley Research Center, Stilwell, Kansas, USA; letter of 9 December 1980 to Dr. Reuver, Bayer AG, Leverkusen, Germany.

Guidelines and GLP: The method employed was that of the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: Only two dose levels were used. The animals were exposed for 80 weeks only and not for the entire lifetime (or at least 24 months). In low- and high-dose males and high-dose females, the selected dose levels caused marked depression of body weight gain and seemed to reach the maximum tolerated dose. The concurrent control group consisted of 10 male and 10 female rats only.

The study is considered supplementary.

Material and Methods: Groups of 50 male and 50 female 35 days old Osborne-Mendel rats (source: Battelle Memorial Institute, Columbus, Ohio, USA; body weight range not specified) received azinphos-methyl (Guthion®, Mobay Chemical Corp., Kansas City, Missouri, USA; batch no. not specified, purity 90%) for 80 weeks by admixture in the diet [Wayne® Lab Blox animal mean (Allied Mills Inc., Chicago, IL, USA)] (with 2% corn oil) at concentrations of 125 and 250 ppm [estimated to be equivalent to 6.25 and 12.5 mg/kg bw/d respectively] for males and 62.5 and 125 ppm [estimated to be equivalent to 3.125 and 6.25 mg/kg bw/d respectively] for females. The concentrations offered to male rats were reduced to 62.5 and 125 ppm as from week 21, resulting in time-weighted average concentrations of 78 and 156 ppm. [estimated to be equivalent to 3.9 and 7.8 mg/kg bw/d respectively]. [Dietary analysis was performed using gas-liquid chromatography to ensure compliance with the theoretical azinphos-methyl content] The dose levels were selected on the basis of a previous subchronic feeding study conducted to estimate maximum tolerated doses of azinphos-methyl.

A group of 10 male and 10 female rats served as matched controls; the pooled controls for statistical tests consisted of the matched controls combined with 95 male and 95 female untreated rats from similar bioassays of 10 other test chemicals. [Animals were housed individually in hanging galvanised steel mesh cages. Temperature and humidity were maintained at 20-24°C and 40-70% respectively. There were 10-12 air changes/h with a 10h

light/14h dark cycle maintained throughout the study. Food and water were available *ad libitum*].

Following 80 weeks of exposure, the animals were retained untreated but under observation for a further 34 or 35 weeks. All animals were observed twice daily for signs of toxicity, weighed at regular intervals [unspecified], and palpated for masses at each weighing. All animals found dead, killed [by unspecified means] in a moribund condition or killed at scheduled sacrifice [114 or 115 weeks] were subjected to a gross and microscopic examination of major tissues, major organs, and all gross lesions [skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gall bladder, pancreas, stomach, small and large intestine, kidney, urinary bladder, pituitary gland, adrenals, thyroid gland, mammary gland, prostate or uterus, testis or ovary and brain].

Statistical methods: Survival analysis, Kaplan and Meier (1958), Cox (1972), and Tarone's (1975) extension of Cox's methods. Incidences of lesions, one-tailed Fisher exact test at significance level of 0.05, with Bonferroni inequality correction, and Cochran-Armitage test for linear trend in proportions, as well as time-adjusted analysis when appropriate. Statistical comparisons were made against the matched and pooled control groups.

Findings

[*Dietary analysis:* Analysis of the azinphos-methyl content of the diet indicated that the measured levels of 61.2, 126.0 and 256 ppm complied with the target levels of 31.2, 62.5 and 125.0 ppm].

General examinations: The mean body weights of low- and high-dose male and high-dose female rats were consistently lower than those of matched controls throughout the study, and the depression of body weight gain in males and high-dose females exceeded 10% at most periods of time [no statistical analysis was performed on the body weight data]. Clinical signs (body tremors) were observed in high-dose animals [2 males and 2 females] after one week on study. [At week 34 an unspecified number of azinphosmethyl-treated animals exhibited exophthalmos which lead to unilateral blindness in 10 high-dose females and bilateral blindness in 5 high-dose females. This was subsequently diagnosed as viral conjunctivitis.] In females, a significant positive dose-related trend in mortality ($p = 0.041$) was observed [see Table below].

Effect of dietary-administered azinphos-methyl over 80 weeks on mortality and body weights in rats

Dose group	**Control**	**Low dose**	**High dose**
Sex	**M/F**	**M/F**	**M/F**
No. of animals initially in study	10/10	50/49#	50/50
Natural death	2/1	4/5	9/9
Moribund sacrifice	2/2	11/10	14/16
Terminal sacrifice	6/7	35/34	27/25
Survival rate (%)	60/70	70/68	54/50*
Body weight [g] (week 0) @	155/125	150/125	145/125
Body weight [g] (week 53) @	610/370	540/390	530/330
Body weight [g] (week 110) @	650/440	615/460	595/380

50 animals initially, but 1 animal was found to be a male animal; @ Approximate mean body weight, derived from graphically presented data; * $p = 0.041$ for positive dose-related trend in mortality in females

Pathological examinations: Numerous tumours of the endocrine organs were observed in both dosed male and dosed female rats [see following Tables]. Those of the adrenals in [high] dosed males and females [p = 0.001], the follicular cells of the thyroid in [high and low] dosed females [p = 0.021 and 0.002 respectively], the anterior pituitary in [high and low] dosed males [p < 0.001], and the parathyroid in [high] dosed males [p = 0.009] occurred at statistically significantly [increased] incidences when compared with the pooled controls, but not with the matched controls, and they were not considered to be related to administration of the test compound.

Summary of the incidence of neoplasms in rats that had been administered azinphos-methyl in their diet over 80 weeks

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
No. of animals examined microscopically	10/9	49/48	49/46
No. of animals with primary tumours	7/7	40/37	41/26
No. of animals with benign (b) tumours	6/7	32/32	33/24
No. of animals with malignant (m) tumours	4/2	15/13	15/6
No. of animals with tumours uncertain b or m	1/0	1/0	0/0
No. of animals with secondary tumours	1/0	3/1	3/0

Incidence of neoplasms in rats that had been administered azinphos-methyl in their diet over 80 weeks

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
Skin (@)	10/10	50/49	49/49
- Squamous cell carcinoma	0/0	1/0	0/0
- Fibroma	0/0	1/0	0/0
- Fibrosarcoma	0/0	1/0	1/0
- Keratoacanthoma	0/0	0/0	0/1
- Liposarcoma	0/1	0/0	0/0
- Mast cell sarcoma	0/0	0/0	1/0
Subcutaneous tissue (@)	10/10	50/49	49/49
- Liposarcoma	1/0	0/0	0/0
Lung (#)	10/9	49/48	48/46
- Alveolar/bronchiolar adenoma	0/0	1/1	0/0
- Lymphoma, metastatic	0/0	0/0	1/0
Bone marrow (#)	10/9	49/48	46/46
- Lymphoma, metastatic	0/0	1/0	0/0
Spleen (#)	9/9	49/43	47/41
- Leiomyoma	0/0	1/0	0/0
- Hemangioma	0/0	1/1	0/1
- Hemangiosarcoma	2/0	0/1	4/0
- Malignant lymphoma, NOS	0/0	2/0	1/0
Lymph node (#)	8/9	49/48	44/46
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
- Malignant lymphoma, NOS	0/0	1/0	0/0
Skeletal muscle (@)	10/10	50/49	49/49
- Malignant lymphoma, histiocytic	1/0	0/0	0/0
- Rhabdomyosarcoma	0/0	1/0	0/0
Liver (#)	9/9	49/47	46/45
- Lymphoma, metastatic	0/0	1/0	1/0
- Adenoma, NOS	0/0	1/0	0/0
- Hepatocellular adenoma	1/2	3/2	5/4
- Adenocarcinoma, NOS	0/0	0/0	0/1

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
- Hepatocellular carcinoma	0/0	0/0	0/1
- Hemangiosarcoma, metastatic	0/0	0/1	0/0
Pancreas (#)	9/9	47/47	45/45
- Acinar cell adenoma	0/0	1/0	0/0
- Liposarcoma, metastatic	0/0	1/0	0/0
- Lymphoma, metastatic	0/0	1/0	0/0
Stomach (#)	9/9	47/46	47/44
- Leiomyosarcoma	0/0	0/0	1/0
- Hemangiosarcoma	0/0	0/1	0/0
Small intestine (#)	9/9	47/48	48/46
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
Kidney (#)	10/9	49/48	47/45
- Transitional cell carcinoma	0/0	1/0	0/0
- Liposarcoma	0/0	2/0	0/0
- Multiple polyposis	0/1	0/0	0/0
Heart (#)	10/9	48/48	47/46
- Lymphoma, metastatic	0/0	0/0	1/0
- Fibrosarcoma, metastatic	0/0	1/0	0/0
- Hemangiosarcoma, metastatic	0/0	0/0	1/0
Pituitary (#)	9/8	46/44	43/41
- Adenoma, NOS	0/0	0/0	3/1
- Chromophobe adenoma	4/2	21/14	13/12
- Chromophobe carcinoma	0/0	0/0	2/0
- Cystadenoma, NOS	0/0	0/0	2/1
- Adenocarcinoma, NOS	0/0	0/8	0/1
Adrenal (#)	9/9	45/45	46/41
- Adenocarcinoma, NOS	0/0	1/0	3/0
- Cortical adenoma	1/1	3/4	7/8
- Pheochromocytoma	0/0	0/0	1/2
- Lymphoma, metastatic	0/0	0/0	1/0
Thyroid (#)	9/9	44/45	43/38
- Adenoma, NOS	0/1	2/2	2/1
- Adenocarcinoma, NOS	0/0	3/1	3/0
- Follicular cell adenoma	1/0	1/0	0/0
- Cystadenoma, NOS	0/0	7/4	10/3
- Cystadenocarcinoma, NOS	0/0	1/0	0/0
- Papillary adenocarcinoma	0/0	0/0	0/1
- Papillary cystadenocarcinoma, NOS	0/1	0/1	1/1
Parathyroid (#)	5/7	26/31	24/19
- Adenoma, NOS	1/0	0/0	4/1
Pancreatic islets (#)	9/7	47/41	45/39
- Islet cell adenoma	0/2	1/1	4/1
- Islet cell carcinoma	0/0	0/0	2/0
Mammary gland (@)	10/10	50/49	49/49
- Cystadenocarcinoma, NOS	0/0	0/1	1/0
- Fibroma	0/0	0/0	2/0
- Cystfibroadenoma	1/0	0/0	0/0
- Adenoma, NOS	0/0	0/1	0/0
- Adenocarcinoma, NOS	0/0	0/2	0/0
- Papillary cystadenocarcinoma, NOS	0/0	0/0	0/1
- Lipoma	0/0	0/1	0/0
- Leiomyosarcoma	0/0	0/1	0/0
- Fibroadenoma	0/2	0/9	0/9
Prostate(#)	10	47	45
- Papillary adenoma	0	1	0
Testis (#)	10	49	48
- Interstitial cell tumour	0	0	1

Dose group	Control	Low dose	High dose
Sex	**M/F**	**M/F**	**M/F**
Uterus (#)	9	43	41
- Endometrial stromal polyp	1	3	0
- Hemangioma	0	1	0
Ovary (#)	9	47	42
- Adenocarcinoma, NOS	0	1	0
- Papillary adenocarcinoma	0	1	0
Brain (#)	10/9	49/48	48/46
- Glioblastoma, multiforme	0/0	1/0	0/0
Rib (@)	10/10	50/49	49/49
- Hemangiosarcoma	0/0	0/0	1/0
Abdominal cavities (@)	10/10	50/49	49/49
- Hemangioma	0/0	0/0	1/0
Parietal peritoneum (@)	10/10	50/49	49/49
- Leiomyosarcoma, metastatic	0/0	0/0	1/0
Tunica vaginalis (@)	10	50	49
- Mesothelioma, NOS	0	1	0
Multiple organs (@)	10/10	50/49	49/49
- Liposarcoma, metastatic	1/0	0/0	0/0

No. of animals examined microscopically; @ No. of animals necropsied; NOS = not otherwise specified

In male rats, the results of the Cochran-Armitage test for positive dose-related trend on the combined incidence of islet-cell adenomas or carcinomas of the pancreas (pooled controls 2/92 [2%], matched controls 0/9, low-dose 1/47 [2%], high-dose 6/45 [13%]) is significant, using either the pooled ($p = 0.008$) or matched ($p = 0.033$) controls [see Table above]. The result of the Fisher exact test comparing the incidence in the high-dose group with that in the pooled controls was also significant ($p = 0.015$). Time-adjusted tests, eliminating animals that died before week 52 on study (pooled control 2/88 [2%], matched controls 0/9, low-dose 1/47 [2%], high-dose 6/44 [14%]) resulted in essentially the same statistics as those of the non-adjusted tests. Since, however, the spontaneous incidence of this lesion in male Osborne-Mendel rats at the performing laboratory varied from 0% to 22%, with a mean of 2%, the incidence found in the high-dose male rats cannot be clearly implicated as a treatment-induced effect.

In male rats, the results of statistical tests using the pooled-control animals on the incidences of benign thyroid tumours (follicular-cell adenomas, adenomas, or cystadenomas), malignant thyroid tumours (adenocarcinomas, cystadenocarcinomas, or papillary cystadenocarcinomas), or the combined benign and malignant follicular-cell tumours are all significant [see Table below]. In each analysis, the result of the Cochran-Armitage test is significant using the pooled controls, and the results of the Fisher exact comparisons of the incidences in any of the dosed groups with the pooled-control group show probability levels less than 0.025. The results of the Fisher exact test comparing the incidence in the matched-control group with that in each dosed group are not significant. Time-adjusted analyses on the incidences of all follicular-cell tumours, eliminating animals that died before week 52 on study (pooled controls 7/82 [9%], matched controls 1/9 [11%], low-dose 14/44 [32%], high-dose 16[1]/43 [37[1]%]), resulted in essentially the same statistics as those of the non-adjusted tests. Since, however, the spontaneous incidence of these neoplasms varied in male Osborne-Mendel rats at the performing laboratory from 0% to 43%, with a mean of 7%, the incidence found in the high-dose male rats cannot be clearly implicated as a treatment-induced effect.

[1] This number was amended due to an error in the original study document

Statistical analysis of the incidence of neoplasms of the pancreas and the thyroid in male rats that had been administered azinphos-methyl in their diet over 80 weeks

Dose group	Pooled control	Matched control	Low dose	High dose
Pancreas (islet-cells): adenoma or carcinoma	2/92 (2%) [p = 0.008#]	0/9 (0%) [p = 0.033#]	1/47 (2%) [N.S.@]	6/45 (13%) [p = 0.015@]
†Thyroid (follicular cells): benign tumours	7/86 (8%) [p = 0.002#]	1/9 (11%) [N.S.#]	10/44 (23%) [p = 0.022@]	12/43 (28%) [p = 0.004@]
‡Thyroid (follicular cells): malignant tumours	0/86 (0%) [p = 0.008#]	0/9 (0%) [N.S.#]	4/44 (9%) [p = 0.012@]	4/43 (9%) [p = 0.011@]
Thyroid (follicular cells): benign and malignant tumours	7/86 (8%) [p = < 0.001#]	1/9 (11%) [N.S.#]	14/44 (32%) [p = 0.001@]	[16]/43 (33%) [p = 0.001@]

N.S. = not significant (when p > 0.05); # Cochran-Armitage test for linear trend; @ Fisher exact test (comparison of the dosed group with the pooled control); † = adenoma, follicular cell adenoma and cystadenoma; ‡ adenocarcinoma, cystadenocarcinoma and papillary cystadenocarcinoma

Conclusion: The NOEL for dietary administration of azinphos-methyl to Osborne-Mendel rats over 80 weeks (then observed for 34-35 weeks) was < 78 ppm in males and 62.5 ppm in females [estimated to be equivalent to < 3.9 and 3.125 mg/kg bw/d respectively]. Clinical signs, decreased body weight gain, and increased mortality were observed at higher dose levels. Azinphos-methyl was not shown to be carcinogenic in female rats. In male rats, neoplasms of the thyroid follicular cells and pancreatic islet cells occurred at significant incidences when compared with pooled controls. Since, however, the incidences were within the historical control ranges, they can not be clearly implicated as a treatment-induced effect.

The study authors concluded that under the conditions of this bioassay, neoplasms of the thyroid and pancreatic islets suggest but do not provide sufficient evidence for the carcinogenicity of azinphos-methyl in male Osborne-Mendel rats.

[*Comment:* No individual animal data were provided. The low number of matched controls was a considerable deficiency in this study.]

♦ ***Schmidt WM (1987) R1582 (common name: azinphos-methyl) Study of chronic toxicity and carcinogenicity to Wistar rats (Administration in the feed for up to 2 years). Report No. 16290. Study No. T 2015169. Lab & Sponsor: Bayer AG, Toxicology Department, Wuppertal, Germany. Study duration: December 1982 - December 1984. Report date: 10 December 1987.***

Guidelines and GLP: The method employed conformed with OECD guideline 453, adopted 12 May 1981. The study is GLP compliant [40 CFR part 1 (FIFRA) and OECD principles of GLP c(81) 30 (final) Annex 2 (Paris, May 1981). This study was quality assured].

The study is considered acceptable.

Material and Methods: Groups of 60 male and 60 female 5-6 week old Wistar rats (strain: Bor:WISW (SPF Cpb), source: Winkelmann, Borchen, FRG, body weight range: 73-89 g for males and 69-91 g for females) received R1582 technical ai (batch no. 79-R-225-42, purity

87.2%) [Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, MO. USA] in the diet [Altromin® 1321 Meal (Altromin GmbH, in Lage, FRG)] (with 1% peanut oil) for 24 months at concentrations of 0, 5, 15 and 45 ppm [estimated to be equivalent to 0, 0.25, 0.75 and 2.25 mg/kg bw/d. Diet was prepared weekly with random samples analysed for active ingredient content, homogeneity and contaminants (unspecified means). Rats were acclimatised prior to the study for 7-8 days then randomly divided into groups based on body weight.] The dose levels were established on the basis of a previous 4-week range-finding study (Eiben *et al* 1983).

[One animal was housed per cage (Type II Makrolon® cages, unspecified source). Food and tap water were available *ad libitum* throughout the study. Room temperature and humidity were maintained at 22 $\pm$ 2°C and approximately 50%, respectively. A 12 h light/12 h dark cycle was maintained throughout the study. Animals were observed twice daily for clinical signs with detailed observations performed weekly with the following evaluated: external surface areas, orifices, posture, general behaviour, respiration and excretory products. Body weights were recorded initially, weekly to week 13 and then fortnightly for the remainder of the study. Food consumption per rat was recorded weekly and water consumption at week 32, 41, 51, 61, 71, 82, 91 and 101 also recorded.]

Ten [randomly selected] rats/sex/group were killed and necropsied after 12 months [with ophthalmological examinations performed on these animals and 10 males and 10 females randomly chosen from the control and 45 ppm groups at 24 months]. All animals on study, whether dying or being killed in a moribund condition during the study, or killed at scheduled sacrifice [rats were anaesthetised with diethyl ether and sacrificed by exsanguination], were subjected to a thorough gross examination [at the earliest possible time] and their tissues preserved.

[Blood and urine samples were collected at 3, 6, 12, 18 and 24 months. Blood samples were collected from either the venae caudales of fasted rats (for determination of blood glucose levels) or from the retro-orbital venous plexus of fed rats that had been anaesthetised with ether. Fasted urine samples were collected from rats that had been allowed drinking water *ad libitum* overnight a few days prior to blood collection. Plasma and RBC ChE levels were measured at 1, 3, 6, 12, 18 and 24 months in 10 rats/group. Brain ChE levels were measured in animals sacrificed during the interim necropsy (12 mo) and at 24 months in 10 rats/group.

The following haematology parameters were analysed: differential leukocyte count, RBC morphology, RBC, Hb, Hct, WBC, MCH, MCHC, MCV and thrombocyte count. The following clinical chemistry parameters were analysed: AP, AST, ALT, glucose, bilirubin, creatinine, total protein, urea, triglycerides, inorganic P, Ca, K, Na and Cl. The following urinary parameters were analysed: bilirubin, urobilinogen, sediment, specific gravity, and volume. The following organs were weighed during the 12 and 24 necropsies: brain, testes, liver, kidneys and adrenals. The following organs/tissues were histopathologically examined: adrenals, aorta, bone marrow, brain, caecum, colon, duodenum, epididymis, oesophagus, eyes, femur, heart, ileum, jejunum, kidneys, lungs, liver, lymph nodes (mesenteric and cervical), ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spleen, sternum, stomach, testes, thymus, thyroid, trachea, urinary bladder and uterus.]

Statistical methods: Intergroup differences by U-test of Mann and Whitney and Wilcoxon, at the significance level α = 5% and α = 1% (two-tailed). In the case of remarkable differences,

Fisher's exact test was used at the same levels of significance. Neoplastic lesions were evaluated according to Peto *et al* (1980) using the test for positive trend with respect to dose rates.

Findings

[*Dietary analysis:* Analysis of the azinphos-methyl content of the diet indicated that the measured levels of 5.6, 15.3 and 47.0 ppm complied with the target levels of 5, 15 and 45 ppm. The azinphos-methyl content was homogenous within a tolerance of ± 10% relative to the mean. The dietary content of azinphos-methyl was also stable for 10 days when a tolerance range of ± 20% of the nominal concentration was taken into consideration.]

General examinations: The average doses ingested at 5, 15 and 45 ppm were equal to 0.25, 0.75 and 2.33 mg/kg bw/d for males and 0.31, 0.96 and 3.11 mg/kg bw/d for females, respectively.

Specific clinical signs were not observed, but animals (particularly females) receiving 45 ppm exhibited a higher incidence of alopecia than the controls [see Table below]. Mortality was unaffected by treatment at all dose levels [see Table below]. The feed consumption of females receiving 45 ppm was slightly increased (about 10%), but there was no effect on the water consumption of any group. The body weights of males receiving 45 ppm were markedly lower [than the control; $p < 0.01$] during the entire period of the study (up to approximately 10%) [see Table below]. [A Statistically significant decrease in body weight occurred at 5 and 15 ppm. The magnitude of this decrease was small and there was no strong dose-response relationship, and as such these findings were not to considered to be related to treatment. There was no treatment-related effect on the incidence or location of palpable masses.]

Effect of dietary administration of azinphos-methyl over 2 years on mortality, body weight, food consumption and clinical signs in rats

Dose level (ppm) **Sex**	**0** **M/F**	**5** **M/F**	**15** **M/F**	**45** **M/F**
[n	60/60	60/60	60/60	60/60]
Mortality (%), 0-24 mo	18/22	14/20	12/26	14/26
Alopecia (%), 0-24 mo	13/30	7/37	8/43	25/83
Feed intake (g/kg bw/d), 0-24 mo	49.0/61.7	50.1/62.3	49.8/63.7	51.7/69.0
Body weight (g),12 mo	401/242	389*/239	387*/239	367**/229**
Body weight (g), 24 mo	429/274	421/275	424/279	407**/273

* $p < 0.05$; ** $p < 0.01$

Clinical laboratory tests: Clinical chemistry, haematology and urinalysis at 3, 6, 12, 18 and 24 months revealed no evidence of any treatment-related impairment or effect on functions of the organs and metabolism, or of any impairment of the blood or haemapoietic tissues, with the exception of ChE [see Table below]. At 45 ppm, there was [biologically and statistically] marked inhibition of ChE in plasma and RBC (at 1, 3, 6, 12, 18 and 24 months) and in the brain (at 12 and 24 months). [There was no perturbation in brain ChE in high-dose males at 1 yr.] At 15 ppm, ChE activity in RBC was [significantly] decreased in both sexes at most intervals whereas ChE activity in plasma and brain was decreased significantly in females only [at 2 years]. No treatment-related changes occurred at 5 ppm. [There was a statistically

significant increase in brain ChE activity at 5 and 15 ppm at 1 yr but in the absence of a dose-response effect this effect was not considered to be treatment-related. At 5 ppm plasma and RBC ChE activities were, on occasion, reduced compared with the control but these changes were not considered toxicologically significant.]

Effect of dietary administration of azinphos-methyl over 2 years on ChE activity (% of control) in rats

Dose level (ppm)	0	5	15	45
Sex	**M/F**	**M/F**	**M/F**	**M/F**
[Plasma, 1 mo]	[100/100]	[93/88]	[95/88]	[62**/44**]
Plasma, 3 mo	100/100	91/88	102/65**	60**/35**
[Plasma, 6 mo]	[100/100]	[88/92]]	[95/71*]	[57**/34**]
Plasma, 1 yr	100/100	84/90	87/65**	54**/33**
[Plasma, 1.5 yr]	[100/100]	[87/100]	[90/74*]	[55**/46**]
Plasma, 2 yr	100/100	113/102	88/81	51**/38**
[RBC, 1 mo]	[100/100]	[97/105]	[84**/87]	[76**/74*]
RBC, 3 mo	100/100	101/110**	88**/88**	77**/72**
[RBC, 6 mo]	[100/100]	[97/109*]	[90/86**]	[80**/77**]
RBC, 1 yr	100/100	102/101	82*/81**	73**/69**
[RBC, 1.5 yr]	[100/100]	[96/94]	[83**/80**]	[73**/73**]
RBC, 2 yr	100/100	88**/98	78**/84**	63**/71**
Brain, 1 yr	100/100	130**/112	137**/90	109/50**
Brain, 2 yr	100/100	117/102	112/79**	68**/45**

* $p < 0.05$; ** $p < 0.01$

Pathological examinations: Gross pathology and organ weight analysis revealed no treatment-related changes after 12 and 24 months of treatment. Histopathological examination of animals from the control and treated groups revealed predominantly inflammatory or degenerative changes after 12 and 24 months that were typical spontaneous lesions of conventionally housed rats of the age and strain employed. The nature, incidence and distribution of these non-neoplastic lesions did not suggest an effect of treatment.

The nature, incidence and time of appearance of benign, malignant and multiple neoplastic changes in all test groups showed only a slight variation, and there was no shift in the normal spectrum of neoplasms in any of the R1582-treated groups [see Tables below]. Thus, there was no indication of a carcinogenic effect.

Summary of the incidence of neoplasms in rats that had been administered azinphos-methyl in their diet up to 2 years

Dose level (ppm)	0	5	15	45
Sex	**M/F**	**M/F**	**M/F**	**M/F**
Animals examined (no.)	50/50	49/50	50/50	50/49
Total neoplasms (no.)	40/48	38/50	26/42	38/41
Benign neoplasms (no.)	34/40	30/40	20/34	33/33
Malignant neoplasms (no.)	6/8	8/10	6/8	5/8
Animals with neoplasms (no.)	28/34	28/34	20/32	23/29
Animals with multiple neoplasms (no.)	7/10	6/13	4/9	11/9

Incidence of neoplasms in rats that had been administered azinphos-methyl in their diet up to 2 years

Dose level (ppm) Sex		0 M/F	5 M/F	15 M/F	45 M/F
Brain (#)		40/39	39/40	40/39	38/38
- Granular cell tumour	(b)	0/0	0/0	1/0	1/0
- Sarcoma, unclassified	(m)	1/0	0/0	0/0	0/0
- Neurinoma	(m)	0/0	1/0	0/0	0/0
- Astrocytoma	(m)	0/0	2/0	0/0	0/0
- Schwannoma	(m)	0/0	1/0	0/0	0/0
Eyes (#)		50/47	47/49	47/48	49/46
- Iridic leiomyoma	(b)	0/1	0/0	0/0	1/0
- Neurinoma	(b)	0/0	0/0	0/0	1/1
Lungs (#)		50/49	49/50	50/49	48/48
- Metastasis/carcinoma	(m)	1/0	0/4	0/1	0/0
- Metastasis/sarcoma	(m)	0/0	1/0	0/0	0/0
Pituitary gland (#)		48/49	48/47	50/48	47/47
- Adenoma	(b)	13/15	9/18	4/19	7/15
Adrenal glands (#)		50/49	48/50	50/48	48/48
- Pheochromocytoma	(b)	6/1	3/1	3/2	7/0
- Pheochromocytoma	(m)	0/0	1/0	1/0	0/0
- Ganglioneuroma	(b)	0/0	0/0	0/1	0/0
Thyroid gland (#)		49/49	48/49	49/47	48/45
- C-cell adenoma	(b)	8/7	9/9	9/3	10/4
- Follicular adenoma	(b)	0/0	0/0	1/0	0/0
- Medullary carcinoma	(m)	0/0	2/0	0/0	1/0
Liver (#)		50/49	49/50	50/49	48/48
- Hepatocellular adenoma	(b)	0/1	0/0	0/1	0/0
- Hepatocellular carcinoma	(m)	0/0	0/0	0/0	1/0
- Colangiocarcinoma	(m)	0/0	0/0	0/0	1/0
Pancreas (#)		49/48	49/49	48/49	48/47
- Islet cell carcinoma	(m)	0/0	0/0	1/0	0/1
Stomach (#)		49/48	49/49	48/48	47/45
- Adenocarcinoma	(m)	0/0	0/1	0/0	0/0
Hemolymphoret. syst. (no. exam.)		50/49	49/50	50/49	48/48
- Histiocytoma	(m)	2/0	0/1	0/0	0/1
- Malignant lymphoma	(m)	0/0	0/1	1/1	0/0
Lymph nodes (#)		49/49	48/50	49/46	47/46
- Hemangioma	(b)	0/0	1/0	0/1	0/0
- Hemangioendothelioma	(m)	0/0	0/0	1/0	1/0
Spleen (#)		50/49	49/49	50/49	48/48
- Hemangioendothelioma	(m)	0/0	0/0	0/0	0/1
- Sarcoma, unclassified	(m)	0/0	0/0	1/0	0/0
Kidneys (#)		50/49	49/50	50/49	48/48
- Adenocarcinoma	(m)	0/0	0/0	0/1	0/0
Urinary bladder (#)		50/48	49/49	50/48	48/48
- Carcinoma	(m)	0/0	0/0	1/0	0/0
Testes (#)		50/-	49/-	50/-	48/-
- Leydig cell tumour	(b)	4/-	5/-	1/-	3/-
- Mesothelioma	(m)	1/-	0/-	0/-	0/-
Ovaries (#)		-/49	-/50	-/48	-/47
- Granul. theca cell tumour	(b)	-/0	-/0	-/1	-/0
Uterus (#)		-/49	-/50	-/48	-/47
- Adenocarcinoma	(m)	-/4	-/7	-/2	-/3
- Squamous cell papilloma	(b)	-/0	-/1	-/0	-/0
- Granular cell tumour	(m)	-/1	-/0	-/0	-/0

Dose level (ppm) Sex		0 M/F	5 M/F	15 M/F	45 M/F
- Endometrial polyp	(b)	-/9	-/6	-/3	-/10
- Leiomyosarcoma	(m)	-/0	-/0	-/1	-/0
Other locations (@)					
Adipose tissue (#)		0/0	0/1	0/0	0/1
- Lipoma	(b)	0/0	0/0	0/0	0/1
Body cavities (#)		0/1	0/2	0/0	1/2
- Hemangioendothelioma	(m)	0/0	0/0	0/0	1/0
Mammary glands (#)		1/8	0/6	0/12	0/13
- Fibroadenoma	(b)	0/3	0/3	0/3	0/2
- Adenocarcinoma	(m)	0/2	0/1	0/1	0/2
Skin (#)		4/9	1/5	1/5	6/5
- Lipoma	(b)	0/0	0/1	0/0	0/0
- Fibroma	(b)	0/0	0/0	0/0	1/0
- Squamous cell papilloma	(b)	0/0	0/0	0/0	1/0
- Trichoepithelioma	(b)	1/1	0/0	0/0	0/0
- Neurinoma	(m)	0/0	0/0	0/1	0/0
- Squamous cell carcinoma	(m)	1/1	0/0	0/1	0/0
Clitoral glands (#)		0/1	0/0	0/0	0/0
- Adenoma	(b)	0/1	0/0	0/0	0/0

No. of examined organs; @ tissues not systematically examined; (b) benign; (m) malignant

Conclusions: The NOEL for dietary administration of azinphos-methyl to rats over 2 years was 5 ppm, equivalent to 0.25 mg/kg bw/d in males and 0.31 mg/kg bw/d in females. A dose related depression of ChE activity [plasma, RBC and brain] was observed at 15 ppm and above, and decreased body weight gain [and body weight in males], increased food consumption and alopecia [females] occurred at 45 ppm. There was no evidence that azinphos-methyl is tumorigenic in rats [up to 45 ppm (estimated to be equivalent to 2.25 mg/kg bw/d)].

2.6.3 Dogs

Noel PRB, Mawdesley-Thomas LE, Cozens D, Kelly J & Street AE (1966) Gusathion (Bayer 17'147) chronic oral toxicity study in dogs. Report No. 1656/66/184. Lab: Huntington Research Centre, Huntington, England. Sponsor: Bayer, Germany. Study duration: 20 April 1964 – 13 July 1966. Report date: 13 July 1966.

No GLP statement or test guidelines provided. This study was not quality assured.

Aim: The aim of this study was to assess the chronic oral toxicity of Gusathion (azinphos-methyl) to dogs over 2 years.

Materials and Methods: A 10% pre-mix of Gusathion (azinphos-methyl) (purity and batch No. unspecified, Farbenfabriken Bayer A/G, Germany) in synthetic silicagel was diluted with Latz FF dog feed (batch and source unspecified) to a concentration of 1%. Further dilution in dog feed yielded dietary levels of 0, 5, 20, 50, 100, 150 and 300 ppm Gusathion (estimated to be equivalent to 0, 0.125, 0.5, 1.25, 2.5, 3.75 and 7.5 mg/kg bw/d). Analysis of the Gusathion content of the diet (by the Biologisches Institut der Farbenfabriken Bayer AG, Germany) was stated by the study authors to indicate that no decomposition of the active ingredient occurred.

Sixteen male and sixteen female vaccinated pedigree Cocker Spaniels (unspecified source), were acclimatised for approximately 2 months prior to commencement of the study. Dogs

were re-inoculated against distemper, canine hepatitis and leptospiral infections approximately 6 weeks before dosing and wormed with 3 doses of anthelmintic, piperazine and adipate. Four males and four females were placed into the following groups: Group 1 (50-300 ppm), Group 2 (20-50 ppm), Group 3 (5 ppm) and Group 4 (0 ppm - control). Group 1 animals received 50 ppm Gusathion for the first 36 weeks, 100 ppm from weeks 37-57, 150 ppm from weeks 58-84 and 300 ppm for weeks 85-105. Group 2 animals received 20 ppm Gusathion for the first 36 weeks and 50 ppm for the remainder of the study. Initial ages were unspecified while initial body weights for males and females ranged from 6637-7450 g and 5687-6800 g respectively. Animals were housed individually in kennels with each dog receiving 400 g of diet per day. Water was available *ad libitum* throughout the study with each dog also receiving 200 mL milk per day during the first 6 months of dosing. Details of laboratory conditions were unspecified (ie room temperature and humidity).

All animals were observed on 3-4 occasions per day for any abnormal occurrences. Body weights were recorded weekly. Food consumption per meal was recorded. Animals dying during the experiment were subjected to a full post-mortem examination. All remaining animals were sacrificed at the end of the study (ie 105 weeks) with an iv injection of sodium pentobarbitone. The carotid arteries were incised and each animal rapidly exsanguinated before a full post-mortem examination was performed. The following organs were weighed and macroscopically examined with the presence of any tumours recorded: brain, pituitary, heart, lungs, liver, spleen, pancreas, thymus, kidneys, uterus/prostate, thyroid, adrenals and gonads. Histopathological examination of the following organs/tissues was performed: spinal cord, eye, sciatic and optic nerves, aorta, salivary gland, oesophagus, stomach, duodenum, jejunum, ileum, colon, gall bladder, lymph nodes, skeletal muscle, skin and bones.

Urinary, haematological and clinical chemistry parameters were analysed once before dosing and after 6, 12, 24, 36, 52, 64, 76, 88 and 100 weeks. The volume and method of blood and urine collection were unspecified. The following haematological parameters were examined: platelet counts, erythrocyte sedimentation rate (ESR), PCV, Hb, RBC, WBC, differential white cell count, reticulocyte count, prothrombin index, MCHC and MCV. The following clinical chemistry parameters were analysed: plasma and RBC ChE, urea, sugar, total protein, serum AP, serum glutamic-pyruvic-transaminase (SGPT), isocitric dehydrogenase (ICD), serum bilirubin, and plasma and RBC ChE. Pre-dose plasma and RBC ChE levels were measured in 5 specimens of blood from each dog. The following urinary parameters were analysed: volume, pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigments, bile salts and urobilinogen. Additionally spun deposits were microscopically examined for epithelial cells, polymorphonuclear leukocytes, mononuclear leucocytes, RBC, organisms and abnormal constituents.

No details of statistical analyses were given although some data appeared to have been analysed using an ANOVA.

Results

Mortalities and clinical observations: A single Group 1 male died during week 94 after receiving 300 ppm Gusathion for 9 weeks. This animal lost 4.25 kg prior to death, despite maintaining a good appetite, and displayed intermittent muscular tremors, weakness of the neck musculature and was less active than the other dogs. In the week prior to death, this dog displayed signs of ataxia, myosis, vomiting, jaundice, a watery discharge from both eyes and

an increased respiratory rate. Following a thorough post-mortem examination the study authors concluded that this dog died of obstructive jaundice due to cholangitis.

Stools of all animals became semi-solid to liquid immediately following commencement of dosing. This change in stool consistency was not due to Gusathion and was likely caused by consumption of the fine powdered diet. Clinical signs were confined entirely to dogs treated with 300 ppm Gusathion (ie Group 1 animals from week 85 onwards) and included muscular tremor and weakness (in the neck and hind limbs), abnormal quietness and sitting posture. These clinical signs appeared to be more frequent in males than females (see Table below).

Effects of dietary-administered Gusathion on dogs over 2 years (n = 4 unless indicated)

Group	1 50-300 ppm		2 20-50 ppm		3 5 ppm		4 0 ppm (control)	
	♂	♀	♂	♀	♂	♀	♂	♀
Clinical Signs†								
Abnormal quietness								
Wk 86	1/4	0/4	-	-	-	-	-	-
Wk 96	2/4	0/4	-	-	-	-	-	-
Wk 104	3/4	1/4	-	-	-	-	-	-
Muscular tremor								
Wk 86	2/4	0/4	-	-	-	-	-	-
Wk 96	3/4	0/4	-	-	-	-	-	-
Wk 104	3/4	2/4	-	-	-	-	-	-
Muscular weakness (neck)								
Wk 86	0/4	0/4	-	-	-	-	-	-
Wk 96	2/4	0/4	-	-	-	-	-	-
Wk 104	2/4	0/4	-	-	-	-	-	-
Muscular weakness (hind limbs)								
Wk 86	1/4	0/4	-	-	-	-	-	-
Wk 96	1/4	0/4	-	-	-	-	-	-
Wk 104	1/4	1/4	-	-	-	-	-	-
Group mean body weight change (g)								
Wk 0-36	4200	3338	5239	3275	3463	3075	4788	2826
Wk 37-57	575	788	-338	138	700	463	188	612
Wk 58-84	625	450	900	337	687	787	912	550
Wk 85-93	-1087	-563	350	675	238	825	375	225
Wk 94-104	‡	225	2500	300	487	313	188	638
Residual food unconsumed/wk (g)								
Wk 0-36	387	1553	345	1418	409	848	401	2302
Wk 37-57	52	1485	5	1169	10	495	72	2364
Wk 58-84	34	2074	5	1322	179	720	41	3171
Wk 85-104	462	2424	33	1317	147	817	110	2923
Mean group dose level (mg/kg bw/d)								
Wk 104	9.40‡	9.83	1.51	1.49	0.15	0.15	0	0

† = signs observed at 300 ppm only; ‡ n = 3; - absence of clinical signs

There was no statistically significant difference in body weight between Gusathion-treated and control dogs, however a transient loss of body weight was observed in both males and females when the Gusathion content fed to Group 1 animals was increased to 300 ppm at week 85 (see Table above). Additionally there was a transient loss of body weight in Group 2

males from week 37-57 which coincides with an increase in dosing from 20 to 50 ppm at week 36.

Generally, Gusathion-treated dogs consumed more food than controls as shown by the lower levels of residual food left by Groups 1-3 compared to Group 4 (see Table above). The exception to this trend was Group 1 males which showed a lower food consumption at weeks 85-104 compared to the control. There was no indication by the study authors whether food consumption by Gusathion-treated animals was statistically different to the control animals. Calculation of mean group dose levels indicated that males and females were dosed with equivalent levels of Gusathion (see Table above).

Clinical chemistry: There was a clear Gusathion-related effect on both plasma and RBC ChE activity (see Table below). During the first 3 months plasma and RBC ChE activities were higher across all treatment groups compared to the control groups. The study authors explanation for this apparent elevation was that control levels fell from their pre-dose levels. From 3 months plasma ChE activities were inhibited in Group 1 animals by 16-48% with a greater effect observed toward the end of the study as the dose level was increased to 300 ppm. The plasma ChE activity of Group 2 was comparable to the control until week 52 and then showed signs of depression for the remainder of the study, however, this inhibition was not biologically significant. There was no effect of 5 ppm Gusathion on plasma ChE activity after 3 months. From 3 months, RBC ChE activity in Groups 1 and 2 was consistently lower than the control group with the effect substantially greater in Group 1 (24-74%) than Group 2 (25-45%). There was no perturbation in RBC ChE activity of Group 3. Although an ANOVA indicated a statistical difference between groups there was no further analysis to ascertain where these differences lay. There was no difference in any other clinical chemistry parameters between Gusathion-treated and control animals.

Effect of dietary-administered Gusathion on plasma and RBC ChE activities in dogs

Group n = 8, males + females	**1** **50-300 ppm**	**2** **20-50 ppm**	**3** **5 ppm**
Plasma ChE (% depression compared to control values)			
Wk 4	+47	+29	+52
Wk 12	+7	+4	+20
Wk 20	-19	-3	+11
Wk 40	-16	0	+7
Wk 60	-18	-19	+5
Wk 80	-21	-19	+5
Wk 100	-48	-16	+5
RBC ChE (% depression compared to control values)			
Wk 4	+8	+41	+42
Wk 12	+24	+12	+32
Wk 20	-41	-29	-10
Wk 40	-24	-25	-8
Wk 60	-58	-35	-13
Wk 80	-49	-39	-8
Wk 100	-74	-48	-8

Haematology and Urinalysis: There was no difference in haematology or urinary parameters between Gusathion-treated and control dogs.

Gross pathology: There was no significant difference in organ weights (expressed as a% of body weight) between Gusathion-treated and control animals. Macroscopic examination of animals at the end of the study indicated no abnormalities that could be attributed to Gusathion. Additionally no tumours were observed.

Histopathology: Histopathological examination indicated that all dogs had chronic pneumonitis which the authors indicated as being 'indigenous' to their dog colony. Evidence of splenic congestion was not observed in controls but was present in 4/8 Group 1 animals, and all animals in Groups 2 and 3 animals. This evidence conflicts with the study authors statement that the incidence of extreme splenic congestion was 'similar throughout all groups'. Furthermore, the study authors concluded that this splenic congestion was the result of administration of large amounts of pentobarbitone to terminate the animals. In females, evidence of kidney inflammation was confined entirely to the treatment groups with 3/4, 1/4 and 2/4 Group 1-3 animals affected respectively. However, it is difficult to conclude that these histopathological abnormalities were due to Gusathion as the sample sizes were small and a clear dose-response effect was not evident. There was no histopathological evidence that azinphos-methyl was carcinogenic.

Conclusions: When Cocker Spaniel dogs were fed Gusathion (azinphos-methyl) at a dietary concentration of up to 300 ppm (estimated to be equivalent to 10 mg/kg bw/d) for up to 2 years, toxicological effects included depressed plasma and RBC levels, and clinical signs such as muscle tremors and weakness, and inactivity. This study also indicated that Gusathion is non-carcinogenic as no Gusathion-related effect on tumour incidence was observed up to 50-300 ppm (estimated to be equivalent to 1.25-7.5 mg/kg bw/d). The NOEL was 5 ppm (estimated to be equivalent to 0.125 mg/kg bw/d) based on depressed plasma and RBC ChE activities at 20-50 ppm (estimated to be equivalent to 0.5-1.25 mg/kg bw/d).

Comments: A number of deficiencies in the study were noted. Of concern was the small sample sizes (n = 4). The absence of standard deviation or errors from most data made it difficult to judge the variability of some observations. Statistical analyses were inadequately described or justified. For example, an ANOVA was utilised to analyse ChE data between all groups. The subsequent F values indicated the presence or absence of statistical differences between groups but did not indicate where these differences lay. Two methods for determining ChE levels were utilised, with the method of Michel (1949) used until week 52 and then changed due to the insensitivity of this method to the method of Williams and Frawley (1957). The increased dose administered to Groups 1 and 2 in the absence of any observable acute toxicological effects did not consider any delayed or cumulative effects of the test compound.

♦ ***Worden AN, Wheldon GH, Noel PRB & Mawdesley-Thomas LE (1973) Toxicity of Gusathion for the rat and dog. Lab: Huntingdon Research Centre, Huntingdon PE18 6ES, England. Dates of experimental work: not specified in detail (start of acclimatisation: February 1964). Published in: Tox Appl Pharmacol 24:405-412.***

This published paper is a summary of data from Noel *et al* 1966 (see preceeding report).

Guidelines and GLP: The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 452. When the study was performed, GLP was not compulsory.

Main deviations from current OECD guidelines: The test procedures are not described in detail. The 8-page report (publication) is not very detailed (missing data for food consumption and body weight, for clinical, laboratory and pathological examinations; missing raw data).

The study is considered supplementary.

Material and methods: Groups of 4 male and 4 female young pure-bred Cocker spaniels (source, age and body weight not specified) were administered Gusathion (source and purity not specified) mixed with their diet over a period of two years. The initial concentrations were 0, 5, 20 or 50 ppm [estimated to be equivalent to 0, 0.125, 0.5 and 1.25 mg/kg bw/d]. The concentration in the high-dose group was increased from 50 to 100 ppm [estimated to be equivalent to 2.5 mg/kg bw/d], and that in the mid-dose group from 20 to 50 ppm [estimated to be equivalent to 1.25 mg/kg bw/d] starting at week 37. Two further increases of the test substance concentration in the high-dose group took place; one after week 57 (from 100 to 150 ppm) [estimated to be equivalent to 3.75 mg/kg bw/d] and the other after week 84 (from 150 to 300 ppm) [estimated to be equivalent to 7.5 mg/kg bw/d]. Blood and urine samples were taken at regular intervals [pre-dose, 6, 12, 24, 36, 52, 64, 76, 88 and 100 weeks] and examined almost according to OECD guideline 452 [for haematology, serum AP, SGPT, ICD, plasma bilirubin, urea, total reducing substances, total protein, electrophoretic pattern and urinalysis. Plasma and RBC ChE levels were measured pre-dose and at monthly intervals thereafter]. The pathological examinations were not described.

Findings: The average doses ingested at 5, 20-50 and 50-300 ppm were 0.15-0.24, 0.72-1.77 and 1.64-8.65 mg/kg bw/d for males and 0.16-0.26, 0.73-1.79 and 2.06-9.42 mg/kg bw/d for females, respectively.

In the high-dose group, decreased motility, tremor of the body musculature, muscular weakness and abnormal sitting posture were registered starting at week 85 (after increase from 150 to 300 ppm). A slight body weight loss was observed in 3 animals of this group, and one male dog died as a result of cholangitis.

A depression of plasma ChE activity was observed in the mid and high-dose groups, starting at week 37. Erythrocyte ChE activity was depressed to about 50% of the control values at 100 ppm and to about 25% of the control values at 150 ppm.

The results of the other examinations conducted (haematology, clinical biochemistry, organ weights, and histopathology including that of the central and peripheral nervous systems) showed no treatment-related changes.

Conclusion: The NOEL for dietary administration of azinphos-methyl to dogs over 2 years was 5 ppm, equivalent to 0.15-0.24 mg/kg bw/d in males and 0.16-0.26 mg/kg bw/d in females. A depression of plasma and erythrocyte ChE activity was observed at 50 ppm and above, and clinical signs occurred at 300 ppm [estimated to be equivalent to 7.5 mg/kg bw/d].

Allen TR, Frei Th, Janiak T, Luetkemeier H, Vogel O, Biedermann K & Wilson J (1990) 52-week oral toxicity (feeding) study with Azinphos-methyl (E 1582) in the dog. Report No. R 5064. Lab: RCC Research and Consulting Company AG, Itingen, Switzerland. Sponsor: Bayer AG, Fachbereich Toxikologie, Wuppertal, Germany. Study duration: 29 March 1988 – 4 April 1989. Report date: 31 May 1990.

GLP: Conforms to OECD (No. 452, 1981) and EPA (Series 83-1, P.B. 86–108958, November 1984) GLP principles/standards. This study was quality assured.

Aim: The aim of this study was to assess the toxicity of azinphos-methyl that had been incorporated into the diet up to 125 ppm, and fed to Beagle dogs for 52 weeks.

Materials and Methods: Azinphos-methyl (91.9% purity, other components unspecified. Batch No. 233 896 032, unspecified source) was dissolved in acetone (unspecified volume and source) and mixed with granulated feed [Kliba (Klingentalmuhle AG, Switzerland)] to yield dietary levels of 0, 5, 25 and 125 ppm (estimated to be equivalent to 0, 0.125, 0.625 and 3.125 mg/kg bw/d). The diet was prepared fortnightly and the stability, homogeneity and content of azinphos-methyl was determined prior to dosing and then at 3 monthly intervals by HPLC. The feed was also analysed for chemical contaminants using AAS, GC, GC-MS and HPLC.

Sixteen male and sixteen female pure-bred Beagle dogs (Laboratory Research Enterprises Inc., Kalamazoo, MI, USA), that had been dewormed and vaccinated against distemper, leptospirosis, contagious hepatitis, rabies, bordetella, parainfluenza and parvovirosis, were acclimatised for at least 12 weeks prior to commencement of the study. The initial age and body weight of dogs was 4-4.5 months and 5.4-8.5 kg respectively. Four males and four females were placed into the following groups: Group 1 (0 ppm - control), Group 2 (5 ppm), Group 3 (25 ppm) and Group 4 (125 ppm). Diet and tap water were available *ad libitum* throughout the study. The tap water was analysed for biological and chemical contaminants at least yearly. Dogs were housed individually in kennels. Temperature and humidity were maintained at 20 $\pm$ 3°C and 40-70% respectively with a 12 h light/12 h dark cycle maintained throughout the study. At least 8 h of music was played during the light period. There were 20 air changes per h.

Dogs were observed twice daily for mortalities and clinical signs. The body weight of each dog was recorded weekly. Food consumption was recorded daily. Ophthalmoscopic examination and hearing tests were performed on all animals prior to dosing and then at 13, 26 and 52 weeks. Blood samples (unspecified volumes) were collected from the jugular vein of each dog between 7-9.30 am prior to dosing and then at 4, 13, 26 and 52 weeks. Urine (unspecified volume) was collected using a catheter from each dog prior to dosing and then at 4, 13, 26 and 52 weeks. The following haematology parameters were analysed: RBC, Hg, Hct, MCV, MCH, MCHC, platelet count, reticulocyte count, nucleated erythrocytes normoblasts, Heinz bodies, methohaemoglobin, WBC, differential WBC, RBC morphology, thromboplastin time and partial thromboplastin time. The following clinical chemistry parameters were analysed: glucose, urea, creatinine, total bilirubin, total lipids, total cholesterol, triglycerides, AST, ALT, LDH, creatinine kinase, AP, GGT, ornithine carbamyl-transferase, Ca, P, Na, K, Cl, total protein, total triiodothyronine (T3), total thyroxine (T4), protein electrophoresis, plasma ChE and RBC ChE.

Liver samples were collected from each animal at necropsy for the determination of cytochrome P-450, N-demethylase and O-demethylase. Samples of brain were also collected at necropsy for ChE determination. The following urinary parameters were analysed: colour, appearance, pH, protein, glucose, ketone, bilirubin, blood, urobilinogen and sediment.

Dogs surviving the duration of the study (52 weeks) were administered an iv injection of Narcoren (Iffa Merieux, Laupheim, Germany) and then killed by exsanguination. All animals were subjected to a thorough post-mortem examination with the following organs weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, prostate, gland, spleen, testes and para/thyroid. A histopathological examination was performed on the following organs/tissue: adrenals, aorta, bone (femur, sternum, marrow), brain (cerebrum, cerebellum, medulla oblongata/pons), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (retropharyngeal, mesenteric), mammary gland, optic nerves, ovaries, pancreas, pituitary gland, prostate, rectum, spinal cord (cervical, mid thoracic, lumbar), spleen, stomach, testes, thymus, para/thyroid, tongue, trachea, urinary bladder, uterus, vagina and all gross lesions.

Univariate 1-way ANOVA was used to assess statistical differences between the groups. Normally distributed data was analysed using a Dunnett Test to ascertain differences between treated groups and the control. Abnormally distributed data was analysed using the Steel-test

Results

Dietary analysis: The mean concentrations of azinphos-methyl in the diet was 90.7-101.5% of the target concentration. The homogeneity of azinphos-methyl in the diet varied from-5% to + 4% of the mean concentration. Azinphos-methyl was stable in dog feed over a period of 14 days at room temperature. The average dose ingested at 5, 25 and 125 ppm was equal to 0.15, 0.0.69 and 3.84 mg/kg bw/d respectively for males and 0.16, 0.78 and 4.33 mg/kg bw/d respectively for females.

Mortalities and clinical observations: There were no deaths throughout the study. There appeared to be an increase in the incidence of vomiting, vomiting mucus, diarrhoea and faeces with mucus, in Group 3 and 4 males (see Table below) although a clear dose-response trend was not evident as Group 4 showed fewer clinical signs than Group 3. Generally, there was no increase in the incidence of treatment-related effects observed in females due to the high incidence of clinical signs displayed by the control group. The large increase in the incidence of diarrhoea in Group 4 females can be attributed to a single dog with a chronic case of diarrhoea.

There was no difference in food consumption between azinphos-methyl-treated and control dogs. Although there was no statistical difference in body weight between treated and control dogs the% increase in mean body weight to day 367 of Group 4 males was lower than the control group (see Table below). Performance of hearing tests on all dogs indicated no difference between treated and control animals. There were no treatment-related effects on ophthalmoscopy parameters.

Clinical chemistry: Group 2 males had elevated urea levels at week 26 and 52 ($p < 0.05$) and elevated AST, LDH and creatinine kinase levels at week 52 ($p < 0.05$). Group 4 males showed depressed albumin ($p < 0.05$) at week 13, and an elevation in β2-globulin ($p < 0.05$) at week 26. None of these observations, with the exception of LDH, were considered to be

biologically significant as they fell within the 95% confidence limits for age and sex-matched untreated Beagle dogs. Although the elevation in LDH levels in Group 2 males at week 52 was above the range for age-matched untreated Beagles, the absence of an effect in the two higher dose groups suggested that it was not treatment-related.

Effect of dietary-administered azinphos-methyl on Beagle dogs over 52 weeks (n = 4)

Group	1 0 ppm		2 5 ppm		3 25 ppm		4 125 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Clinical Signs† Total days observed (No. dogs affected)								
Vomiting	0(0)	7(4)	6(1)	4(2)	20(3)	9(3)	0(0)	10(3)
Vomiting of mucus	1(1)	9(4)	6(3)	2(1)	17(4)	10(2)	1(1)	11(1)
Diarrhoea	8(4)	54(3)	5(3)	39(4)	68(4)	44(4)	30(3)	264(4)
Faeces with mucus	1(1)	40(4)	0(0)	8(4)	22(4)	17(2)	30(3)	52(4)
% increase in bw (d 0 – d 365)	15.2	19.6	14.5	16.1	17.6	14.7	6.3	15.2
RBC ChE (µmol-SH/mL)								
Pre-test	2.29	2.82	2.67	2.99	2.52	2.97	2.99	2.74
Wk 4	2.18	2.69	2.37	2.39	1.71	2.14	0.75**	0.38**
Wk 13	1.99	2.46	1.84	2.07	1.20**	1.41**	0.26**	0.19**
Wk 26	2.57	3.27	2.37	2.57	1.75	2.03**	0.32**	0.28**
Wk 52	2.86	3.36	3.01	2.86	2.09	2.20*	0.41**	0.47**
RBC ChE‡ (% inhibition)								
Wk 4	0	0	-9	11	22	2	66**	86**
Wk 13	0	0	8	16	40**	43**	87**	92**
Wk 26	0	0	8	21	32	38**	88**	91**
Wk 52	0	0	-5	15	27	35*	86**	86**
Plasma ChE (µmol-SH/mL)								
Pre-test	6.65	5.68	6.06	6.65	6.97	5.36	6.49	5.31
Wk 4	7.51	6.70	6.70	7.88	6.60	5.74	4.72	3.22*
Wk 13	6.60	5.85	5.74	5.95	5.58	4.83	3.11**	2.47**
Wk 26	8.26	8.15	7.08	8.96	7.24	5.47	3.49**	3.54**
Wk 52	8.26	9.71	7.35	8.53	7.24	6.76	3.86**	4.56*
Plasma ChE‡ (% inhibition)								
Wk 4	0	0	11	-18	12	14	37	52*
Wk 13	0	0	13	-2	15	17	53**	58**
Wk 26	0	0	14	-10	12	33	58**	57**
Wk 52	0	0	11	12	12	30	53**	53*
Brain ChE (µmol-SH/mL)								
Wk 52	6.26	6.64	6.21	6.71	5.61	6.55	4.60**	5.33*
Brain ChE (% inhibition)	0	0	1	1	10	1	27**	20*
Cytochrome P-450 (nmol/g)	12.2	15.0	13.6	13.8	14.2	13.8	17.0*	17.2
Spleen:body weight								
Mean	0.63	0.39	0.47	0.30	0.29*	0.37	0.26*	0.37
SD	0.10	0.23	0.28	0.06	0.07	0.23	0.11	0.16
Spleen:brain weight								
Mean	81.01	47.68	55.16	33.11	34.37*	41.86	29.64*	40.70

Group	1 0 ppm		2 5 ppm		3 25 ppm		4 125 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
SD	13.59	26.18	34.14	7.65	7.79	27.84	16.62	17.09
Liver:brain								
Mean	455.2	409.0	353.4**	378.5	373.2*	378.0	369.6*	415.2
SD	24.3	38.4	66.4	33.3	15.6	87.9	28.9	26.4

† summarised from raw data by reviewing toxicologist; ‡ calculated by reviewing toxicologist; * statistically different to the control at $p < 0.05$; ** statistically different to the control at $p < 0.01$.

Group 2 females had depressed T3 and A1-globulin at week 13; depressed T3 and total protein, and elevated LDH at week 26; elevated γ-globulin and depressed albumin at week 52 (all $p < 0.05$ compared to control). Group 3 females showed depressed β2-globulin at week 13, depressed T3 at week 26, and depressed serum-β, β1 and β2-globulin at week 52 (all $p < 0.05$ compared to control). Group 4 females exhibited a depression in total protein and ornithine carbamyl-transferase at week 26 and 52 respectively ($p < 0.05$). None of these observations were considered to be treatment-related as they fell within the 95% confidence limits for age and sex-matched untreated Beagle dogs.

There was a clear treatment-related effect on RBC, plasma and brain ChE activity (see Table above). While there was no treatment-related effect on ChE activity in Group 2 animals, a transient depression (40%; $p < 0.01$) in RBC ChE activity was observed in Group 3 males at week 13, and Group 3 females showed consistently depressed RBC ChE activity (35-43%; $p < 0.01$-0.05) from week 13. A 30-33% inhibition in plasma ChE occurred in Group 3 females in the later half of the study but this was not statistically significant. In Group 4 males RBC and plasma ChE activities were significantly depressed (66-88 and 53-58% respectively; $p < 0.01$) from week 4 and 13 respectively, while the RBC and plasma ChE activity of Group 4 females was significantly depressed (86-92 and 52-57% respectively; $p < 0.01$-0.05) from week 4. Brain ChE activity in Group 4 animals was also significantly lower than the control (27% and $p < 0.01$ for males; 20% and $p < 0.05$ for females).

Cytochrome P-450 activity was elevated in Group 4 animals but only males were determined to have a statistically higher acticity ($p < 0.05$) than their controls (see previous Table).

Haematology: There were no haematological abnormalities that could be attributed to the administration of azinphos-methyl. Group 4 males showed an elevated reticulocyte count at week 13 ($p < 0.05$), a depressed methhaemaoglobin concentration at week 26 and an elevated platelet count at week 52 ($p < 0.05$). Group 4 females showed a depressed prothrombin time ($p < 0.05$) at week 13, and Group 2 females showed a depressed RBC, Hb and Hct ($p < 0.05$) at week 26. None of these observations were considered to be toxicologically significant as they fell within the 95% confidence limits for age and sex-matched untreated Beagle dogs. Group 4 females also showed an elevated MCV ($p < 0.05$) at week 13 but this reflected a difference that was detected at pre-dose.

Urinalysis: There were no treatment-related effects on urinary parameters.

Gross pathology: Although there was no effect on absolute organ weights, there was evidence of a treatment-related effect on spleen:body weight and spleen:brain weight ratios in treated males (see Table above). The study authors concluded that the reduction in spleen weight in males could be attributed to a higher proportion of control males showing splenic congestion. Treated males also had statistically lower liver:brain weight ratios ($p < 0.01$-0.05) with Group

4 males also having a statistically lower kidney:brain weight ratio ($p < 0.05$) compared to the control group. There was no difference in organ weights, organ:body weight and organ:brain weight ratios between treated and control females. All other pathology findings were within normal range of spontaneous background alterations which may be encounted in this age and breed of dog. There was no macrosopic evidence of tumour formation.

Histopathology: There was no evidence of any abnormal histopathology in azinphos-methyl-treated dogs. There was no histopathological evidence that azinphos-methyl was carcinogenic.

Conclusions: In this study azinphos-methyl was administered to Beagle dogs at dietary concentrations up to 125 ppm (estimated to be equivalent to 3.125 mg/kg bw/d). Toxicological effects included a depression in RBC, plasma and brain ChE activity, an increase in cytochrome P-450 activity and clinical signs in males (diarrhoea, vomiting mucus, faeces with mucus). The NOEL was 5 ppm (estimated to be equivalent to 0.125 mg/kg bw/d) based on a depression in RBC and plasma ChE activity at 25 ppm (estimated to be equivalent to 0.625 mg/kg bw/d). Azinphos-methyl was not tumorigenic in dogs fed diets containing up to 125 ppm (estimated to be equivalent to 3.125 mg/kg bw/d).

Comments: The low sample size of each group (n = 4) made it difficult to attribute certain observations to the administration of azinphos-methyl. For example, although it appeared that the incidence of diarrhoea was increased in Group 3 and 4 males, 1 Group 4 male showed no clinical signs, suggesting a large degree of biological variability between individuals within the same group.

2.7 REPRODUCTIVE TOXICITY

2.7.1 Mice

Root M, Vesselinovitch D, Meskauskas J & Doull (1965) Effect of Guthion in the diet on the reproduction & lactation of mice. Report No. 16963 Lab: Department of Pharmacology, University of Chicago, Chicago, IL, USA. Sponsor: Chemagro Corp., Kansas City, MO, USA. Study duration: unspecified. Report date: 15 September 1965.

GLP and Test Guidelines: No GLP or test guidelines provided. This study was not quality assured.

Aim: To evaluate the effect of Guthion on the fertility and pre/post-natal development in mice.

Materials and Methods: Guthion (azinphos-methyl) (80% purity, other components unspecified, control no. 803. Chemagre Corp. Kansas City, MO, USA) in Cerelose 2001 (dextrose sugar, unspecified source) was added to ground laboratory food (Rockland Mouse Breeder Diet, unspecified source) to yield dietary levels of 0, 5, 10, 25 and 50 ppm (estimated to be equivalent to 0, 0.75, 1.5, 3.75 and 7.5 mg/kg bw/d). Diets were prepared weekly or more often if necessary. There was no analysis of the homogeneity, stability and ai content of the diet.

Fifty male and two hundred female Carworth Farms CF_1 mice (unspecified source), between 8–10 weeks of age and unspecified body weight, were housed (cage types unspecified) in

groups of one male to four females for 10 days (ie 2 oestrus cycles). Females were then separated and observed for 4 weeks, with any non-pregnant mice rebred or discarded. Breeding and litter size records of individual animals were used to select the following 5 groups consisting of 6 males and 24 females of proven fertility: Group 1 (0 ppm - control), Group 2 (5 ppm), Group 3 (10 ppm), Group 4 (25 ppm) and Group 5 (50 ppm). Each of these groups were then fed test or control diets for 30 days and then mated. During mating, each male was housed with 4 females for 10 days, with pregnant females housed individually thereafter. Pregnant females were kept on the experimental diets until the young were weaned, with the young continued on the experimental diets until mating at 8-10 weeks of age. This procedure was repeated using the F_{1b} mice to obtain two litters of F_2 animals and again repeated to obtain 30-day old male and female mice of the F_{3b} generation. Diet and water were available *ad libitum* throughout the study. Details of laboratory conditions (ie temperature, humidity and light/dark cycle) were unspecified.

Five male and five female F_{3b} mice from each group were sacrificed under ether anaesthesia and a gross pathological examination performed. The following organs/tissues were weighed: heart, kidney, spleen, testis, urinary bladder and thymus. The above organs/tissue and the following were examined histopathologically: adrenals, bone and marrow (sternum), colon, duodenum, ileum and lymph nodes (mesenteric).

No details of the types and frequency of clinical observations were specified.

No statistical analyses were performed on the data.

Results

Clinical signs and fertility: Deaths were observed in F_0 females in Groups 1, 2, 3 and 5 prior to mating (see Table below). The high incidence of deaths in Group 5 F_0 females was likely due to Guthion as an unspecified number of deaths also occurred in Group 5 F_0 males. However, the absence of treatment-related mortality at 25 ppm, although there were apparently comp[ond-related deaths at 10 ppm, was not explained. Due to the apparent toxicity of 50 ppm Guthion no further investigation was performed at this dietary level. All F_0 females that were mated became pregnant except for 2/15 Group 3 mice, with all except 3/22 Group 1 and 1/13 Group 3 mice delivering (see Table below). There was no difference in the average litter size at birth between Guthion-treated and control mice (see Table below). There was no treatment-related effect on the average litter size at birth. In contrast, the average litter size at weaning of Group 5 mice was substantially lower than all other groups (see Table below) probably due to the lack of maternal care or reduced lactation. That is, these data suggested that 50 ppm was maternally toxic but not toxic to foetuses.

Effect of Guthion on reproduction in CF_1 mice

Group	**1** **0 ppm**	**2** **5 ppm**	**3** **10 ppm**	**4** **25 ppm**	**5** **50 ppm**
F_0					
Deaths (♀)	2/24	4/24	9/24	0/24	15/24
No. Pregnant	22/22	20/20	13/15	24/24	9/9
No. Litters	19	20	12	24	9
Mean litter size (d 0)	9.8	9.9	9.8	10.0	9.4
Mean litter size (d 21)	5.7	6.8	6.8	7.0	1.9
F_1					

Deaths (♀)	0/24		0/24		1/24		0/24		-
No. Pregnant	24/24		24/24		24/24		24/24		-
No. Litters	24		24		23		23		-
Mean litter size (d 0)	8.0		8.0		10.4		9.3		-
Mean litter size (d 21)	6.0		7.0		7.9		7.3		-
F_2									
Deaths (♀)	11/24		6/24		0/35		0/36		-
No. Pregnant	13/13		18/18		35/35		35/36		-
No. Litters	10		17		34		34		-
Mean litter size (d 0)	10.7		11.1		9.5		9.4		-
Mean litter size (d 21)	7.1		7.5		6.1		7.0		-
Relative F_{3b} Lung Weight (g)	♂ 1187	♀ 1056	♂ 1112	♀ 1068	♂ 1289	♀ 1321	♂ 750	♀ 832	-
Relative F_3 Testis Weight (g)	♂ 323		♂ 483		♂ 518		♂ 531		-

There was no effect of Guthion on the fertility and mortality of F_1 mice (see Table above). In the F_2 generation, there was a high incidence of mortality in Group 1 and 2 females which proceeded observations of severe diarrhoea in these animals. This observation suggested that the study animals may have been of poor health unrelated to Guthion administration. In anticipation of a similar occurrence in the other groups, additional animals were conscripted but as deaths did not follow, Groups 3-5 had larger sample sizes than Groups 1 and 2. There did not appear to be an effect of Guthion on the fertility of F_2 mice (see Table above). Calculation of fertility, gestation and lactation indices by the study authors confirmed that Guthion up to 25 ppm had no effect on fertility.

Gross Pathology: The relative lung weight of Group 4 F_{3b} offspring was lower than all other groups (see Table above). It was unclear whether this result was significant as no statistical analysis was performed and the next lowest dose appeared to show an increase in relative lung weight. There appeared to be a dose-response trend with regard to an increase in relative testis weight in Guthion-treated F_{3b} males (see Table above), however, the study authors indicated that the average testis weight of Group 1 was less than two thirds of that usually obtained with age-matched controls. There was an incidental increase in relative spleen weight in Group 2 but this was not considered to be related to the test compound as there were no effects at the 2 higher dose levels. No body weight data was given. The study authors indicated that there was large variability in the organ weight data but no evidence was given to support this statement.

Gross pathology and histopathology: There were no gross pathological or histopathological abnormalities observed in F_{3b} offspring that could be attributed to the administration of Guthion.

Conclusions: Azinphos-methyl was toxic as shown by the high incidence of mortalities in F_0 animals at 50 ppm and substantially lower survival in their offspring. There was no evidence that azinphos-methyl up to 50 ppm (estimated to be equivalent to 7.5 mg/kg bw/d) had any effect on the fertility of CF_1 mice. The NOEL was 50 ppm (estimated to be equivalent to 7.5 mg/kg bw/d) for reproductive toxicity based on the absence of any effect on fertility at this dose. The NOEL was 25 ppm (estimated to be equivalent to 3.75 mg/kg bw/d) for parental and post-natal toxicity based on mortality in both adults and pups at 50 ppm (estimated to be equivalent to 7.5 mg/kg bw/d).

Comments: The study and report had a number of deficiencies and deviations from OECD test guidelines 415 and 416 (adopted 26 May 1983). There was no analysis of the homogeneity, stability and ai content of the diet. 1:4 male:female matings were employed whilst the OECD guidelines suggest 1:1 or 1:2 matings. The sexes of the pups were not recorded. Apart from recording mortalities, there appeared to be no clinical observations made such as mating behaviour. Food consumption was not measured. Although relative organ weights were given no body weight or individual animal data were provided. Histopathological examination was performed on F_{3b} offspring only, not on all animals that died during the study. More detailed macro/microscopic examination of the reproductive organs of mice would have been useful. There was no measurement of ChE activity. No statistical analyses were performed on the data. No indication of the variability of the data was included such as standard deviations or errors.

2.7.2 Rats

♦ ***Eiben R & Janda B (1987) R1582 (common name: azinphos-methyl, the active ingredient of Guthion): Two-generation study on rats. Report No. R3956. Study No. T 6006415. Lab & Sponsor: Institute of Toxicology, Industrial Chemicals, Toxicology Department, Bayer AG, Wuppertal, Germany. Study duration: September 1982 - February 1984. Report date: 10 March 1987.***

Guidelines and GLP: The test method employed conformed with OECD guideline 416, adopted 26 May 1983. The study is GLP compliant [40 CFR Part 160 (2 May 1984)]. This study was quality assured.

The study is considered acceptable.

Material and methods: Groups of 12 male and 24 female 5-6 week old Wistar rats [acclimatised for 6 d] (strain: Bor:WISW (SPF-Cpb); source, Winkelmann, Borchen, FRG; body weight range: 78-87 g) received R1582 (batch no. 79/R225/42, purity 87.2%) admixed in the diet [Altromin 1321 and Ssniff R pulverised feed (1:1) (unspecified batch and source)] at concentrations of 0, 5, 15 and 45 ppm [equivalent to 0, 0.33–0.42, 1.02–1.22 and 3.46–7.37 mg/kg bw/d for males and 0, 0.48–0.67, 1.48–2.02 and 4.84–10.27 mg/kg bw/d for females] throughout the entire test period, including mating, gestation, and raising of the pups (2 generations, 2 litters per generation). The dose levels were selected based on the results of a 4-week toxicity study (Eiben *et al* 1983). [HPLC was used to determine the stability and homogeneity of azinphos-methyl in the diet prior to the study, and the ai content at regular intervals throughout the study.

The 12 males and 24 females were randomly assigned to the following test groups based on body weight: Group 1 (0 ppm – control), Group 2 (5 ppm), Group 3 (15 ppm) and Group 4 (45 ppm). A summary of the experimental design is provided in Table below.]

Summary of experimental design

Event	Period
Pre-mating exposure F_0 ♂ and ♀	100 d
1st mating	20 d
Gestation	22 d
Lactation of F_{1A} pups	28d

Sacrifice of F_{1A} pups	
Waiting period	14 d
2nd mating F_0	20 d
Gestation	22 d
Raising F_{1B} pups Sacrifice F_0 adults after weaning Reduction to 8 pups/litter	100 d
1st mating F_{1B}	20 d
Gestation	22 d
Lactation of F_{2A} pups Sacrifice of F_{2A} pups	28 d
Waiting period	14 d
2nd mating F_{1B}	20 d
Gestation	22 d
Lactation of F_{2B} pups Sacrifice F_{1B} and F_{2B} adults	28 d

During the 3-week mating period, two females were caged together with one male. The male rats were interchanged each week so that essentially each female was together with 3 different males. [Rats were housed in Type II Makrolon cages (unspecified source) prior to and after mating. Room temperature and humidity were maintained at approximately 22°C and 60% respectively. There were approximately 10-15 air changes per h and a 12 h light/ dark cycle maintained throughout the study. Food and tap water were available *ad libitum*. Tap water was of drinking water quality (Drinking Water Ordinance, Federal Law Gazette, part 1, Z 199 7A, 31 Jan 1975).

All rats were observed for clinical signs and mortalities daily with a detailed physical examination performed weekly (all body orifices, hair coat and faeces). Body weights were recorded every week before and after the mating period. Inseminated females were weighed at 1, 6, 15 and 20 days post-insemination. Weekly food consumption of each group up to the first mating was determined. The insemination rate and gestation period were determined for each female based on the presence of a vaginal plug, or sperm in a vaginal smear. Pup and litter weight were determined after birth, on day 5, after reduction [culling], and then at week 1, 2, 3 and 4. The total numbers of pups born live and still born, and the ratios of males to females were determined at birth. Fertility, gestation, viability, lactation and insemination indices were calculated by the study authors.

All pups (ie live and dead) were examined for any gross abnormalities immediately after birth and during the lactation period. All rats that died during the experiment or sacrificed when moribund were also subjected to a gross pathological examination. All F_0 and F_{1B} parental rats were anaesthetised with ether and exsanguinated 1–3 weeks after weaning their pups. A thorough gross pathological examination was subsequently performed on these parental rats.]

Additional pathology: The livers, kidneys, testes, and ovaries of the F1B parental rats were weighed. The histopathological examinations were performed for the brain, pituitary, liver, kidneys, and the organs of the reproductive system of all F0 and F1B parental rats of the 0 ppm, 15 ppm, and 45 ppm groups and all fixed organs [brain, pituitary, liver, kidneys, testes, epididymides, seminal vesicles, prostate, ovaries, uterus and vagina] of the rats of the 5 ppm group that died during the experiment.

[*Reproductive indices*: Reproductive indices were calculated by the study authors using the following formulas. Fertility index = no. pregnant females/no. inseminated females x 100.

Gestation index = no. females with live litters/no. pregnant females x 100. Viability index = no. live pups after 5 days/no. live pups born x 100. Lactation index = no. live pups after 4 weeks/no. live pups at day 5 after culling x 100. Insemination index = no. inseminated females/no. mated females x 100.]

Statistical methods: Comparisons of test population with controls by means of U-test of Mann, Whitney, and Wilcoxon at the significance level of alpha = 5%, and alpha = 1%. Comparison of indices at 95% and 99% confidence limits calculated according to Clopper and Pearson, and intergroup differences compared by Fisher's exact test at the significance level of alpha = 5%, and alpha = 1%. The mean pup weights per dose level were calculated from the mean pup weight of each individual litter. Each calculation of litter size was based on the number of females that had been pregnant.

Findings

[*Dietary analysis:* Analysis of the azinphos-methyl content of the diet indicated that the measured levels of 5.4, 16 and 46.6 ppm complied with the target levels of 5, 15 and 45 ppm. The distribution of azinphos-methyl in the diet was homogenous within a tolerance range of $\pm$ 10% relative to the target concentration. Azinphos-methyl was stable in the diet for 10 days at ambient temperature within a tolerance range of $\pm$ 20% of the target concentration.]

F_0 (F_{1A}/F_{1B}) generation: Increased alopecia, unpreened hair coat, poor general physical condition, inflamed areas of the eye, sporadic convulsions and increased mortality of dams were observed at 45 ppm. [At this dose, a single F_0 male and 5 F_0 females died (see Table below). The actual incidences of unpreened hair coats and convulsions in females at 45 ppm were unspecified and the incidence of alopecia in females was twice that of the control (approximately 50% of animals affected). Inflammation of the eye was detected in only a single F_0 female at 15 ppm, and a single male and female at 45 ppm and thus it is tenuous to conclude that it was due to azinphos-methyl. Tremor was observed in 3/19 F_0 females at 45 ppm. Clinical signs observed in all other treatment groups occurred at no greater incidence than in the controls.]

Effect of azinphos-methyl on the mortality, feed intake (FI) and body weight (bw) in F_0 and F_{1B} parental rats

Dose level (ppm)	**0**	**5**	**15**	**45**
F_0	**M/F**	**M/F**	**M/F**	**M/F**
Mortality (no.)	0/0	0/2	0/1	1/5
FI (g/animal)	2298/1877	2200/1971	2091/2017	2430/2118
FI (g/animal/d)	19/16	18/17	18/17	20/18
bw at 0 wk (g)	84/78	86/79	86/80	87/79
bw at 13 wk (g)	323/197	337/199	309/197	315/188*
F_{1B}	M/F	M/F	M/F	M/F
Mortality (no.)	0/1	0/0	0/2	0/0
FI (g/animal)	1438/1372	1338/1360	1191/1386	2214/2019
FI (g/animal/d)	21/20	19/19	17/20	32/29
bw at 5 wk (g)	78/69	76/69	69/67	73/52*
bw at 13 wk (g)	318/182	319/184	287*/190	265**/166*

* $p < 0.05$; ** $p < 0.01$

Feed intake was increased and body weight gain was decreased in females at 45 ppm. [There was a trend towards increased food consumption in treated F_0 females however this result

was not statistically significant (see Table above). Food consumption of treated F_0 males was comparable to the control group. The body weight gain of treated F_0 parents was comparable to the control at all dose levels with the exception of females administered 45 ppm azinphos-methyl which were statistically lighter than the control group at week 13 ($p < 0.05$) (see Table above).]

There was a[n apparent] decrease in fertility, particularly at the 2nd mating and a decrease of the number of pups born at 15 and 45 ppm. [However, no statistical difference in mating behaviour, gestation and fertility indices between F_0 treated and control groups in either of the first or second matings (see Table below).

Effect of azinphos-methyl on the fertility of F_0 and F_{1B} rats

Dose level (ppm)	0	5	15	45
F_0	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**	**Mating 1/2**
Mated females (no.)	24/24	24/24	24/23	23/19
Insemination index (%)	100/100	95.8/100	91.6/91.3	95.6/94.7
Fertility index (%)	91.7/91.7	95.7/95.8	90.0/85.7	86.4/83.3
Gestation index (%)	100/100	100/100	100/100	100/93.3
Gestation period (d)	22.5/22.3	22.5/22.7	22.5/22.6	22.8/22.8
F_{1B}	Mating 1/2	Mating 1/2	Mating 1/2	Mating 1/2
Mated females (no.)	24/24	24/24	24/23	5/5
Insemination index (%)	100/100	100/100	100/100	100/100
Fertility index (%)	91.7/87.5	100/91.7	87.5/95.7	100/80.0
Gestation index (%)	100/95.2	100/100	100/95.5	100/75.0
Gestation period (d)	22.1/22.2	22.3/22.6	22.6/22.2	22.4/22.3

There was an increased number of stillborn F_{1A} pups observed at 15 and 45 ppm compared to the control (see Table below) however this was not observed in the F_{1B} generation. There was no indication by the study authors whether the relatively high incidence of stillbirths at 15 and 45 ppm in the F_{1A} generation was statistically different to the control or whether they were comparable to historical control data. There was an incidental increase in the number of stillborn F_{1B} pups at 5 ppm, but in the absence of an effect at the two higher doses, was not considered to be treatment-related. There was no difference in the ratio of males to females in treated vs control groups in F_{1A} and F_{1B} pups. Although the litter size of each treatment group was comparable to the control at day 0, by day 5 the average litter size of F_{1A} pups at 15 and 45 ppm was significantly lower ($p < 0.05$ and 0.01 respectively) than the control (see Table below).]

Effect of azinphos-methyl on pup parameters

Dose level (ppm)	0	5	15	45
F_{1A}				
Live pups at birth/dead (no.)	252/1	247/0	204/8	197/9
Pups m/f (%)	52/48	53/47	49/51	52/48
Litter size at 0 d/5 d (no.)	11.5/11.1	11.2/10.5	10.1/8.7*	10.1/3.9**
Viability index (%)	96.8	93.9	86.6**	38.7**
Pups at 5 d#/4 wk (no.)	175/169	167/156	139/134	62/17
Lactation index (%)	96.6	93.4	96.4	27.4**
Body weight at 0 d/3 wk (g)	5.8/36.7	5.7/37.5	5.9/35.9	5.4/25.8**
F_{1B}				
Live pups at birth/dead (no.)	235/1	236/11	175/1	133/0

Dose level (ppm)	**0**	**5**	**15**	**45**
Pups m/f (%)	52/48	50/50	51/49	55/45
Litter size at 0 d/5 d (no.)	10.6/10.5	9.8/9.5	9.7/9.7	8.9/2.8**
Viability index (%)	98.3	97.3	98.9	31.6**
Pups at 5 d#/4 wk (no.)	165/161	164/162	128/117	39/18
Lactation index (%)	97.6	98.8	91.4*	46.2**
Body weight at 0 d/3 wk (g)	5.7/39.9	5.8/39.2	5.9/37.8	5.2**/27.2**
F_{2A}				
Live pups at birth/dead (no.)	259/3	270/0	230/0	43/0
Pups m/f (%)	55/45	55/45	54/46	51/49
Litter size at 0 d/5 d (no.)	11.7/11.5	11.2/10.8	11.0/10.7	8.6*/7.0*
Viability index (%)	98.1	95.9	97.8	81.4**
Pups at 5 d#/4 wk (no.)	176/173	185/174	152/134	29/21
Lactation index (%)	98.3	94.1	88.7**	72.4**
Body weight at 0 d/3 wk (g)	5.7/37.3	5.7/35.6	5.7/36.0	5.4/22.4**
F_{2B}				
Pups at birth/dead (no.)	223/1	244/2	214/3	25/0
Pups m/f (%)	51/49	55/45	49/51	56/44
Litter size at 0/5 d (no.)	10.6/10.1	11.0/10.0	9.6/8.5	6.2/6.2
Viability index (%)	95.5	90.1*	88.6*	100
Pups at 5 d#/4 wk (no.)	143/133	165/138	137/123	22/20
Lactation index (%)	93.0	83.6*	89.8	90.9
Body weight at 0 d/3 wk (g)	5.8/40.2	5.9/39.6	5.6/37.8	5.8/27.0**

After reduction; * $p < 0.05$; ** $p < 0.01$

The mean litter size was [significantly] reduced at 45 ppm (F_{1B}) [but only at day 5 ($p < 0.01$) (see Table above)]. The viability index was markedly decreased at 45 ppm and slightly at 15 ppm in the F_{1A} generation [with both statistically lower than the control ($p < 0.01$) (see Table above)]. (The study authors, however, state that the viability index of the 15 ppm group (F_{1A}) was within the range of normal laboratory variation (range for viability index: 78.7-100%; historical control values 1978-1984) and this deviation is considered of no relevance.) [The decrease in the viability index of F_{1B} pups at 45 ppm was also statistically significant ($p < 0.01$) (see Table above).]

[The F_{1A} generation treated with 45 ppm azinphos-methyl had a significantly lower lactation index ($p < 0.01$) than the control group (see Table above).] The lactation index was markedly decreased at 45 ppm [$p < 0.01$] and slightly at 15 ppm [$p < 0.05$] in the F1B generation [(see Table above)]. (The study authors, however, state that the lactation index of the 15 ppm group (F_{1B}) was within the range of normal laboratory variation (range for lactation index: 79.8-100%; historical control values 1978-1984) and this deviation is considered of no relevance.) As a consequence of these effects, at 45 ppm only 5 females were available for mating in the F_{1B} generation.

[At birth, the mean body weight of treated F_{1A} pups was comparable to the control but at week 3 the body weight of pups treated with 45 ppm azinphos-methyl was significantly lower than the control ($p < 0.01$) (see Table above).] At birth, pups of the 45 ppm group were in some cases significantly lighter and smaller (F1B). [the mean birth weight of F_{1B} pups at 45 ppm was significantly lower ($p < 0.01$) than control pups (see Table above).] During the 4-week lactation period, significantly lower pup body weights were recorded at 45 ppm [$p < 0.05$].

No malformations were observed up to and including 45 ppm.

F_{1B} (F_2A/F_{2B}) generation: Impairment of general physical condition, unpreened hair coat, and, in some rats, convulsions were observed at 45 ppm. [The sample size of this group was very low (n=5) in the F_{1B} generation which made it difficult to conclude that the increase incidence of alopecia was due to azinphos-methyl. The incidences of unpreened hair coats and convulsions were unspecified. Clinical signs observed in all other treatment groups occurred at no greater incidence than in the control.

A statistically depressed ($p < 0.05$) body weight was detected at 45 ppm in F_{1B} females at week 5 (see Table above).] Body weights of [F_{1B}] males at 15 ppm [wk 13 ($p < 0.05$)] and both sexes at 45 ppm were significantly lower than in controls [($p < 0.01$ for males and $p < 0.05$ for females) (see Table above)]. Feed intake was increased at 45 ppm (but this is not evaluated as a toxic effect by the study authors) [(see Table above). The statistical significance of this finding was unspecified by the study authors.]

After the 2nd mating, the indices for fertility and gestation were slightly lower at 45 ppm. (According to the study authors, the relevance of these differences from control is questionable, however, because of the small number of rats in this group. In addition, all females of the 45 ppm group had live pups at least one time. The study authors state that no effect on fertility can be inferred from these results.) [There was no statistically significant difference in fertility parameters between treated and control F_{1B} rats (see Table above).

The was no treatment-related effect on the incidence of stillbirths or the proportion of male and female pups in both F_{2A} and F_{2B} generations (see Table above).] There was a decrease of the number of pups born at 15 and 45 ppm and a decrease of the mean litter size at 45 ppm (F_{2A}) [at day 0 and 5 ($p < 0.05$) (see Table above)]. The viability index was [significantly] decreased at 45 ppm in the F_{2A} generation [$p < 0.05$] and at 5 and 15 ppm in the F_{2B} generation [see Table above]. (The study authors, however, state that the reduction in viability of F_{2A} pups at 45 ppm was evaluated as a random occurrence, since all F_{2B} pups at 45 ppm survived the first 5 days. The viability indices at 5 and 15 ppm (F_{2B}) were within the range of normal laboratory variation (range for viability index: 78.7-100%; historical control values 1978-1984) and the deviations were considered of no relevance by the study authors.)

The lactation index was significantly decreased at 15 and 45 ppm in the F_{2A} generation [see Table above] (The study authors, however, stated that the lactation indices up to and including 15 ppm were within the range of normal laboratory variation (range for lactation index: 79.8-100%; historical control values 1978-1984).) [There was an incidental decrease in the lactation index of Group 2 F_{2B} pups and although this result was statistically different to the control group ($p < 0.05$) it was not considered to be treatment-related as there was no effect at the next two higher doses.]

[There was no significant difference in the birth weight of F_{2A} and F_{2B} pups between treatment and control groups.] During the 4-week lactation period, significantly lower pup body weights were recorded at 45 ppm [in both the F_{2A} and F_{2B} generations ($p < 0.01$) (see Table above3)].

No malformations were observed up to and including 45 ppm.

Gross pathology, histopathology: The majority of the F_0 rats at 45 ppm that died had dark red discolouration of the lungs [(5/5) and lungs filled with fluid (3/5). There were no gross pathological abnormalities detected in treated F_0 and F_{1B} parental rats that were sacrificed as scheduled.] Necropsy and organ weight determinations of the F_{1B} rats indicated no specific treatment-related organ damage up to and including 45 ppm (but males at 45 ppm that exhibited marked delay in body weight gain had also significant reductions in absolute organ weights) [Although absolute liver, kidney and testes weights of F_{1B} males treated with 45 ppm azinphos-methyl were significantly lower than the control group ($p < 0.01$, 0.01 and 0.05 respectively) there was no significant effect of azinphos-methyl on relative organ weights]. Microscopic examination of the F_0 and F_{1B} rats revealed no evidence of treatment-related organ changes. [Although there was no strong evidence of microscopic abnormalities in treated rats there were some abnormal findings that were present at 45 ppm in F_0 females but absent in the control group such as: slight formations in the brain (4/24); mild formations in the liver that were of moderate size and frequently present (4/24); and slight to moderate formations in the pituitary (5/23).]

Conclusion: The NOEL for dietary administration of azinphos-methyl to rats over 2 generations was 5 ppm with respect to parental toxicity and reproduction, equivalent to a dose level of 0.33-0.42 mg/kg bw/d in males and 0.48-0.67 mg/kg bw/d in females. At dietary concentrations of 15 ppm and above, body weight gain, fertility (fertility index, number of delivered pups) and pup viability were affected. *[In an addendum to the current study (28 April 2000), the German BgVV considered that the reduction in pup viability was a systemic toxic effect (associated with ChE inhibition) and not a specific reproductive toxic effect, since it occurred at dose levels where a toxicological significant inhibition of brain ChE has been observed in the dams. The slight decrease in fertility which has been observed in the 2-generation study at dose levels of 15 ppm and above has not been confirmed in the supplementary 1-generation study using a higher number of animals and is therefore considered not to be treatment-related.]*

Australian regulatory authorities considered that the NOEL for parental toxicity was 15 ppm (equivalent to 1.02–1.22 mg/kg bw/d in males and 1.48-2.02 mg/kg bw/d in females) based on decreased body weight gain in both F_0 and F_{1B} parental animals at 45 ppm (equivalent to 3.46-7.37 and 4.84-10.27 mg/kg bw/d males and females respectively). The NOEL for peri/post-natal toxicity was also considered to be 15 ppm based on decreased pup body weight and viability at 45 ppm. The NOEL for reproductive toxicity was > 45 ppm (equivalent to 3.46-7.37 and 4.84-10.27 mg/kg bw/d in males and females respectively), the highest dose tested, based on the absence of any reproductive effects at this dose.

Comments: ChE activity was not measured in this study. Historical control data were provided for viability and gestational indices only. There was no clear treatment-related effects on reproduction parameters. The decrease in fertility index was not statistically significant although the litter size at day 0 in F_{2A} high-dose animals was statistically decreased. Other pup findings (eg decreased viability and reduction in pup weight after parturition) were probably signs of developmental effects due to poor maternal care or reduced lactation but no data was available on the ai in milk.]

Van Goethem DL (1987) Azinphos-methyl, the active ingredient of Guthion®: Correlation of the findings in the two-generation study with the results of a 28-day toxicity study and a chronic toxicity study. Report No. 94817. Report: Mobay Corporation, Health, Environment and Safety, Corporate Toxicology – Data Evaluation Department, Stilwell,

KS, USA. Sponsor: Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO, USA. Report date: 27 August 1987.

GLP and Test Guidelines: Data requirement - US EPA/FIFRA, Section 158.135 Guidelines 83-4. This report was quality assured.

Aim: The purpose of this report was to correlate the findings in the previous 2-generation reproduction study (Eiben and Janda 1987) with ChE data from 28-day (Eiben *et al* 1983) and chronic toxicity studies (Schmidt 1987).

Materials and Methods: Refer to Eiben and Janda (1987) and Eiben *et al* (1983) for details of the 2-generation reproduction and 28-day toxicity studies respectively. Details of the chronic toxicity study were unspecified in the current document, however the study author indicated that the report was in preparation as of 27 August 1987. Details of the chronic study were subsequently reported by Schmidt (1987).

Results: Evidence presented in the previous evaluation (Eiben and Janda 1987) indicated that the NOEL for dietary administration of azinphos-methyl to rats over 2 generations was 5 ppm (determined to be 0.33–0.42 and 0.48–0.67 mg/kg bw/d for males and females respectively) with respect to parental toxicity based on a decrease in body weight gain, fertility (fertility index, number of delivered pups) and pup viability at 15 ppm (estimated to be equivalent to 0.75 mg/kg bw/d). According to the study author, the rationale for the current report was that this previous reproduction study could not be accurately assessed in the absence of ChE data.

In a 28-day feeding study (Eiben *et al* 1983) there was a statistically ($p < 0.01$) and biologically significant inhibition of plasma (61%) and brain (54%) ChE activity in virgin female Wistar rats fed 50 ppm azinphos-methyl (see Table below). During this same study, RBC ChE was statistically ($p < 0.01$) depressed (27–35%) at 20 ppm and above. In the reproduction study, clinical signs of cholinergic inhibition were observed in females at 45 ppm. Thus while ChE activity was not measured, it was likely that ChE activity was inhibited in these high-dose rats during the 2-generation reproduction study. The mid-dose group in the 28-day study received a higher (by 5 ppm) level of azinphos-methyl than the mid-dose group in the reproduction study and as such are not directly comparable. From the data (see Table below), the NOEL in the 28-day study was 5 ppm (estimated to be equivalent to 0.25 mg/kg bw/d) based on inhibition of RBC ChE at 20 ppm (estimated to be equivalent to 1 mg/kg bw/d). Brain ChE activity was inhibited by 18% although this was not statistically significant compared to the control.

% Inhibition of plasma, RBC and brain ChE activities in virgin females rats fed azinphos-methyl in their diet for 28 days (Eiben *et al* 1983)

Dose Level (ppm)	Plasma % Inhibition	RBC % Inhibition	Brain % Inhibition
0	0	0	0
5	5	1	5
20	-3	27*	18
50	61*	35*	54*

*statistically different to the control ($p < 0.01$)

In a chronic feeding study in virgin female Wistar rats (Schmidt 1987) the same dose-levels were investigated as in the previous 2-generation reproduction study. During the chronic

study, plasma ChE activity was statistically ($p < 0.01–0.05$; 29–76%) depressed at 15 ppm and above (see Table below). Significant dose-related inhibition of RBC ChE was detected at 15 and 45 ppm (see Table below).

% Inhibition of plasma, RBC and brain ChE activities in virgin females rats fed azinphos-methyl in their diet for up to 12 months (Schmidt 1987)

Dose Level (ppm)	1 mo	3 mo	6 mo	12 mo
Plasma ChE				
0	-	-	-	-
5	12	12	8	10
15	29**	35**	29*	35**
45	56**	65**	66**	67**
RBC				
0	-	-	-	-
5	-	-	-	-
15	13	12**	14**	19**
45	26*	28**	23**	31**
Brain				
0	-	-	-	-
5	-	-	-	-
15	-	-	-	10
45	-	-	-	50**

* statistically different to the control ($p < 0.051$); ** statistically different to the control ($p < 0.01$)

The effect at 15 ppm ranged from 12-19% and at 45 ppm from 21–31%. While the RBC ChE inhibition did not reach 20% at 15 ppm, the effect was considered to be treatment-related. The dietary levels of 15–20 ppm appeared to be a threshold for the inhibition of RBC ChE activity, with no inhibition at lower doses and clear inhibition at higher doses. Brain ChE was inhibited at 45 ppm ($p < 0.01$; 50%). These data suggest, but do not prove, that plasma and RBC ChE activity was inhibited at 15 ppm during the previous 2-generation reproduction study. The NOEL in the chronic study was 5 ppm (estimated to be equivalent to 0.25 mg/kg bw/d) based on inhibition of plasma and RBC ChE activity at 15 ppm (estimated to be equivalent to 0.75 mg/kg bw/d).

Conclusions: It was probable that inhibition of plasma, RBC and brain ChE activities occurred in rats treated with 45 ppm azinphos-methyl during the previous 2-generation reproduction study. It was also probable that plasma and RBC ChE activities were inhibited in rats treated with 15 ppm azinphos-methyl in this same study. The NOELs determined during the 28-day and chronic toxicity studies (based on ChE inhibition) corresponded to the NOEL of 5 ppm determined for the 2-generation reproduction study (based on clinical signs).

Comments: The main criticism of this report was the practice of correlating observations from different studies as there can be variation between batches of diet, animals etc. This may not be problematic at high-doses where treatment-related effects are often well-defined, but at mid-doses where effects are often borderline then this variation can have a significant impact on the presence/absence of experimental findings. However, the results from the 28-day (Eiben *et al* 1983) and chronic feeding studies (Schmidt 1987) lend support to the study authors' assumption that ChE inhibition occurred at the mid and high-dose levels in the reproduction study (ie 15 and 45 ppm). Another weakness in extrapolating effects from the 28-day study was that the mid-dose level used was 5 ppm higher than that used during the reproduction study (ie 20 ppm vs 15 ppm) and as such is not directly comparable. The report only correlated ChE activity in females and ignored males, however, in the reproduction

study, females appeared to be more sensitive to the effects of azinphos-methyl administration than males.

♦ ***Holzum B (1990) E1582 (R1582) (c.n. azinphos-methyl): Investigation of inhibition of cholinesterase activity in plasma, RBC and brain in a 1-generation study. Report No. 19594. Study No. T0027362. Lab & Sponsor: Bayer AG, Toxicology Department, Wuppertal, Germany. Study duration: 18 February 1988 - 8 August 1988. Report date: 8 October 1990.***

Guidelines and GLP: Since the objective of this study was to investigate whether the slight effect on fertility at 15 ppm in a previous 2-generation study (Eiben and Janda, 1987) could be confirmed, and, if reproducible, to determine whether the effect was attributable to treatment of the males or females, no particular method is applicable. The study is GLP compliant [OECD principles of GLP, Bundesanzeiger No. 42a of 02.03.1983 and Bundesanzeiger I of 22.03.1990. This study was quality assured.]

The study is considered acceptable.

Material and methods: Groups of 18 male and 46 female 6-8 weeks old Wistar rats (strain: Bor:WISW (SPF-Cpb); source: Winkelmann, Borchen, FRG; body weight range: 92-146 g) received E1582 technical (batch no. 233796036, purity 92.0% or 91.7%) [Bayer AG, Wuppertal, Germany] admixed in the diet [Altromin® 1321, Altromin GmbH, Lage, Germany] at concentrations of 0, 5, 15 and 45 ppm [(equivalent to 0, 0.43, 1.30 and 3.73 mg/kg bw/d for males and 0.55, 1.54 and 4.87 mg/kg bw/d for females). The homogeneity of the diet was assessed using HPLC prior to the commencement of the study. HPLC analysis of the ai content and stability of the diet was performed at the start of the study, at 3 months and at the end of the study. Rats were randomly divided the following groups based on weight: Group 1 (0 ppm males and females-control); Group 2 (5 ppm males and females); Group 3 (15 ppm males and females) and Group 4 (45 ppm males and females).] Additional groups of 10 treated males (5, 15 and 45 ppm) were paired with groups of 20 untreated females [Group 5 (5 ppm males, 0 ppm females); Group 6 (15 ppm males, 0 ppm females) and Group 7 (45 ppm males, 0 ppm females). A summary of the experimental design is provided in the Table below]. After 14 weeks of treatment females [6-8 week old] were mated to males [6-8 week old] on a 2:1 basis for a period of 16 days. [Females which showed no signs of pregnancy (no insemination or weight gain) were remated up to 4 times with a male from the same group which had successfully mated.] Five days after birth, F_1 litters were reduced [sacrificed with carbon dioxide], where necessary, to 8 pups. Rearing of the F_1 pups ended on day 28 post-partum. for groups with both sexes dosed [Groups 1-4] and on day 5 post-partum for groups with males only treated [Groups 5-7].

Summary of experimental design

Event	Period
Pre-mating exposure F_0 parents Group 1-4 ♂and ♀ (18 ♂ and 46 ♀/group) Group 5-7 ♂ (10 ♂/group) F_0 ♀: determination of ChE activity	14 wk
Mating (1:2 ♂:♀) F_0 ♂ sacrificed: determination of ChE	16 d

activity; macroscopic and histopathological examination	
Gestation F_0 ♀: determination of ChE activity @ d 11	22 d
Reduction to 8 pups/litter	5 d after birth
Rearing of F_1 pups Groups 1-4 Groups 5-7 F_0 ♀ sacrificed (Groups 1-4 @ d 28; Groups 5-7 @ d 5): determination of ChE activity; macroscopic and histopathological examination F_1 pups sacrificed @ d 5 and 28: brain ChE determination; macroscopic and histopathological examination	 28 d 5 d

[Prior to and after mating animals were kept individually in Makrolon® type III cages (unspecified source). Room temperature and humidity were maintained at 22 ± 2°C and approximately 50% respectively. There were at least 10 air changes/h and a 12 h light/dark cycle maintained throughout the study. Diet and tap water (drinking water quality; Statute on Drinking water and Water for the Food Industry, 22.05.86, Bundesgesetzblatt I, p 760) were available *ad libitum* throughout the study. Fresh food and water were given once a week until insemination was established with inseminated females receiving fresh food and water on day 0, 7, 14 and 20 post-coitum and day 0, 5, 7, 14, 21 and 28 after the birth of the pups.

All animals were inspected twice daily for any clinical signs. A detailed examination was performed weekly including an assessment of general condition, behaviour, coat and body orifices, and changes in excretory products. The body weight of F_0 rats was recorded at the start of the study and then weekly until post-mortem for males, and until insemination for females. Following insemination, females were weighed at day 0, 7, 14 and 20 post-coitum and on day 0, 5, 7, 14, 21 and 28 after the birth of the pups. Food consumption was measured weekly up until the end of pre-treatment in males, and until the establishment of insemination in females and thereafter at day 0, 7, 14 and 20 post-coitum and on day 0, 5, 7, 14, 21 and 28 after the birth of the pups. The insemination rate and gestation period were calculated based on the presence of a vaginal plug and/or a positive sperm test. Insemination, fertility and gestation indices were calculated by the study authors. The sex and number of live pups were determined immediately after birth. Mortalities during the 4-wk rearing period were recorded. Pups were weighed at day 0, 5, 7, 14, 21 and 28 and examined for any visible signs of malformation. Live birth, viability and lactation indices were calculated by the study authors.]

ChE activities [plasma, RBC and brain] were determined [from randomly selected animals] in all groups with both sexes dosed, in F_0 males at the end of the mating period (10 animals), and in F_0 females at the end of pre-treatment phase, on day 11 post-coitum, and on days 5 and 28 p.p (10 animals each). [Blood was collected from the retro-orbital venous plexus (unspecified volume) for measurement of plasma and RBC ChE activity. In F_0 rats the left hemisphere of the brain was removed for measurement of brain ChE activity.] In F1 animals brain ChE activity was determined in 5 and 28 day old pups (5 animals each). [The entire brain of 5 d-old pups and the left hemisphere of 28 d-old pups were utilised for ChE measurements.

Wherever possible, a gross pathological examination was performed on any rats/pups found dead or that were sacrificed moribund. All remaining F_0 females were anaesthetised with ether and sacrificed by exsanguination, and examined at the same times appointed for ChE measurement. F_0 males were sacrificed and macroscopically examined at the end of the mating period. Pups sacrificed at day 5 and 28 were also macroscopically examined.] Macroscopically changed organs [and tissues] of F_0 animals and pups were fixed for microscopic examination.

[*Reproductive indices*: Reproductive indices were calculated by the study authors using the following formulas: Insemination index = no. inseminated females/no. mated females x 100; Fertility index = no. pregnant females/no. inseminated females x 100; Gestation index = no. females with live litters/no. pregnant females x 100; Live birth index = no. live pups at birth/no.pups born x 100; Viability index = no. live pups after 5 days/no. live pups born x 100; Lactation index = no. live pups after 4 weeks/no. live pups at day 5 after culling x 100.]

Statistical methods: U-test after Mann and Whitney, or after Wilcoxon for parental and litter data; Fisher's exact test for reproduction indices at the significance levels alpha = 5% and alpha = 1%; F-test and t-test after Welch for ChE values, the significance levels corresponding to the results of the t-test.

Findings

[*Dietary analysis:* Analysis of the ai content of the diet indicated that the mean analytical levels of 5.1, 15 and 42 ppm complied with the target levels of 5, 15 and 45 ppm. Dietary levels of azinphos-methyl were stable at ambient temperature, within a tolerance range of $\pm$ 20% of the target concentration, for 17 and 21 days at concentrations of 45 and 5 mg/kg respectively.] Analysis of deposit feed samples showed non-homogeneous distribution of the test compound in the 45 ppm group in the 3rd, 4th and 6th week of the study. As a result of modifying the mixing process at the end of the 6th week, the homogeneity of the test substance was within the permitted tolerances thereafter. The mean test compound intake at the dose levels of 0, 5, 15 and 45 ppm was estimated to be 0, 0.43/0.44, 1.30/1.32 and 3.73/3.83 mg/kg bw/d in the males (respectively) [Groups 1-4/Groups 5-7] and 0, 0.55, 1.54 and 4.87 mg/kg bw/d in the females (respectively).

The 45 ppm level led to non-specific signs (poor general condition, bloody nose, inertia, stumbling gait) and mortality in the F_0 females, whereas males of the same dose level were not affected. Five F_0 females died [during the 14-week pre-treatment period] between weeks 3 and 6, and 2 were killed in a moribund condition in weeks 3 and 10. [None of the females that died during the study exhibited any abnormal clinical signs while the two that were sacrificed moribund exhibited clinical signs consistent with cholinergic effects such as poor general condition, bloody noses, inertia and a stumbling gait. There were no deaths in F_0 males during the study.] These effects [on mortality and clinical signs in F_0 females] were probably due to non-homogeneous distribution of the test substance in the diet [as suggested by the study authors].

[There was no treatment-related effect on body weight, food consumption or food efficiency in F_0 rats.] Body weights and feed efficiency were marginally lower at 45 ppm during the pre-treatment phase [although not statistically significant], whereas the feed intake in females was reduced during lactation [d 7-14] at 15 ppm and above (marginal at 15 ppm, statistically significant at 45 ppm [$p < 0.01$]). [There were frequent statistical differences detected

between treatment and control groups with regard to food efficiency, but in the absence of any dose-response relationship the study authors concluded that they were due to chance events and were not toxicologically significant.]

[There was a clear treatment-related effect on the inhibition of ChE activity in both F_0 males and females, and F_1 pups (see Tables below).] The activity of ChE was inhibited in the F0 males at 5 ppm in RBC, at 15 ppm in plasma, and at 45 ppm in the brain. [There was a clear dose-response trend with regard to the inhibition of both plasma and RBC ChE activities in F_0 males. A statistically significant inhibition of plasma ChE was detected at 15 and 45 ppm ($p < 0.001$; 43% at 45 ppm compared to 14% at 15 ppm). A statistically ($p < 0.01$-0.001; 19-94%) significant depression in the RBC ChE activity of F_0 males was seen in all treatment groups. Brain ChE activity was inhibited at 45 ppm in F_0 males ($p < 0.01$; 19%).]

Effect of dietary administration of azinphos-methyl on ChE activity (plasma, RBC: kU/l, brain: U/g) in F_0 animals and F_1 pups

	F_0 males	F_0 females				F_1 pups	
	End of mating	End of pre-treat.	Day 11 p.c.	Day 5 p.p.	Day 28 p.p.	Day 5	Day 28
Plasma							
0 ppm	0.44	1.84	1.44	1.34	0.80	-	-
5 ppm	0.43	1.84	1.55	0.99*	0.76	-	-
15 ppm	0.38***	1.39*	1.18	0.72***	0.49**	-	-
45 ppm	0.25***	0.70***	0.57***	0.45***	0.30***	-	-
RBC							
0 ppm	0.48	0.24	0.21	0.67	0.62	-	-
5 ppm	0.39**	0.25	0.21	0.50**	0.33***	-	-
15 ppm	0.15***	0.13***	0.10***	0.17***	0.10***	-	-
45 ppm	0.03***	0.07***	0.04***	0.06***	0.07***	-	-
Brain							
0 ppm	1.72	1.79	2.39	2.11	2.04	1.79	2.49
5 ppm	1.71	1.62*	2.49	2.12	1.80	1.92	2.84
15 ppm	1.89*	1.72	1.88**	1.30**	1.06***	1.77	2.14
45 ppm	1.40**	0.80***	0.74***	0.72***	0.66***	1.49*	1.34***

$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; p.p. = post-partum; p.c. = post-coitum

[Effect of dietary administration of azinphos-methyl on the% inhibition of plasma, RBC and brain ChE activity in F_0 animals and F_1 pups

	F0 males	F0 females				F1 pups	
	End of mating	End of pre-treat.	Day 11 p.c.	Day 5 p.p.	Day 28 p.p.	Day 5	Day 28
Plasma							
0 ppm	0%	0%	0%	0%	0%	-	-
5 ppm	2.3%	-	-	26%*	5%	-	-
15 ppm	14%***	25%*	18%	46%***	39%**	-	-
45 ppm	43%***	62%***	60%***	66%***	63%***	-	-
RBC							
0 ppm	0%	0%	0%	0%	0%	-	-
5 ppm	19%**	-	-	25%**	47%***	-	-
15 ppm	69%***	46%***	52%***	75%***	84%***	-	-
45 ppm	94%***	71%***	81%***	91%***	89%***	-	-
Brain							
0 ppm	1.72	0%	0%	0%	0%	0%	0%

	F0 males	F0 females				F1 pups	
	End of mating	End of pre-treat.	Day 11 p.c.	Day 5 p.p.	Day 28 p.p.	Day 5	Day 28
5 ppm	1%	10%*	-	-	12%	-	-
15 ppm	-	4%	21%**	38%**	48%***	1%	14%
45 ppm	19%**	55%***	69%***	66%***	68%***	17%*	46%***

p < 0.05 ; ** p < 0.01 ; *** p < 0.001; p.p. = post-partum; p.c. = post-coitum]

In F_0 females, inhibition of ChE activity was noted at 5 ppm and above in plasma and RBC and at 15 ppm and above in the brain. [There was a clear dose-response trend with regard to the inhibition of both plasma and RBC ChE activities in F_0 females at all sampling periods at 15 ppm and above. The level of inhibition of plasma ChE ranged from 18–46% at 15 ppm, and 60–63% at 45 ppm. A transient depression in plasma ChE activity was detected at day 5 post-partum at 5 ppm ($p < 0.05$; 26%). The level of inhibition of RBC ChE ranged from 46-84% at 15 ppm, and 71–91% at 45 ppm. At 5 ppm a statistically significant depression in RBC ChE activity was observed at day 5 ($p < 0.01$; 25%) and 28 post-partum ($p < 0.001$; 47%). At 45 ppm, brain ChE was inhibited at all sampling periods ($p < 0.001$; 55–68%). At 15 ppm brain ChE was also lower than the control group from day 11 post-coitum ($p < 0.01$–0.001; 21–48%). There was no significant inhibition of brain ChE at 5 ppm.

In the pups, brain ChE activity was inhibited at 45 ppm ($p < 0.001$ and $p < 0.05$; 46 and 17% inhibition at day 28 and 5 respectively) (see Table above).

There were no treatment-related effects on fertility parameters (insemination, fertility and gestation indices, and gestation period) (see Table below)].

Effect of azinphos-methyl on fertility parameters

Dose level, m-f (ppm)	0-0	5-5/5-0	15-15/15-0	45-45/45-0
Mated females (no.)	36	36/20	36/20	31/20
Insemination index (%)	100	100/100	100/100	100/100
Fertility index (%)	97.2	94.4/100	100/95.0	96.8/100
Gestation index (%)	88.5	100/100	100/100	95.5/95.0
Gestation period (d)	22.8	22.4/22.6	22.6/22.7	22.7/22.5

The reproduction parameters investigated were not affected by treatment of male and female parent animals with 5 ppm. At 15 ppm and higher, when males and females were treated, the viability index and the body weight of the pups during the rearing period were reduced. However, after treatment of male parental animals only, reproduction parameters remained unaffected up to and including 45 ppm. [There was no treatment-related effect on the number of pups at birth, the number of still born pups, the ratio of male:female pups, litter size, the lactation index and pup birth weight (see Table below).

Effect of azinphos-methyl on F_1 pup parameters

Dose level, m-f (ppm)	0-0	5-5/5-0	15-15/15-0	45-45/45-0
Pups at birth (no.)	240	293/216	275/196	214/211
Pups dead at birth (no.)	13	4/2	3/1	3/3
Live birth index (%)	94.6	98.6*/99.1*	98.9**/99.5**	98.6*/98.6*
Male pups (%)	45	48.8/52.3	48.7/48.5	49.5/65.4
Litter size (no.)	9.9	11.1/10.7	10.5/10.3	10.0/10.9
Viability index (%)	93.4	92.4/98.1*	86.0*/90.8	48.3**/95.7

Lactation index (%)	62.1	74.2/-	69.8/-	57.7/-
Body weight, 0 d (g)	5.9	5.7/5.8	5.8/5.9	5.9/5.7
Body weight, 5 d (g)	9.2	8.7/8.9	9.0/9.3	7.8*/8.7
Body weight, 14 d (g)	24.4	22.5/-	21.4/-	19.9/-
Body weight, 28 d (g)	52.4	52.7/-	49.5/-	46.3/-

* $p < 0.05$; ** $p < 0.01$

The live birth index was statistically higher across all treatment groups compared to the control most likely due to the high number of stillborn pups in the control group (see Table above). The viability index of pups whose parents were both treated with 45 ppm azinphos-methyl was significantly lower ($p < 0.01$) than the control group (see Table above). This effect on the viability index was also evident at 15 ppm however it was of a lower magnitude and statistically less significant ($p < 0.05$) than that occurring at 45 ppm. In contrast, the viability index of pups whose father only was fed azinphos-methyl was the same or marginally higher than the control group. F_1 pups whose father was given 5 ppm exhibited an incidentally higher viability index than the control group but this was not considered to be treatment-related as there was no effect at the two higher doses. The mean body weight of 5-day old pups whose parents were both treated with 45 ppm azinphos-methyl was statistically lower than the control ($p < 0.05$). The mean body weight of most treated pups at day 14 and 28 was marginally lower than the control but no statistical significance was evident.]

[There were no treatment-related clinical signs observed in F_1 pups at birth or during the 4-week rearing period.]

No treatment-related pathological changes were observed in routinely sacrificed male and female F_0 animals and in the pups at doses up to and including 45 ppm. Some female F_0 rats which died or were sacrificed moribund were found to have dark red or red-brown lungs, clear demarcation of the liver lobules or a pale spleen, but microscopic examination revealed no indication of test compound induced organ damage. [There were no treatment-related effects on the brain weight of F_0 rats and F_1 pups, however, the brain weight of 45 ppm F_1 pups was statistically lower ($p < 0.01$) than the control at day 5 post-partum but not at day 28. The study authors concluded that this was likely due to the relatively low body weight of this group.]

Conclusion: The NOEL for dietary administration of azinphos-methyl to rats over 1 generation was < 5 ppm with respect to parental toxicity, equivalent to a dose level of < 0.43-0.44 mg/kg bw/d in males and < 0.55 mg/kg bw/d in females. Plasma and RBC ChE activity was inhibited at all dose levels, whereas brain ChE activity was depressed at 15 ppm and above in females and at 45 ppm in males. [In F_0 males, RBC ChE activity was inhibited across all dose levels while plasma ChE activity was inhibited at 15 ppm and above. In F_0 females, plasma and RBC ChE activity was inhibited across all dose levels at day 5 and 28 post-partum while only at 15 and 45 ppm after pre-treatment and at day 11 post-coitum] The NOEL with respect to reproduction was 5 ppm, based on reduced viability index and retardation of growth in F_1 pups at 15 ppm and above [when both F_0 parents were treated with azinphos-methyl. Significant reduction in pup body weight were only seen at 45 ppm on day 5.] At 45 ppm, brain ChE activity in pups was also inhibited. Treatment of F_0 male animals and subsequent mating with untreated females elicited no effects on reproduction parameters or on the progeny [which suggested that the treatment of F_0 females with azinphos-methyl was responsible for the observed toxicological effects in F_1 pups.]

The slight effect on fertility (fertility index, number of delivered pups) observed in the previous 2-generation study (Eiben & Janda 1987) at and above 15 ppm was not confirmed in this study.

[*Comments:* Although 3 extra groups were conscripted to determine whether any effect on fertility was attributable to the treatment of male or female animals, ChE measurements were not performed on these additional groups. The rearing of Groups 1–4 pups ended at day 28 whereas for Groups 5–7 it ended on day 5. Relative organ weights (eg brain weight) were not calculated. Besides brain, no other organ weights were recorded. Only brain ChE activity was measured in F_1 pups while it would have been preferable to also measure plasma and RBC ChE activity as the NOEL for F_1 parents was set based on the inhibition of RBC ChE activity. Unlike the 2-generation study, no marked pup mortality was detected on day 0–5 or 5–21 at 15 ppm, or day 5–21 at 45 ppm]

2.8 DEVELOPMENTAL TOXICITY

2.8.1 Mice

Kavlock RJ, Chernoff N & Rogers EH (1985) The effect of maternal toxicity on fetal development in the mouse. Teratogenesis, Carcinogenesis, and Mutagenesis. 5: 3 – 13.

Guidelines and GLP: No GLP or test guidelines were provided. This study was not quality assured.

Materials and Methods: Azinphos-methyl (technical grade, batch no. 411-0229, Chemagro Inc., Kansas City, MO, USA) was administered by intubation in corn oil (unspecified concentration) to primiparous CD-1 mice weighing 29–33 g (Charles River Breeding Laboratories, North Wilmington, MA, USA). Details of mating conditions were unspecified although mice were checked for pregnancy by the presence of a vaginal plug. On day 8 of gestation, mice were given a single PO dose of azinphos-methyl at 0 (n = 20), 16 (n = 20) or 20 mg/kg bw azinphos-methyl (n = 40). The dose-selection was based on a toxicity study in which five groups of 10 nulliparous mice were dosed with azinphos-methyl at concentrations bracketing the anticipated LD_{50} value. Doses were chosen that would induce a low or moderate degree of maternal lethality.

Five mice were housed per plastic cage. A 12 h light/dark cycle was maintained throughout the study with food (Wayne Lab Blox, unspecified source) and water (unspecified source) available *ad libitum*. No further feeding and housing conditions were specified. At day 18 of gestation, mice were sacrificed by decapitation and the uteris removed and weighed. Maternal (the difference in overall weight gain and gravid uterus weight) and foetal weights were recorded. Foetuses were examined for gross malformations, then fixed and stained for determination of skeletal abnormalities and maturity.

Statistical analyses: Overall treatment-related effects were ascertained by an ANOVA while post hoc t-tests were used to compare individual treatment groups.

Results

Maternal effects: A clear dose-response trend was evident with regard to maternal deaths (see Table below). The percentage of viable litters was also reduced at 20 mg/kg bw although no statistical significance was reported (see Table below). There was no treatment-related effect on the incidence of whole litter resorptions or maternal body weight gain.

Maternal and foetal effects of a single PO dose of azinphos-methyl

	0 mg/kg bw	16 mg/kg bw	20 mg/kg bw
Maternal Effects			
n	15	20	40
% Deaths	0	5	53
% Viable litters	87	95	68
Foetal Effects			
No of litters	13	18	13
Weight (g) (Mean ± 1 SEM)	1.11 ± 0.04	1.05 ± 0.03	0.99 ± 0.03*

* Statistically different to the control ($p < 0.05$)

Foetal effects: There was no treatment-related effect on the number of litters, prenatal mortality, the number of sternal and caudal ossifications, and the percentage of enlarged cerebral ventricles and renal pelvises. The mean foetal weight of the high-dose group was statistically lower than the control ($p < 0.05$) (see Table above). Graphically presented data illustrated a statistically significant increase ($p < 0.05$) in the incidence of supernumerary ribs (SNR) at both doses (approximately 23% at 16 mg/kg bw and 58% at 20 mg/kg bw compared to 17 % for the control). The effect at 16 mg/kg bw fell within the historical control range of 3–25% and thus may not have been treatment-related. The effect at 20 mg/kg bw/d was considered to be treatment-related, but as SNR can be induced in CD-1 mice by maternal stress, this may have been the result of a maternally toxicity dose. There were no further malformations detected at either dose.

Conclusions: These observations indicated that azinphos-methyl was maternally and foetally toxic at 20 and 16 mg/kg bw respectively. Maternal toxicity was evidenced by mortalities at both 16 and 20 mg/kg bw and thus the NOEL was < 16 mg/kg bw. The NOEL for foetal toxicity and developmental toxicity was < 16 mg/kg bw/d due to a statistically significant decrease in foetal weight ($p < 0.05$) at 20 mg/kg bw and a statistically significant increase in the incidence of SNR at 16 and 20 mg/kg bw. The presence of SNR was a likely result of maternal stress due to the administration of a maternally toxic dose.

Comments: There were numerous deficiencies in this study. Individual animal data were not provided. Mating details were not specified. Deviations from OECD guidelines included: 15 control animals instead of at least 20; housing 5 animals/cage instead of individually; 2 dose levels were used instead of 3; maternal mortality (50%) was higher than 10%; use of a single dose instead of dosing throughout organogenesis; lack of clinical observations; lack of food consumption data; lack of foetal sex characterisation; lack of statistical analysis

♦ ***Short RD, Minor JL, Unger TM & Lee C-C (1978) Teratology of Guthion. Report No. EPA-600/1-78-056. Lab: Midwest Research Institute, Kansas City, MO, USA. Sponsor: US Environmental Protection Agency, Research Triangle Park, NC, USA. Study duration: unspecified. Report Date: August 1978.***

♦ ***Short RD, Minor JL, Lee C-C, Chernoff N & Baron RL (1980) Developmental toxicity of Guthion in rats and mice. Lab: Midwest Research Institute, Kansas City, MO, USA. Sponsor: US Environmental Protection Agency, Research Triangle Park, NC, USA. Arch. Toxicol. 43: 177-186.***

Guidelines and GLP: The test method employed was the internal standard at the time the study was performed and is in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory. Main deviations from current OECD guidelines: The report is not very detailed (missing raw data). The study is considered supplementary.

Material and Methods: Groups of 22 or 23 inseminated CD®-1 mice (source: Charles River Breeding Laboratories, North Wilmington, MA, USA) [weighing 20–22 g, were acclimatised for at least 7 days and then] received azinphos-methyl (lot no. M007, purity: 90.6%, [Mobay Chemical Corp., Kansas City, MO, USA]) orally in a vehicle of corn oil at dose levels of 0, 1.25, 2.5 and 5.0 mg/kg bw/d from day 6 to day 15 of gestation. [All doses were administered in a volume of 5 mL/kg of corn oil.] The dose levels were selected on the basis of a range

finding study [described in the same report]. The animals were sacrificed on day 18 of gestation for examination of the foetuses. [Mice were housed in animal quarters that were maintained at 20–26°C and 40–60% humidity, with a 12 h light/dark cycle. The types of cages used to house the mice were unspecified. Rodent chow (Wayne Lab-Blox, Allied Mills Inc., Chicago, IL, USA) and tap water were available *ad libitum* throughout the study. A single female was mated with a single male overnight, with the presence of sperm in a vaginal smear, or a vaginal plug indicating a successful mating and designated day 1 of gestation.

Maternal mice were examined for mortalities and clinical signs (unspecified frequency). Maternal body weight and food consumption was recorded (unspecified frequency). For foetal examinations, maternal mice were sacrificed by cervical dislocation and a laparotomy performed. The number of live, dead and resorbed foetuses was determined. Live foetuses were removed, weighed and immediately examined for external anomalies. One half of the live foetuses from each litter were examined for soft tissue anomalies with the other half examined for skeletal anomalies.

All soft tissue anomalies were assigned a rank which reflected the opinion of the study authors' with regard to their value in predicting teratogenic potential. Anomalies with a rank of one were considered to have little value in predicting teratogenic potential as they may have occurred spontaneously or represent an artefact of preparation. Anomalies with a rank of 2 were considered to have intermediate value in predicting teratogenic potential and anomalies with a rank of 3 occur infrequently in controls and are indicative of a disruption in normal development which may compromise survival.]

Statistical methods: [Data were analysed for homogeneity by Bartlett's test.] Homogenous data were analysed by Dunnett's procedure, heterogeneous data by a nonparametric rank test. [Results were considered to be statistically significant when $p < 0.05$.]

Findings: [There were no treatment-related effects on maternal body weight or food consumption.] Signs of ChE inhibition (salivation, urination, lacrimation and tremors) [incidences unspecified] and one death were observed at 5 mg/kg bw/d. There was no effect on litter size, incidence of resorptions or fetal body weights at any of the doses tested.

No external anomalies were observed and none of the individual soft-tissue anomalies increased in a dose-related fashion. However, when all of the anomalies were combined by rank there was a significant increase ($p < 0.05$) in soft-tissue anomalies with a rank of 2 (anomalies with intermediate value in assessing teratogenic potential) or 3 (anomalies most valuable in assessing teratogenic potential) in the groups that received 2.5 and 5 mg/kg bw/d [however, no dose-response trend was evident (see Table below). Additionally, the statistical significance of this result appears to hinge on the incidence of haemorrhage in the pericardium which was only given intermediate value in assessing teratogenic potential (see Table below). These two factors plus the high level of variability in the data, make it difficult to conclude that this result was treatment-related.] In addition, skeletal anomalies with a rank of 2 and the incidence of malaligned sternebrae [followed a dose-response trend and] were significantly increased ($p < 0.05$) at 5 mg/kg bw/d [See Table below]. However, there was no pattern of characteristic defects. According to the study authors, this observation suggests that azinphos-methyl increased the incidence of naturally occurring anomalies without producing specific defects.

Incidence of soft tissue and skeletal anomalies in foetuses whose mothers were administered azinphos-methyl daily by PO gavage from day 6-15 of gestation. [Results are expressed as the mean of the % of foetuses with the indicated anomaly calculated on a per litter basis. Standard errors are contained in square brackets.]

Dose level (mg/kg bw/d)	0	1.25	2.5	5.0
Litters/foetuses inspected	19/80	18/82	17/80	14/64
Hemorrhage in pericardium [2#]	0 [0]	1.1 [1.1]	3.5 [2.0]	3.0 [2.1]
Duodenum enlarged [3#]	0 [0]	0 [0]	0 [0]	1.0 [1.0]
Hydronephrosis, marked [2#]	0 [0]	1.9 [1.9]	1.0 [1.0]	1.0 [1.0]
Small kidney [3#]	0 [0]	0 [0]	0 [0]	1.8 [1.8]
Ectopic kidney [3#]	0 [0]	0 [0]	1.2 [1.2]	0 [0]
Hydroureter [2#]	0 [0]	0 [0]	1.5 [1.5]	0 [0]
Gastroschisis [3#]	0 [0]	1.1 [1.1]	0 [0]	0 [0]
Summary by rank (rank 2 and 3)	0 []	4.1 [2.3]	7.2* [2.4]	6.8* [2.7]
Litters/foetuses inspected	19/87	18/93	17/85	14/67
Interparietal medially curved [2#]	0 [0]	0 [0]	0 [0]	1.4 [1.4]
Sternebrae: unossified [1#]	0 [0]	0 [0]	0 [0]	1.4 [1.4]
incompletely ossif. [1#]	19.4 [5.5]	9.5 [2.7]	7.3 [3.3]	8.7 [2.8]
lobed [2#]	0 [0]	2.0 [1.4]	2.0 [2.0]	2.6 [1.8]
malaligned [2#]	6.4 [2.2]	12.5 [4.4]	19.0 [6.3]	24.3*[4.8]
extra ossif. between [2#]	0 [0]	1.1 [1.1]	0 [0]	0 [0]
Summary by rank (rank 2)	6.4 [2.2]	15.6 [4.7]	21.4 [6.3]	26.9*[4.9]

Rank of anomaly; * Significantly different from control (two-sample rank test)

Conclusion: The NOEL for PO administration of azinphos-methyl to pregnant mice from day 6 to day 15 of gestation was 2.5 mg/kg bw/d with respect to maternal toxicity, based on clinical signs of ChE inhibition at 5.0 mg/kg bw/d. [The actual incidences of these clinical signs were unspecified and as such it is difficult to establish a NOEL for maternal toxicity due to this lack of detail in reporting. In the absence of any other treatment-related effects a definite NOEL for maternal toxicity could not be set for this study.] The NOEL for developmental toxicity was 2.5 mg/kg bw/d, based on an increased incidence of naturally occurring anomalies (malaligned sternebrae) at 5.0 mg/kg bw/d, a dose overtly toxic to the dams. There was no evidence of teratogenicity.

[*Comments:* Maternal animals did not appear to be macroscopically examined. Incidences of clinical signs were inadequately recorded and thus should not be the sole evidence for setting a NOEL. Individual animal data were not provided. No ChE measurements were taken. The high level of variability in the soft tissue data was not reflected in the summary by rank data. No historical control data were provided.]

2.8.2 Rats

♦ ***Short RD, Minor JL, Unger TM & Lee C-C (1978) Teratology of Guthion. Report No. EPA-600/1-78-056. Lab: Midwest Research Institute, Kansas City, MO, USA. Sponsor: US Environmental Protection Agency, Research Triangle Park, NC, USA. Study duration: unspecified. Report Date: August 1978.***

♦ ***Short RD, Minor JL, Lee C-C, Chernoff N & Baron RL (1980) Developmental toxicity of Guthion in rats and mice. Lab: Midwest Research Institute, Kansas City, MO, USA.***

Sponsor: US Environmental Protection Agency, Research Triangle Park, NC, USA. Arch. Toxicol. 43: 177-186.

Guidelines and GLP: The test method employed was the internal standard at the time the study was performed and is, for the teratology study, in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory. Main deviations from current OECD guidelines: The report is not very detailed (missing raw data). The study is considered supplementary.

Material and Methods: Groups of inseminated CD rats (source: Charles River Breeding Laboratories, North Wilmington, MA, USA) [weighing 200–20 g and having been acclimatised for at least 7 days] received azinphos-methyl (lot no. M007, purity: 90.6% [Mobay Chemical Corp., Kansas City, MO, USA]) orally in a vehicle of corn oil at dose levels of 0, 1.25, 2.5 and 5.0 mg/kg bw/d. The dose levels were selected on the basis of a range finding study [(described in the same report). Rats were housed in animal quarters that were maintained at 20–26°C and 40–60% humidity, with a 12 h light/dark cycle. The types of cages used to house the rats were unspecified. Rodent chow (Wayne Lab-Blox, Allied Mills Inc., Chicago, IL, USA) and tap water were available *ad libitum* throughout the study. A single female was mated with a single male overnight, with the presence of sperm in a vaginal smear, or a vaginal plug indicating a successful mating and was designated as day 1 gestation.]

a) In the 1st study (teratology study), groups of 21 rats were treated from day 6 to day 15 of gestation and sacrificed on day 20 of gestation for examination of the foetuses.

b) In the 2nd study (peri-/postnatal toxicity study), groups of 14 or 15 rats were treated from day 6 of gestation until the pups were weaned, 21 days after birth. After weaning, surviving pups of the control, intermediate and high dose group were sacrificed at 30-40 days of age and preserved.

[Maternal rats were examined for mortalities and clinical signs (unspecified frequency). Maternal body weight and food consumption was recorded (unspecified frequency). For foetal examinations, maternal mice were sacrificed by cervical dislocation and a laparotomy performed. The number of live, dead and resorbed foetuses was determined. Live foetuses were removed, weighed and immediately examined for external anomalies. One half of the live foetuses from each litter were examined for soft tissue anomalies with the other half examined for skeletal anomalies.]

Statistical methods: [Data were analysed for homogeneity by Bartlett's test.] Homogenous data were analysed by Dunnett's procedure, heterogeneous data by a nonparametric rank test. [Results were considered to be statistically significant when $p < 0.05$.]

Findings

a) In the teratology study, [significantly] reduced weight gain and feed consumption [$p < 0.05$] during the treatment period [see Table below], signs of ChE inhibition (salivation, urination, lacrimation and tremors) [unspecified incidences] and one death were observed at 5 mg/kg bw/d.[The average body weight change of dams treated with 5 mg/kg bw/d from day 6–15 of gestation, was significantly lower than the control ($p < 0.05$), while no effect was seen at the 2 lower doses (see Table below). The same pattern of effect occurred with regard

to the body weight gain of dams at day 20 that were corrected for uterine weight (see Table below). Food consumption of dams treated with 5 mg/kg bw/d from day 6–15 of gestation was significantly reduced ($p < 0.05$) but this recovered to control levels when treatment ceased (see Table below). There was an incidental increase ($p < 0.05$) of slight lateral and fourth ventricle hydrocephalus at a dose of 1.25 mg/kg bw/d, but in the absence of any effect at the next two doses, this was not considered to be treatment-related.] There was no effect of treatment on the reproduction parameters and no evidence of embryotoxicity, fetotoxicity or teratogenicity was obtained. [There were no treatment-related effects on the incidence of visceral, soft tissue and skeletal abnormalities.]

[Effect of azinphos-methyl administered daily by PO gavage from day 6–15 of gestation on maternal body weight and food consumption

	Guthion (azinphos-methyl) mg/kg bw/d			
	0	**1.25**	**2.5**	**5.0**
Bw change (g)†				
During treatment	61 ± 3	57 ± 3	56 ± 3	29 ± 5 *
After treatment	62 ± 3	56 ± 3	61 ± 2	59 ± 5
Dams corrected ‡	71 ± 4	71 ± 3	67 ± 3	48 ± 5 *
Food Consumption (g/rat/d)				
During treatment	25 ± 1	25 ± 1	24 ± 1	19 ± 1 *
After treatment	28 ± 2	28 ± 1	29 ± 1	29 ± 1

† g/pregnant rat/d/interval (unspecified); ‡ Dam body weight (d 0-20) – uterine weight day 20; * statistically different from the control at $p < 0.05$]

b) Treatment of the dams up to the end of the lactation period resulted in reduced weight gain [at day 16–20 gestation and day 0–4 postpartum ($p < 0.05$)] and feed consumption [at day 6–20 gestation ($p < 0.05$)] at 5 mg/kg bw/d [(see Table below). Feed intake in high-dose dams was marginally lower than the control at day 7–21 post-partum]. At this dose level, dams were more sensitive to treatment later in gestation with the results that deaths and clinical signs of ChE inhibition [unspecified incidences] increased during this time. [On the contrary, results indicated that the number of deaths was relatively constant from day 6–23 and then actually dropped post-partum (see Table below).] The fertility index was not altered by the treatment, however, there was a trend towards a reduced gestation index in the high dose group [although this was not statistically significant. There was no treatment-related effect on the duration of gestation].

Maternal and developmental toxicity parameters in rats treated with azinphos-methyl by daily PO gavage during gestation and lactation

Dose level (mg/kg bw/d)	**0**	**1.25**	**2.5**	**5.0**
Dams treated/pregnant	14/13	14/13	14/12	15/13
Mortality, total/days 6-16	0/0	0/0	0/0	8/3
Mortality, days 16-23/post-partum	0/0	0/0	0/0	4/1
[Food Consumption (g/rat/d)				
Gestational d 6-20	27 ± 1	26 ± 1	28 ± 1	20 ± 1 *
Postpartum d 7-21	68 ± 4	64 ± 5	63 ± 6	47
Bw (g/rat)				
Gestational				
d 0	241 ± 4	241 ± 4	248 ± 4	239 ± 4
d 6	275 ± 4	276 ± 5	286 ± 5	276 ± 5
d 16	331 ± 7	333 ± 7	345 ± 4	293 ± 8 *

d 20	387 ± 9	382 ± 11	400 ± 10	327 ± 13 *
Post-partum				
d 0	311 ± 9	297 ± 7	315 ± 6	260 ± 11*
d 4	304 ± 8	304 ± 7	320 ± 5	257 ± 14 *
d 7	317 ± 8	316 ± 7	332 ± 4	278 ± 21
d 14	346 ± 8	344 ± 7	363 ± 4	340
d 21	342 ± 10	341 ± 6	360 ± 5	346]
Fertility/gestation index (%)	93/100	93/100	86/100	87/46
Viable litters, birth/day 4	13/13	13/13	12/11	6/3
Viable litters, day 7/21	13/13	13/13	11/11	1/1
Pup weight, birth/day 4 (g)	7.1/9.0	6.4/8.8	6.5/8.6	5.7*/5.4*
Pup weight, day 7/21 (g)	12.0/37.0	12.5/37.0	12.2/34.4	7.8/24.4
Pup survival, days 0-4/4-21 (%)	100/96	86/95	87/98	46*/14*

* Significantly different from control (two-sample rank test) [$p < 0.05$]

Pup weight [birth, day 4, day 7, day 21] and pup survival were reduced at 5 mg/kg bw/d [(see Table above). There was a large reduction in the number of viable litters from birth to day 21 in the high-dose group but this result was not statistically significant (see Table above). There was no treatment-related effect on the number pups delivered per dam. At 5 mg/kg bw/d pup weights were significantly lower than the control group ($p < 0.05$) from birth to day 4 but reductions in pup weight at day 7–21 were not statistically significant (see Table above).] One day after weaning, pups in the single surviving litter of the high dose group were observed to maintain their rear legs at right angles to the body and to have muscular incoordination in the use of these legs, muscular tremors in the tail, and upturned snouts. In this litter of 5 pups, these effects were noticeable in 2 pups and of questionable incidence in 2 pups. However, similar signs were also observed in one pup from the control group, which complicated attempts to correlate these observations with azinphos-methyl treatment.

Conclusion: The NOEL for PO administration of azinphos-methyl to pregnant rats from day 6 to day 15 of gestation was 2.5 mg/kg bw/d with respect to maternal toxicity, based on reduced weight gain and feed consumption and clinical signs of ChE inhibition at 5.0 mg/kg bw/d. The NOEL for developmental toxicity was 5.0 mg/kg bw/d, the highest dose tested.

The NOEL for PO administration of azinphos-methyl to pregnant rats from gestational day 6 to postpartum day 21 was 2.5 mg/kg bw/d with respect to maternal toxicity, based on deaths, clinical signs of ChE inhibition and reduced weight gain and feed consumption at 5.0 mg/kg bw/d. The NOEL for reproduction toxicity was 2.5 mg/kg bw/d, based on a reduced gestation index and reduced survival and weight gain of pups at 5 mg/kg bw/d.

[*Comments:* Maternal animals did not appear to be macroscopically examined. Incidences of clinical signs were inadequately recorded and this is a deficiency of the study. However, other treatment-related effects were also seen at the high dose and so this lack of reporting does not prevent the establishment of a NOEL. No individual animal data were provided. No ChE measurements were taken.]

♦ ***Kowalski RL, Clemens GR, Bare JJ & Hartnagel Jr. RE (1987a) A teratology study with azinphos-methyl (Guthion® technical) in the rat. Report no. MTD0043. Lab: Miles Inc., Toxicology Department, Elkhart, IN, USA. Sponsor: Mobay Chemical Corp., Kansas City, MO, USA. Study duration: 7 July 1987 - 7 August 1987. Report date: 22 December 1987.***

Guidelines and GLP: The study was performed according to OECD guideline 414, adopted 12 May 1981, with additional ChE determinations. The study is GLP compliant [40 CFR Part 160 (FIFRA) and 792 (TSCA)]. The study is considered acceptable.

Material and Methods: Groups of 33 [naturally] inseminated Crl:CD BR rats (source: Charles River Breeding Laboratories, Portage, MI, USA) [13-wk old, weighing 209–322 g and having been acclimatised for at least 7 d] received azinphos-methyl (batch no. 79-R-225-42/5FEB87, purity 87.7%, [Mobay Chemical Corp., Kansas City, MO, USA.]) orally, by intubation in a 6% aqueous Emulphor emulsion [daily] from day 6 to day 15 of gestation at dose levels of 0, 0.5, 1.0 and 2.0 mg/kg bw/d. [Rats were mated at a ratio of 1 male to 2 females overnight with the presence of spermatozoa in vaginal smears indicating successful mating, and thus designated day one gestation. Prior to commencement of the study, the concentration, homogeneity and stability of azinphos-methyl in the test vehicle was verified by HPLC]. The dose levels were selected on the basis of a [previous] range finding study. [Test and control samples were administered at a constant dose volume of 10 mL/kg based on the body weight obtained on day 6 of gestation.]

[Following insemination, all dams were housed individually in suspended cages (cage type and source unspecified). Food (Purina Certified Rodent Chow #5002; Ralston Purina Company, St Louis, MO, USA) and water (unspecified source) were available *ad libitum* throughout the study. Room temperature and humidity were maintained at 65–76°F and 40-65% respectively. A 12h light/dark cycle was maintained throughout the study.

From each group, 5 dams were sacrificed [by CO_2 asphyxiation] on gestation day 16 and the remaining dams on day 20. Plasma, RBC and brain ChE activities in dams were determined on days 16 and 20. [Whole blood (unspecified volume) was collected by cardiac puncture.] Brain ChE activity was also determined in 20 foetuses/group on gestation day 20. [Each dam was observed daily for any clinical signs including changes in appearance or behaviour. Food consumption was recorded at day 0, 5, 6, 11, 15 and 19 of gestation. The following parameters were assessed in dams that were sacrificed on day 16: body weight (0, 6, 8, 10, 12, 15 and 16); examination of abdominal and thoracic viscera; pregnancy rates; and gross pathological abnormalities. In the remaining dams that were sacrificed on day 20 gestation, the following parameters were assessed: body weight (on day 0, 6, 8, 10, 12, 15 and 20 of gestation); pregnancy rates; the number of dams with live progeny, corpora lutea; implantations and resorptions; litter size; foetal weights and viability; placental weights; uterine weights; foetal sex ratios; pre- and post-implantation loss; examination of the abdominal and thoracic viscera; and gross pathological anomalies. Approximately half the foetuses from each dam were sacrificed by intracranial injection of barbiturate and examined for gross external, visceral and skeletal dysgenesis.]

Statistical methods: Dunnett's test (body weight, feed consumption, ChE); Fisher's exact test, Kruskall-Wallis and Dunn's test (reproductive data); Chi-square test, Fisher's exact test (fetal skeletal data).

Findings

[*Dietary analysis*: The homogeneity, ai content and stability (at ambient temperature for 27 d) of azinphos-methyl in the vehicle were within a tolerance range of $\pm$ 5% of the nominal concentrations.]

Appearance, behaviour, [mortality], feed consumption, and body weight gain of the dams were not adversely affected by treatment at any of the dose levels employed. [There were no gross pathological abnormalities in dams that could be attributed to the administration of azinphos-methyl.]

Statistically significant ($p \leq 0.05$) and/or biologically relevant (> 20%) inhibition of ChE in all three compartments occurred in the high dose group at day 16. [A biologically significant (37%) though statistically insignificant inhibition of plasma ChE occurred in the high-dose group (see Table below). Both RBC and brain ChE activities were biologically (79 and 39% respectively) and statistically ($p < 0.05$) inhibited at this same dose (see Table below).] Practically complete restitution of the plasma ChE activity was found at day 20; recovery of erythrocyte and brain activities was incomplete. [At day 20, RBC ChE was inhibited by 23% while brain ChE was statistically significantly inhibited by 28% ($p < 0.05$) (see Table below).] The fetal brain ChE activity was not inhibited by the treatment of the dams.

Effect of gavage-administration of azinphos-methyl from day 5-16 of gestation on ChE activity (plasma, RBC: kU/l, brain: U/g, and% of control) in dams and foetuses

Dose level	0 mg/kg bw/d	0.5 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d
Plasma, day 16	1.73 (100%)	1.56 (90%)	1.64 (95%)	1.08 (63%)
RBC, day 16	0.38 (100%)	0.34 (90%)	0.34 (90%)	0.08* (21%)
Brain, day 16	2.53 (100%)	2.70 (107%)	2.55 (101%)	1.55* (61%)
Plasma, day 20	1.44 (100%)	1.47 (102%)	1.40 (97%)	1.32 (92%)
RBC, day 20	0.58 (100%)	0.59 (103%)	0.61 (106%)	0.44 (77%)
Brain, day 20	2.67 (100%)	2.72 (102%)	2.46 (92%)	1.91* (72%)
Fetal brain	1.04 (100%)	0.96 (91%)	1.04 (100%)	1.00 (96%)

* $p < 0.05$

There was no effect of treatment on the reproduction parameters [fertility and gestation indices; number of litters, copora lutea, foetuses; litter size; pre-implantation loss; number of implantations and resorptions; and post-implantation loss] and no evidence of embryotoxicity, foetotoxicity or teratogenicity was obtained. [The percentage of treated dams exhibiting resorptions was marginally higher (52.5, 63.0 and 61.5% at 0.5, 1 and 2 mg/kg bw/d respectively) than the control group (25.0%) but this was not considered to be biologically significant as the incidences fell within the range for historical controls (39.1–72.7%). There was an incidental decrease in the combined median weight of viable foetuses at 0.5 mg/kg bw/d, and although this result was statistically different to the control ($p < 0.05$), it was not considered to be treatment-related as there was no effect at 1.0 and 2.0 mg/kg bw/d. There was a significant increase ($p < 0.05$) in median post-implantation loss in dams treated with 1.0 mg/kg bw/d azinphos-methyl, but in the absence of a dose-response relationship this was not considered to be toxicologically significant.

The foetuses from maternal rats that were sacrificed at day 20 of gestation showed no treatment-related effects on mortality, foetal/placental weights, sex ratios or incidences of external and visceral anomalies. There were no treatment-related malformations, changes in the frequency of common variations, or delayed ossification of the foetal skeleton. There was a statistically higher incidence ($p < 0.01$) of incomplete ossification of certain vertebrae and sternebrae at 0.5 mg/kg bw/d compared to the control, but in the absence of an effect at 1.0 and 2.0 mg/kg bw/d this was not considered to be biologically significant. There was incomplete ossification in the 4th sternebral in treated foetuses, with the result statistically higher than the control at 0.5 and 2.0 mg/kg bw/d ($p < 0.01$ and 0.05 respectively) and

marginally higher at 1.0 mg/kg bw/d azinphos-methyl. In the absence of a clear dose-response effect and relevant historical data the toxicological significance of this result was unclear.]

Conclusion: The NOEL for PO administration of azinphos-methyl to pregnant rats was 1.0 mg/kg bw/d with respect to maternal toxicity, based on inhibition of RBC and brain ChE activity at 2.0 mg/kg bw/d. The NOEL for developmental toxicity was 2.0 mg/kg bw/d, the highest dose tested.

Kowalski RL, Clemens GR, Bare JJ & Hartnagel Jr. RE (1987b) Addendum: A teratology study with azinphos-methyl (Guthion® technical) in the rat. Report No. 94987. Lab: Miles Inc., Toxicology Department, Elkhart, IN, USA. Sponsor: Mobay Chemical Corp., Kansas City, MO, USA. Study duration: 7 July 1987 - 7 August 1987. Report date: 22 December 1987.

This is an addendum to the previous teratology study and identifies the vehicle used as Emulphor® (EL-719) (GAF Corp. New York, NY, USA). Emulphor® was used to dissolve azinphos-methyl during dose preparation and studies have shown that it has an acute PO LD_{50} of 70 cc/kg (Farm Chemicals Handbook, 1988).

Rubin Y & Nyska A (1988). Cotnion - M Teratogenicity study in the rat. Report no. R4678. Lab: Life Science Research Israel Ltd, Ness Zion Israel, Project no. MAK/124/AZM. Sponsor: Makhteshim Chemical Works Ltd., Beer Sheva, Israel. Study duration 19 July 1987 – 25 August 1987. Report date: 10 February 1988.

Guidelines and GLP: The study was performed according to OECD guideline 414, adopted 12 May 1981, and US EPA Pesticide Assessment Guidelines Subdivision F, Section 83-3. The study is GLP compliant [40 CFR Part 160 (FIFRA), OECD 1982, and US FDA Title 21, Part 58]. A Quality Assurance Report was issued for this study.

Materials and methods: Two batches of sexually-mature female Sprague-Dawley-derived CD rats (Charles River, UK; 56 to 64 days old (180-200 g) or 47 to 50 days old (150-170 g)) were acquired and then acclimatised for a 12-day period before being paired one-to-one with males (same breeding stock). Where ejected mating plugs were found, a vaginal smear was prepared and a semi-quantitative estimate made of sperm in the vaginal tract. Females that displayed unequivocal evidence of mating were then used in the study, and males were re-used for subsequent matings after a minimum 2-day rest period. Animals were housed individually throughout the study, and were fed a commercial pelleted rodent diet (Altromin 1314) and drinking water *ad libitum*. The animal room had its own supply of filtered air (at least 14 room air changes per hour) and temperature and humidity (targeted values 21 $\pm$ 2 degrees C and 55 $\pm$ 15%, respectively) were recorded daily. Mated females were allocated to treatment groups in order of mating and with a similar weight distribution between groups. Azinphos-methyl (Makhteshim; 92.7% purity; batch 50287) was given by PO gavage (maize oil vehicle; dose volume 2 mL/kg bw) to females at doses of 0 (vehicle control), 0.4, 1.2, and 3.6 mg/kg bw/d from day 6 to day 15 post coitum inclusive, with 22 animals per dose group. The short-term (4h) stability of the test material in maize oil was confirmed (analytical method not specified, limit of detection 0.01 mg/mL).

Observations: All females were observed daily for signs of ill health or reaction to treatment, and were weighed on days 0, 3, 6-15, 17 and 20 of gestation (post coitum), and food

consumption was measured twice weekly. Animals were killed by CO_2 asphyxiation on day 20 post coitum, and the following was recorded for each animal: any macroscopic abnormality of the reproductive tract, number of corpora lutea in each ovary, weight of the gravid uterus, distribution of live and dead foetuses and distribution of resorption sites, individual placental weights, individual foetal weight, crown-rump length and sex, and external foetal anomalies. The thoracic and abdominal contents of approximately half of each litter were dissected and examined, and then these foetuses were fixed in ethanol for skeletal staining (alizarin red) and evaluation. The remaining foetuses were fixed in Bouin's fluid for preparation of free-hand sections and examination of the visceral organs. Macroscopic lesions were preserved and subjected to histopathological examination at the discretion of the Study Director.

Statistical analyses: Maternal gross observational signs were tested using the Fischer exact test. The Student's T-test using pooled within group error variance was used to test data on maternal food consumption and body weight, uterine, foetal and placental weights, foetal length, numbers of corpora lutea and live foetuses, and percent pre- and post-implantation loss (Freeman-Tukey transformed data). Foetal data (observations at necropsy, at free-hand sectioning and at skeletal examination) were tested using the Chi-square or Fischer exact test. Litter mean data were tested non-parametrically using the Mann-Whitney U-test.

Pre- and post-implantation loss: Pre-implantation loss included losses due to non-fertilisation of ova and very early post-implantation deaths (ie those occurring up to days 9-10 of gestation). Post-implantation loss included losses during the period between days 8 or 9 and 20 of gestation, but not those that occurred during the first 3-4 days post-implantation.

Results

In-treatment observations: No deaths or treatment-related clinical signs were noted during the study. Food consumption during gestation was similar in control and treated females. The group mean body weight of females at 3.6 mg/kg bw/d was slightly lower than controls during the period prior to treatment, but bodyweight and bodyweight gain were unaffected by treatment with azinphos-methyl.

Litter observations: Mean placental weights and foetal lengths and body weights were similar in control and treated groups. There were no treatment-related changes in the gravid uterine weights, the number or sex of live foetuses, or the number of resorptions. There was a slight increase in the number of litters with one or more resorption, but this effect was not statistically significant, and was not considered to be treatment-related (see Table below).

Number of Litters with One or More Resorption

Group	1	2	3	4
Dosage (mg/kg/d)	0	0.4	1.2	3.6
Litters examined	21	21	22	21
Litters affected	8	10	12	13

The incidences of pre- and post-implantation loss at 1.2 and 3.6 mg/kg bw/d were statistically significantly different to controls, but the relationship between these effects and treatment is questionable. Pre-implantation losses include losses that occurred prior to treatment and losses that occurred up to day 3 or 4 of treatment. However, the incidence of pre-implantation loss at 3.6 mg/kg bw/d was reduced compared with controls and so in the

absence of a dose-response for this finding the increased incidence of pre-implantation loss at 1.2 mg/kg bw/d was considered to be unrelated to treatment.

The incidence of post-implantation loss was increased at 1.2 and 3.6 mg/kg bw/d ($p<0.05$, Student's t-Test), but there was no dose-response relationship for this effect and the incidence at both these doses fell within the historical control range for this testing laboratory (mean $\pm$ SD percentage for post-implantation losses ranged from 4.1 $\pm$ 0.9 to 7.0 $\pm$ 2.1). In addition, these findings were not statistically significantly increased compared with controls under non-parametric assumptions (Mann-Whitney U-test). As such, the increases in post-implantation loss at 1.2 and 3.6 mg/kg bw/d were not considered to be related to azinphos-methyl treatment. A summary of the litter data is provided in the Table below.

Group Mean Litter Data on Day 20 of Gestation

Dose (mg/kg)		Corpora lutea	Weight of gravid uterus (g)	1.1.1.1.1Live foetuses			Resorptions			Implantation loss		Mean foetal wt. and SD (g)	Mean CRL and SD (mm)	Mean placental wt. and SD (g)
				M	F	Total	Early	Late	Total	pre-(%)*	post-(%)*			
0.0	Mean	16.2	73.3	6.3	7.7	14.0	0.7	0.0	0.7	6.3	4.9	3.36	36.4	0.48
	SD	2.6	10.0	2.3	2.4	2.0	1.0	0.0	1.0	4.3	1.6	0.21	0.8	0.03
	N = 21													
0.4	Mean	16.1	74.2	7.4	6.9	14.3	0.5	0.0	0.5	5.4	4.8	3.32	36.2	0.48
	SD	2.5	9.8	1.9	1.8	2.1	0.6	0.0	0.6	3.6	1.1	0.22	0.8	0.04
	N = 21													
1.2	Mean	16.6	72.5	7.5	6.6	14.0	1.0	0.0	1.0	9.0[b]	6.9[c]	3.34	36.6	0.48
	SD	1.8	10.9	2.1	2.7	2.1	1.3	0.2	1.2	2.2	2.2	0.20	0.6	0.05
	N = 22													
3.6	Mean	15.8	73.7	7.2	7.1	14.3	0.8	0.1	0.9	4.3[a]	6.8[c]	3.37	36.4	0.47
	SD	1.9	6.8	2.7	2.4	1.8	0.8	0.3	0.8	1.1	1.4	0.21	1.0	0.05
	N = 21													

[a] Significantly different from contol, $p < 0.05$, Students t-test
[b] Significantly different from contol, $p < 0.01$, Students t-test
[c] Significantly different from contol, $p < 0.001$, Students t-test
* Freeman – Turkey arcsine transformed data

Foetal observations: A single high-dose foetus was severely runted, and presented with multiple external malformations, including ectrodactyly, acaudia, and hind limb arthrogryphosis. Single incidences of hydroureter (3.6 mg/kg bw/d) and interrupted aortic arch (1.2 mg/kg bw/d) were also reported. The low incidence and sporadic nature of foetal anomalies were not suggestive of a relationship with treatment.

Free-hand sectioning did not reveal any findings that could be attributed to treatment. A single foetus in the high-dose group that had multiple external malformations was found to have multiple internal malformations also, including severe hydrocephalus with apparent agenesis of olfactory lobes, generalised oedema, apparent agenesis of the thyroid gland, agenesis of the left kidney, ovary and adrenal gland, agenesis of the urinary bladder, and double right kidney. This isolated instance of a foetus with malformations was not considered to be treatment-related.

Skeletal observations revealed some retardation of ossification in foetuses at 3.6 mg/kg bw/d, and this reduced or absent ossification was seen in the supraoccipital, pubic and hyoid bones. An increased incidence of supernumerary ribs (14^{th} lumbar) was also observed at 3.6 mg/kg bw/d (4.7%; $p<0.01$), and the incidence of this finding was outside the historical control range from the testing laboratory (0 to 3.1%). These effects are consistent with treatment-related delayed development, and occurred in the absence of any notable signs of maternal toxicity. Reduced or incomplete ossification of the pubic bones was also noted at 0.4 mg/kg bw/d, but as the incidence of this effect was not increased at 1.2 mg/kg bw/d this finding at the low dose was not considered to be treatment-related.

The significant skeletal observations are summarised in the Tables below.

Skeletal observations – number (percent) of affected foetuses

Dose (mg/kg bw/d)	0.0	0.4	1.2	3.6
Number of foetuses examined	147	150	157	149
Skull				
Supraoccipital bone: reduced or incomplete ossification	24 (16.3)	34 (22.7)	24 (15.3)	40 (26.8)*
Hyoid bone unossified	17 (12.1) (N=140)	27 (18.9) (N=143)	16 (10.7) (N=149)	37 (27.4)** (N=135)
Spinal Column & Thorax				
14^{th} (lumbar) rib present unilaterally	0 (0.0)	1 (0.7)	1 (0.6)	7 (4.7)***
Appendicular Skeleton				
Pubis: reduced or incomplete ossification / unossified	4 (2.7)	12 (8.0)****	6 (3.8)	18 (12.1)***

* significantly different from control, $p < 0.05$, Chi-square test; ** significantly different from control, $p < 0.01$, Chi-square test; *** significantly different from control, $p < 0.01$, Fisher exact test; **** significantly different from control, $p < 0.05$, Fisher exact test

Skeletal observations – litter distribution of affected foetuses

Dose (mg/kg bw/d)	0.0	0.4	1.2	3.6
Number of litters examined	21	21	22	21
	N (Mean %)			
Skull				
Hyoid bone unossified	10 (13.1)	12 (17.6)	10 (11.8)	14 (28.1)*
Spinal Column & Thorax				
14th (lumbar) rib present unilaterally	0 (0.0)	1 (0.7)	1 (0.6)	7** (4.9)#
Appendicular Skeleton				
Pubis: reduced or incomplete ossfication / unossified	3 (2.6)	8 (8.2)*	4 (3.8)	9*** (11.8)*

* significantly different from control, $p < 0.05$, Mann-Whitney U-test; ** significantly different from control, $p < 0.01$, Fisher exact test; *** significantly different from control, $p < 0.05$, Fisher exact test; # significantly different from control, $p < 0.01$, Mann-Whitney U-test

Conclusions: The NOEL for maternal toxicity was 3.6 mg/kg bw/d, with no effects seen at any dose in this study. The NOEL for developmental toxicity was 1.2 mg/kg bw/d, based on increases in the incidence of supernumerary ribs (14th, lumbar) and delayed ossification (pubic, hyoid, and supraoccipital bones) in foetuses at 3.6 mg/kg bw/d. No structural malformations were observed in foetuses at any dose. ChE activity was not measured in this study.

2.8.3 Rabbits

♦ ***Machemer L (1975) R1582 (active ingredient of Gusathion®). Studies for embryotoxic and teratogenic effects on rabbits following oral administration. Report no. 5455. Lab & Sponsor: Bayer AG, Toxicology Department, Wuppertal-Elberfeld, Germany. Study duration: January 1975 - June 1975. Report date: 3 June 1975.***

Guidelines and GLP: The test method employed was the internal standard at the time the study was performed. The study is in general compliance with OECD guideline 414, adopted 12 May 1981. When the study was performed, GLP was not compulsory. Main deviations from current OECD guidelines: Only 11 pregnant animals were used in the control group. The selected dose levels were too low to induce some overt maternal toxicity. The study is considered supplementary.

Material and Methods: Groups of 11 or 12 inseminated Himalayan rabbits [weighing 2–2.5 kg, acclimatisation period unspecified] (source: Dr. Karl Thomae GmbH, Biberach, Germany) received azinphos-methyl (batch no. not specified, rcvd. 1/73, purity 92.4%, [source unspecified]) orally, by intubation in a 0.5% aqueous Cremophor emulsion from day 6 to day 18 of gestation at dose levels of 0, 0.3, 1.0 or 3.0 mg/kg bw/d. [The applied volume being constant at 5 mL/kg body weight in each group.] The dose levels were selected on the basis of a [previous] range finding study [in non-pregnant rabbits. Each doe was mated with 1 buck under observation with repetition of copulation occurring after approximately 1 h]. Caesarian section was carried out on day 29 of gestation. [Rabbits were housed individually in cages made of perforated plate (48 x 39 x 32 cm), and received HOING Rabbit Food 222 (HOING, Kraftfutterwerk Niedersachsen, Verder/Aller) and tap water *ad libitum*. Room temperature and humidity were maintained at 20–23°C and 60% respectively. A 12 h light/dark cycle was maintained throughout the study.

Maternal rabbits were observed for any clinical signs and mortalities (unspecified frequency). Maternal body weights were recorded (unspecified frequency). Rabbits were sacrificed by an unspecified means. Litter and average foetus weights, the number of stunted foetuses (weighing < 25 g) and the sex of all foetuses was recorded. All foetuses were examined for external malformations. The abdominal and thoracic organs were examined for any abnormalities. The presence of brain and skeletal malformations was also assessed in all foetuses.]

Statistical methods: U test of Wilcoxon, Mann and Whitney (weight gain, no. of implantations, foetuses and resorptions, foetus weight, placenta weight), Chi-square test (no. of foetuses with alterations or malformations), Chi-square test or Fisher's exact test (quotas of fertilized and pregnant does). [Results were considered to be statistically significant when $p < 0.05$.]

Findings: Azinphos-methyl had no adverse effect on the appearance, behaviour and body weight gain of the animals at any of the dose levels employed, and all does survived to caesarean section. At 1 mg/kg bw/d, 2 does resorbed all foetuses. At 3 mg/kg bw/d, one doe also resorbed the foetuses and another doe aborted. The observed frequency is within the normal range for the rabbit strain used in this study. There were no detectable effects on the number of foetuses, number of resorptions, foetal weight, placental weight, number of stunted foetuses, and number of foetuses with slight alterations in bone development or with malformations. [The study authors indicated that the statistically higher ($p < 0.05$) average foetus weight observed at 3 mg/kg bw/d was a random effect (40.39 g vs 37.64, 39.54 and 37.08 g for the control, 0.3 and 1.0 mg/kg bw/d groups respectively). There was no treatment-related effect on the sex ratio of foetuses. No malformations were revealed upon external examination, examination of the skeleton or dissection of the head.]

Conclusion: The NOEL for PO administration of azinphos-methyl to pregnant rabbits was 3.0 mg/kg bw/d. The tested dose levels did not induce maternal toxicity and had no detectable effects on embryonic nor foetal development.

However, the study can not be regarded as satisfactory by OECD standards since the selected dose levels were too low to induce some overt maternal toxicity.

[*Comments:* A thorough macroscopic examination of maternal animals was not performed. Maternal food consumption was not recorded. Measurement of ChE activity did not occur. Analysis of the ai, stability and homogeneity of azinphos-methyl in the vehicle was not performed.]

♦ ***Clemens CR, Bare JJ & Hartnagel Jr RE (1988) A teratology study in the rabbit with azinphos-methyl (Guthion® technical). Report no. MTD0070. Lab: Miles Inc., Toxicology Department, Elkhart, IN, USA. Sponsor: Mobay Chemical Corp., Kansas City, MO, USA. Study duration: 22 September 1987 - 23 October 1987. Report date: 27 June 1988.***

Guidelines and GLP: The study was performed according to OECD guideline 414, adopted 12 May 1981, with additional ChE determinations. The study is GLP compliant [40 CFR Part 160 (FIFRA) and 792 (TSCA)]. The study is considered acceptable.

Material and Methods: Groups of 20 [artificially] inseminated American Dutch rabbits (source: Langshaw Farms, Augusta, MI, USA) [that weighed 2.22–3.16 g (acclimatisation

period unspecified)] received azinphos-methyl (batch no. 79-R-225-42/5FEB87, purity 87.7%, [Mobay Chemical Corp., Kansas City, MO, USA.]) orally, by [daily] gavage in a 7% aqueous Emulphor emulsion from day 6 to day 18 of gestation at dose levels of 0, 1, 2.5 and 6 mg/kg bw/d. The dose levels were selected on the basis of a range finding study. [The concentration, homogeneity and stability of azinphos-methyl in the test vehicle was verified by HPLC. The applied volume was 4 mL/kg body weight in each group based on the body weight obtained on day 6 of gestation. Randomly selected does were artificially inseminated over a 4-day period with semen collected from bucks of proven fertility.

All rabbits were housed individually in stainless steel cages and allowed water *ad libitum* and 130 g Purina Certified Rabbit Chow #5322 (Ralston Purina Company, St Louis, MO, USA) per day except during the time when food consumption was monitored. Room temperature and humidity were maintained at 64–69°F and 40–70% respectively. The type of light/dark cycle was unspecified.

Does were observed daily for clinical signs, changes in behaviour and appearance. Body weights were recorded on day 0, 6, 10, 14, 18, 21 and 28 of gestation. Food consumption was recorded on day 1, 6, 7, 12, 15, 19, 23 and 28 of gestation.] Caesarian section was performed on day 28 of gestation. Plasma and RBC ChE activities were determined on day 19 and 28 of gestation, and in brain on day 28 of gestation. [Blood samples (unspecified volume) were obtained from the auricular artery. All does were sacrificed by iv barbiturate overdose on day 28 of gestation. The following parameters were evaluated: pregnancy rates, does with live progeny, copora lutea, uterine horn weight, implantations, resorptions, litter size, foetal weights, foetal viability, placental weights, foetal sex ratios, and pre- and post-implantation loss. A gross pathological examination was performed on all does at necropsy. All foetuses were examined for gross external, visceral and skeletal abnormalities.]

Statistical methods: Dunnett's test (body weight, feed consumption, ChE); Fisher's exact test, Kruskall-Wallis and Dunn's test (reproductive data); Chi-square test, Fisher's exact test (fetal skeletal data), Healy's test (fetal weight).
Findings

[*Dietary analysis*: The homogeneity, ai content and stability (at ambient temperature for 27 days) of azinphos-methyl within the vehicle were within a tolerance range of $\pm$ 5% of the nominal concentrations.]

Although no animals were found dead during the study, a single doe from the mid-dose group and 2 does from the high-dose group were sacrificed (unspecified times), each due to an apparent broken back.] At 6 mg/kg bw/d, ataxia was noted in 4 does and tremors in 2 of these same animals. [A single control doe had soft, little or no stools and a red-coloured discharge prior to aborting 10 progeny on day 27. Two mid-dose does aborted progeny on day 15 or day 27 but showed no detectable changes in appearance or behaviour.] Body weight and feed consumption were unaffected by treatment at all dose levels. [There were no gross pathological abnormalities observed in does at necropsy that could be attributed to the administration of azinphos-methyl. The 2 does from the mid-dose group that aborted exhibited pulmonary changes indicative of dosing trauma or a respiratory tract infection.]

At both the intermediate and high dose levels, statistically significant ($p < 0.05$) and/or toxicologically meaningful (> 20%) inhibition of plasma and erythrocyte ChE activity was noted on day 19 of gestation [(see Table below). At 6 mg/kg bw /d both plasma and RBC

ChE activities were statistically ($p < 0.05$; 22 and 50% respectively) lower than the control group. The effect at 2.5 mg/kg bw/d was less definitive with a 13% depression in plasma ChE activity detected at day 19 of gestation ($p < 0.05$). In the mid dose group there was a 20% depression in RBC ChE activity which was not statistically significant.] The values returned to normal near term at day 28 of pregnancy. Brain ChE activity was inhibited to a statistically significant extent at 6 mg/kg bw/d [($p < 0.05$; 12%) (see Table below)].

Effect of daily administration of azinphos-methyl by PO gavage from day 6-18 of gestation on maternal ChE activity (plasma, RBC: U/l, brain: U/g, and% of control)

Dose level	0 mg/kg bw/d	1 mg/kg bw/d	2.5 mg/kg bw/d	6 mg/kg bw/d
Plasma, day 19	396 (100%)	404 (102%)	345* (87%)	307* (78%)
RBC, day 19	615 (100%)	530 (86%)	489 (80%)	307* (50%)
Plasma, day 28	200 (100%)	240 (120%)	212 (106%)	229 (115%)
RBC, day 28	564 (100%)	592 (105%)	595 (105%)	493 (87%)
Brain, day 28	2.21 (100%)	2.11 (96%)	2.08 (94%)	1.94* (88%)

* $p < 0.05$

Administration of azinphos-methyl produced no meaningful adverse effect on any maternal reproductive or fetal parameter studied. [There were no treatment-related effects on fertility and gestation indices, the number of litters, the number of corpora lutea, the total number of implantations, post-implantation loss, the sex ratio of foetuses, the total number and viability of foetuses, the number of resorption sites, and the number of resorptions. The median litter size of the high-dose group was statistically lower ($p < 0.05$) than the control group but fell within the performing laboratory's historical control range.] There was, however, a statistically significant increase [$p < 0.01$–0.05] in pre-implantation loss for all 3 treatment groups, when compared with the control [see Table below]. The median values for the low- and mid-dose group fell within the laboratory's historical control range (0-13.3%).

According to the study authors, the increased pre-implantation loss in the high-dose group is not believed to be treatment-related but, rather, an incidental finding, resulting from a decrease in number of implantations compared with the number of corpora lutea for this group. In the range-finding study, at a dose level of 7 mg/kg bw/d, and in a prior study in rabbits, at a dose level of 6 mg/kg bw/d, pre-implantation loss was not increased. [Pre-implantation loss would, in any case, have occurred prior to initiation of treatment.]

Due to a slight but statistically significant reduction in litter size (within the laboratory's historical control range), fetal and placental weights were [significantly] increased in the high-dose group [$p < 0.01$ and 0.05 respectively (see Table below). The effect on viable foetal weight was not considered to be toxicologically relevant as it fell within the upper limit for the historical control range (34.7–39.8 g). The effect on median placental weight was not strongly significant as it fell just outside the historical control range (5.1–5.6).]

There were no external and visceral abnormalities on foetuses at termination on day 28 that could be attributed to the administration of azinphos-methyl. Additionally there were no treatment-related effects on the development of the foetal skeleton or on the incidence of malformations or variations.

Effect of daily administration of azinphos-methyl by PO gavage from day 6-18 of gestation on reproductive efficiency and foetal weight

Dose level (mg/kg bw/d)	0	1	2.5	6
Pregnant/total does	18/20	18/20	20/20	20/20
Litter size, mean/median	7.4/7.0	6.2/7.0	7.0/7.0	5.5/6.0*
Pre-implantation loss, mean/median (%)	1.5/0.0	23.0/11.3**	14.8/12.5*	28.0/30.3**
Post-implantation loss, mean/median (%)	2.4/0.0	3.0/0.0	4.3/0.0	7.2/0.0
Weight of viable foetuses, median (g)	37.1	38.2	36.1	39.4**
Placenta weight, median (g)	5.4	5.4	5.1	6.0*

* $p < 0.05$; ** $p < 0.01$

Conclusion: The NOEL for PO administration of azinphos-methyl to pregnant rabbits was 1.0 mg/kg bw/d with respect to maternal toxicity, based on inhibition of RBC and plasma ChE activity at 2.5 mg/kg bw/d and above and clinical signs [ataxia, tremor] and inhibition of brain ChE activity at 6 mg/kg bw/d. [At 6 mg/kg bw/d, the depression of brain ChE activity was only 12% while RBC and plasma ChE activities were unaffected.] The NOEL for developmental toxicity was 6 mg/kg bw/d, the highest dose tested.

Gal N, Rubin Y, Nyska A & Waner T (1988) Cotnion - M Teratogenicity study in the rabbit. Report no. R4935. Lab: Life Science Research Israel Ltd, Ness Zion Israel, Project no. MAK/126/AZM. Sponsor: Makhteshim Chemical Works Ltd., Beer Sheva, Israel. Study duration: 10 August 1987 – 20 October 1987. Report date: 15 June 1988.

Guidelines and GLP: The study was performed according to OECD guideline 414, adopted 12 May 1981, and US EPA Pesticide Assessment Guidelines Subdivision F, Section 83-3. The study is GLP compliant [40 CFR Part 160 (FIFRA), OECD 1982, and US FDA Title 21, Part 58]. A Quality Assurance Report was issued for this study.

Materials and methods: Sexually-mature female New Zealand White rabbits (Charles River, Italia; 4-5 months old; 3.0-3.2 kg) were acquired and then acclimatised for a 20-day period before being paired one-to-one with males (same breeding stock). Following mating, each female was administered with 51 IU chorionic gonadotrophin by iv injection to ensure ovulation took place. The day of mating was defined as gestation day 0, but the success of mating was not otherwise determined. Animals were housed individually throughout the study, and were fed a commercial pelleted rabbit diet (Altromin 2113) and drinking water *ad libitum*. Mated females were randomly allocated to treatment groups prior to mating. Azinphos-methyl (Makhteshim; 92.7% purity; batch 50287) was given by PO gavage (maize oil vehicle; dose volume 2 mL/kg bw) to females at doses of 0 (vehicle control), 1.5, 4.75, and 15.0 mg/kg bw/d from day 7 to day 19 post coitum inclusive. The formulated dosing solutions were analysed twice (methos of analysis not stated; limit of detection 0.01 mg/mL) to verify achieved concentration and short-term (4 h) stability of the formulated test compound. The group size was initially 15/dose level, but additional animals were conscripted into some of the groups to ensure that sufficient pregnant animals were available at term after any non-pregnancies, deaths and/or abortions. The group sizes were adjusted to 16, 18, 15, and 18, at 0, 1.5, 4.75, and 15.0 mg/kg bw/d, respectively, and each group ultimately consisted of at least 12 pregnant animals at terminal sacrifice.

Observations: All females were observed daily for signs of ill health or reaction to treatment, and were weighed on days 0, 3, 7-19, 22, 25 and 29 of gestation, and food consumption was

measured twice weekly. Any animal found dead or killed in extremis was necropsied. Animals were killed by pentobarbitone sodium injection on day 29 post- coitum, and the following was recorded for each animal: any macroscopic abnormality of the reproductive tract, number of corpora lutea in each ovary, weight of the gravid uterus, distribution of live and dead foetuses and distribution of resorption sites, individual placental weights, individual foetal weight, crown-rump length and sex, and external foetal anomalies. Each foetus was killed by pentobarbitone sodium injection, then animals were dissected and the contents of the abdominal and thoracic cavities were examined. The sex of each foetus was recorded, and the skull of each foetus was sectioned transversely through the frontal-parietal suture and the brain was examined in section. Each foetus was fixed in ethanol for skeletal staining (alizarin red) and evaluation.

Pre- and post-implantation loss: Pre-implantation loss included losses due to non-fertilisation of ova and very early post-implantation deaths (ie. those occurring up to days 10-11 of gestation). Post-implantation loss included losses during the period between days 10-11 and 29 of gestation, but not those that occurred during the first 3-4 days post-implantation.

ChE activity: ChE activity in RBC and plasma was measured for each animal before pairing and again after 11 days of dosing. Acetyl ChE activity was measured using a test kit, after the method of Ellman.

Results

In-treatment observations: Some slight reductions in group mean bodyweight gain were noted during treatment in dams at 4.75 and 15.0 mg/kg bw/d, but the magnitude of this effect was not great and group mean bodyweights were similar in all groups during the study. No consistent, treatment-related clinical signs were observed, but deaths occurred in all groups, including controls (one control animal, two animals at 1.5 mg/kg bw/d, one animal at 4.75 mg/kg bw/d, and three animals at 15 mg/kg bw/d). Death was attributed to intercurrent disease (controls, low and mid-doses) or lung dosing (high dose). A low incidence of abortions was observed in several groups, including controls, but this finding was not attributed to treatment. Food consumption was similar in control and treated groups.

ChE activity: After 11 days of dosing, RBC ChE activity was statistically significantly decreased ($p < 0.05$) by 27% at 15 mg/kg bw/d compared with controls. Statistically significant decreases in plasma ChE activity compared with controls ($p < 0.05$ or $p < 0.01$) were seen at all dose levels after 11 days of treatment. However, there was no consistent dose-response relationship for this effect, and the magnitude of the inhibition of activity was not great (22%, 29%, and 26%, at 1.5, 4.5 and 15 mg/kg bw/d) and so the toxicological significance of this finding is not clear. The pre-treatment plasma ChE activity in the 1.5 mg/kg bw/d group was 11% lower than the control group mean, and so the decrease in plasma ChE activity during the 11 days of dosing was only about 11% compared with controls. These data are summarised in the Table below.

ChE Activity: Percentage Inhibition

Dose (mg/kg bw/d)	Before Mating		After 11 ± 1 days dosing	
	Plasma ChE	RBC ChE	Plasma ChE	RBC ChE
1.50 N = 13	11	+21	22*	+8
4.75 N = 12	3	+11	29**	4
15.00 N = 14	3	+16	26*	27*

* Significantly different from control; $p < 0.05$, Student's t-test; ** Significantly different from control; $p < 0.01$, Student's t-test; + indicates increase in activity compared with controls

Litter observations: No statistically significant effects were observed on gravid uterus weight, number of live foetuses, resorptions, foetal weights, placental weights or crown-rump lengths. Statistically significant ($p < 0.001$) increases in pre-implantation loss were observed at 4.75 mg/kg bw/d only. Historical control data were not provided for this effect, and it is not possibly to differentiate between pre-implantation losses that arose before or after the initiation of treatment. However, in the absence of any dose-response relationship and with no increase in pre-implantation loss at the high dose, this finding was not considered to be treatment-related. In the absence of a dose-response relationship, the statistically-significant ($p<0.05$) changes in post-implantation losses were not considered to be treatment-related, with a decrease in the incidence at 1.5 mg/kg bw/d, an increase in incidence at 4.75 mg/kg bw/d, and no change at the high dose level. Litter observations are summarised in the Table below.

Summary of litter observations

Dose (mg/kg)		Corpora lutea	Weight of gravid uterus (g)	1.1.1.1.2Live foetuses			Resorptions			Implantation loss		Mean foetal wt. and SD (g)	Mean CRL and SD (mm)	Mean placental wt. and SD (g)
				M	F	Total	Early	Late	Total	pre-(%)*	post-(%)*			
0.0	Mean	10.3	529.6	4.7	3.6	8.4	0.4	1.1	1.5	5.6	13.0	44.3	95.2	5.8
	SD	1.9	109.6	1.5	1.7	2.1	1.3	2.0	2.2	1.5	5.4	4.6	4.4	0.9
	N = 14													
1.5	Mean	10.7	601.6	4.8	4.2	9.1	0.4	0.5	0.9	7.2	9.0[a]	46.9	97.2	6.4
	SD	2.1	120.9	1.8	1.6	2.2	1.4	0.8	1.4	1.9	3.5	5.3	2.5	0.9
	N = 13													
4.75	Mean	10.8	425.1	4.2	2.9	7.1	0.6	0.8	1.4	16.9[b]	16.8[a]	46.1	97.7	6.4
	SD	2.2	187.6	2.8	2.1	3.8	1.2	1.3	1.6	15.2	4.4	9.8	8.5	1.8
	N = 12													
15.00	Mean	11.0	512.4	4.5	4.2	8.7	0.6	0.8	1.4	8.6	14.3	42.6	93.9	6.0
	SD	2.3	110.8	1.4	1.7	2.7	1.1	1.0	1.3	3.4	3.5	7.1	4.6	1.4
	N = 14													

[a] Significantly different from control, $p < 0.05$, Students t-test
[b] Significantly different from control, $p < 0.001$, Students t-test
* Freeman – Turkey arcsine transformed data
CRL=Crown Rump Length

Foetal observations: A statistically significant ($p < 0.05$) increase in the incidence of small foetuses (< 30 g in weight) was reported at 15 mg/kg bw/d. This incidence (8.2%) fell just within the testing facility's range of historical control mean values (0.7 – 8.4%), but may be indicative of delayed development resulting from treatment. No major foetal malformations were observed in the high-dose group. A small number of foetuses in the other groups, including controls, presented with malformations. These included a control foetus with a markedly enlarged aorta, rudimentary pulmonary trunk, absent subclavian artery, and hydrothorax. A single foetus in the 4.75 mg/kg bw/d group had multiple external and internal malformations, including brachygnathia with protruding tongue, right forelimb arthrogryposis, hydronephrosis, distended urinary bladder, distended ureter, hydrocephalus, and right retro-oesophageal aortic arch. Other minor structural variations occurred sporadically among treatment and control groups and in the absence of any dose-related increase in visceral malformations, these findings were considered to be incidental to treatment.

Skeletal examination revealed statistically-significant ($p < 0.001$) increases in the incidence of reduced ossification of long bone epiphyses in foetuses at 4.75 and 15 mg/kg bw/d, and the incidence of this finding (42.4 and 44.3%, respectively) was outside the range of historical control mean values of 10.7-35.9%. This finding may be indicative of delayed foetal development resulting from treatment with azinphos-methyl. At 15 mg/kg bw/d there was also an increased incidence of foetuses with asymmetric pelvic articulation (ilium articulating with first or first and second sacral vertebra unilaterally), and while the incidence of this finding (8.2%) was not statistically significantly different to controls, it was outside of the range of historical control mean values (0.9-7.6%). An increased incidence of reduced or incomplete ossification of the pubic bone was seen at 4.75 and 15 mg/kg bw/d, but this was statistically significantly different to controls only at the mid-dose level. An increased incidence of unossified pubic bones and reduced or incompletely ossified hyoid bones (high dose only) may also have been an indication of delayed foetal development. A single control foetus was found to have multiple spino-thoracic malformations, but no other treatment-related major skeletal malformations were observed at other doses. Significant foetal observations are summarised in the Table below.

Foetal Observations at Necropsy – Number (Percent) of Affected Foetuses

Dose (mg/kg bw/d)	**0.00**	**1.50**	**4.75**	**15:00**
Number of foetuses examined	117	118	85	122
External Observations				
Small foetus (<30.0g)	3 (2.6)	1 (0.8)	3 (3.5)	10 (8.2)*
Skull				
Interparietal-supra-occipital suture open	30 (25.6)	17 (14.4)**	14 (16.5)	17 (13.9)**
Reduced or incomplete ossification of hyoid bone	11 (9.4)	9 (7.6)	7 (8.2)	16 (13.1)
Spinal Column & Thorax				
Scoliosis associated with vertebral dysgenesis	1[#] (0.9)	1 (0.8)	0 (0.0)	0 (0.0)
Multiple spino-thoracic malformations: Atlas bipartite; 8th (supernumeracy) cervical vertebra present; thoracic vertebral centra 8 and 9 bipartite; 4th and 5th ribs (right) fused proximally with bony projection; 6 lumbar vertebrae only present; agenesis of	1[#] (0.9)	0 (0.0)	0 (0.0)	0 (0.0)

Dose (mg/kg bw/d)	0.00	1.50	4.75	15:00
sacral vertebrae 3 and 4 and all caudal vertebrae; manubrium unossified				
Appendicular Skeleton				
Reduced ossification of long bone epiphyses	21 (17.9)	30 (25.4)	36 (42.4)***	54 (44.3)***
Ilium articulating with 1st + 2nd sacral vertebra (unilaterally)	4 (3.4)	4 (3.4)	6 (7.1)	10 (8.2)
Pubes: reduced or incomplete ossification	1 (0.9)	3 (2.5)	5 (5.9)*	5 (4.1)

* Significantly different from Control; p<0.05, Fisher exact test; ** Significantly different from Control; p<0.05, Chi-square test; *** Significantly different from Control; p<0.001, Chi-square test; # These observations occurred in the same foetus

Conclusions: The NOEL for developmental effects was 1.5 mg/kg bw/d, based on retarded ossification of the long bones at 4.75 and 15 mg/kg bw/d, and other skeletal variations (asymmetric pelvic articulation) and reduced foetal size at 15 mg/kg bw/d. No frank maternal toxicity was observed at any dose (NOEL 15 mg/kg bw/d). Statistically-significant decreases in plasma cholinesterase activity were seen in dams at all dose levels, but the lack of a dose-response relationship and the magnitude of this effect (22-29% reduced compared with controls) makes the toxicological significance of this finding equivocal. The NOEL for inhibition of erythrocyte cholinesterase activity was 4.75 mg/kg bw/d, based on a 27% reduction in activity at 15 mg/kg bw/d after 11 days of treatment. The lack of any demonstrated signs of maternal toxicity (reductions in body weights, clinical signs) was a deficiency of this study.

2.9 GENOTOXICITY

A summary of submitted and published findings of genotoxicity studies with azinphos-methyl is shown in the Table below.

Summary of Mutagenicity Testing with Azinphos-methyl

Assay	Bacterial strain or Cell type	Concentration (or Dose)	Metabolic activation	Results	Reference
Gene Mutation					
S. typhimurium	TA98 TA100 TA1535 TA1537 TA1538	0-160 µg/plate	+,-	-,-	Evenchik (1987)
	TA 98 TA 100 TA 1535 TA 1537	4-2500 µg/plate	+,- +,- +,- +,-	-,- -,- -,- -,-	♦Herbold (1978)
	TA 98 TA 100 TA 1535 TA 1537	75-9600 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	♦Herbold (1988) (GLP)
	TA 97 TA 98 TA 100 TA 102	NS	+, - +, - +, - +, -	-, - -, - -, - -, -	Hrelia *et al* (1990)†
	TA 98 TA 100 TA 1535 TA 1537 TA 1538	33-4000 µg/plate	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	♦Lawlor (1987) (GLP)
	TA 98 TA 100 TA 1535 TA 1537 TA 1538	1 µg-10 mg/plate	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Sandhu *et al* (1985)†
	TA 100 TA 1535 TA 1537 TA 1538	1-1000 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	Simmon (1978)
	TA 98 TA 100 TA 1535 TA 1537 TA 1538	NS	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	♦Waters *et al* (1982)†
E. coli	WP2 (uvrA)	Up to 10 mg/plate	+, -	-, -	Sandhu *et al* (1985)†
	WP2 ($uvrA^-$)	1-1000 µg/plate	+, -	-, -	Simmon (1978)
		NS	+, -	-, -	♦Waters *et al* (1982)†
S. cerevisiae	S138 and S211α	33.3-10000 µg/mL	+,-	-, -	♦Hoorn (1983)
	D7	10-50 mg/mL	+, -	-, -	Sandhu *et al* (1985)†
	D7	NS	+, -	-, -	♦Waters *et al* (1982)†

Assay	Bacterial strain or Cell type	Concentration (or Dose)	Metabolic activation	Results	Reference
S. pombe	SP-198	3-95 mM	+, -	+, +2	Gilot-Delhalle *et al* (1983)†
Mammalian cells	Mouse lymphoma L5178Y	NS	+, -	+, +	Sandhu *et al* (1985)†
		NS	+, -	+, -	♦Waters *et al* (1982)†
DNA Damage and Repair					
^{32}P-postlabeling	Calf thymus DNA	1 mM3	+	+	Shah *et al* (1997)
Differential toxicity	*E. coli* W3110 *E. coli* p3478	625-10000 µg/plate	+, -	-, -	♦Herbold (1984)
		1 mg/disc	-	-	Simmon (1978)
	B. subtilis H17 *B. subtilis* M45	1 mg/disc	-	-	Simmon (1978)
Unscheduled DNA synthesis	NS	NS	+, -	-, -	Hrelia *et al* (1990)†
	Rat hepatocytes	0.25-50.3 µg/mL DMSO vehicle	-	-	♦Myhr (1983)
	Human lung fibroblasts (WI-38)	NS	+, -	-, -	Sandhu *et al* (1985)†
		10^{-7}-10^{-3} M	+, -	+, -	Simmon (1978)
		NS	+, -	-, -	♦Waters *et al* (1982)†
Recombination	*S. typhimurium* SL 4700 (rec$^-$/rfa$^-$) SL 4525 (rec$^+$/rfa$^-$) TA 1978 (rfa$^-$) TA 1538 uvrB$^-$/rfa$^-$)	NS	- - - -	- - - -	Sandhu *et al* (1985)†
		NS	- - - -	- - - -	♦Waters *et al* (1982)†
	S. cerevisiae D3	Up to 50 mg/mL	+, -	+, +4	Sandhu *et al* (1985)†
		4.5 and 5%	+, -	+, +	Simmon (1978)
		NS	+, -	+, +	♦Waters *et al* (1982)†
Reversion, gene conversion and crossing over	*S. cerevisiae* D7	500-25000 µg/mL	+, -	-, +	Bianchi *et al* (1994)
		NS	+, -	+	Hrelia *et al* (1990)†
		NS	+, -	-, -	Sandhu *et al* (1985)†
		NS	+, -	-, -	♦Waters *et al* (1982)†

Assay	Cell type	Concentration (or Dose)	Metabolic activation	Results	Reference
Chromosomal Effect Assays *(in vitro)*					
Sister Chromatid Exchange	Chinese hamster lung cells (V79)	5-25 µg/mL	+, -	-, -	♦Chen *et al* (1982a)
		2.5-20 µg/mL	-	-	♦Chen *et al* (1982b)

2 The number of mutants was approximately 2-fold higher in the absence of metabolic activation
3 Experiment performed on 'metabolites' of Guthion (azinphos-methyl) which were extracted with diethyl ether from a reaction mixture of Guthion and rat liver S9 homogenate.
4 The number of mutants was approximately 2-fold higher in the absence of metabolic activation

	Chinese hamster ovary cells	NS	+, -	-, -	Sandhu *et al* (1985)†
		NS	+, -	-, -	♦Waters *et al* (1982)†
	Human lymphocytes	2, 4, 8, 10, 20 and 30 ppm	-	-	Gomez-Arroyo *et al* (1987)
		NS	+, -	-, -	Hrelia *et al* (1990)†
Clastogenicity	Chinese hamster ovary cells-(K1)	60-120 µg/mL	-	+	♦Alam *et al* (1974)
	WI-38 (diploid)	0, 120, 140 and 160 µg/mL	-	+	Alam & Kasatiya (1975 and 1976)
	HEp-2 (heterodiploid)	0, 140 and 160 µg/mL	-	+	
	Human lymphocytes	5-500 µg/mL (+ S9) 1-100 µg/mL (- S9) DMSO vehicle	+, -	-, -	♦Herbold (1986) (GLP)
Micronucleus test	Cytokinesis-blocked human lymphocytes	0.06, 0.6 and 6 µg/mL in DMSO	-	-	Bianchi-Santamaria *et al* (1997)

Assay	Species	Dose	Result	Reference
Chromosomal Effect Assay *(in vivo)*				
Micronucleus test	Mouse (Hsd/Win: NMRI)	2 x 2.5 and 2 x 5 mg/kg bw PO	-	♦Herbold (1979a)
		5 mg/kg bw, IP	-	♦Herbold (1995) (GLP)
	Mouse (Swiss-Webster)	NS, PO or IP	-	Sandhu *et al* (1985)†
		NS, PO or ip	-	♦Waters *et al* (1982)†
Mammalian bone marrow cytogenetic test	CD albino rats	6.28 mg/kg bw, PO gavage	-	Henderson *et al* (1988)
	Rat (NS)	NS, IP	-	Hrelia *et al* (1990)†
Dominant lethal	Mouse (Charles River strain)	125 or 250 µg/kg, IP	-	Arnold (1971)
	Mouse (NS)	NS, diet over 7 wk	-	Sandhu *et al* (1985)†
	Mouse (NMRI)	1 x 4 mg/kg bw, PO	-	♦Herbold (1979b)
	Mouse (ICR/SIM)	20, 40, 80 mg/kg of diet over 7 wk	-	Simmon (1978)
		NS, diet over 7 wk	-	♦Waters *et al* (1982)†
Recessive lethal	Drosophila melanogaster	0.25-1.0 ppm	-	Sandhu *et al* (1985)†
		NS	-	♦Waters *et al* (1982)†

Results (+, positive; -, negative) are expressed relative to the presence (+) or absence (-) of metabolic activation; NS = not specified; † = study report contained inadequate methodological and/or observational detail for regulatory purposes; ♦ studies were derived from the German BgVV monograph on azinphos-methyl with any additional Australian regulatory conclusions and comments enclosed in square brackets [ ].

2.9.1 Gene Mutation Assays

2.9.1.1 Salmonella typhimurium *reverse-mutation assay*

♦ Herbold, BA (1978) R1582 (Gusathion M active ingredient) Salmonella/microsome test to evaluate for point mutation. Report no. 7965. Lab & Sponsor: Bayer AG, Toxicology

Department, Wuppertal, Germany. Study duration: November 1978 – November 1978. Report date: 4 December 1978.

Guidelines and GLP: The test method employed was according to Ames *et al* (1975) and the scientific standard at the time the study was performed. In part it is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983).

Deviation: The test with the metabolising system was performed with the highest dose level of the test substance and the positive control substances only. When the study was performed, GLP was not compulsory. The study is considered supplementary only since it does not fully comply with current standards.

Material and Methods: Azinphos-methyl (batch no.: 230705148/201-300; purity: 92.3% [source unspecified]) was tested on quadruplicate cultures of *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 [source unspecified] at concentrations of 0, 4, 20, 100, 500, 2500 µg/plate, in the presence of S-9 mix. Positive control substances were Endoxan [batch 7320, source unspecified], 725 µg/plate (only TA1535 and TA100) and Trypaflavine [batch 028995, source unspecified], 250 µg/plate (only TA1537 and TA98). The positive and negative control substances and the highest dose level of the test substance were also tested in the absence of S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance.

For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single ip dose of Aroclor 1254, 500 mg/kg bw dissolved in peanut oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.

[Bacterial suspensions were obtained from 24 h cultures in nutrient broth incubated at 37°C and plated onto unspecified media. Following incubation at 37°C for 48 h the number of mutants per plate were counted. Two plates per group were used for determination of the total bacterial count.]

Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.

Findings

Cytotoxicity test: Doses of azinphos-methyl up to 2500 µg/plate had no bacteriotoxic effect. However, at the highest dose of azinphos-methyl precipitation on the plates occurred.

Reverse mutation assay: None of the strains used showed a dose-related increase of mutants in comparison to the negative control. Conversely, the positive controls clearly increased the mutant count to far over double that of the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.

Conclusion: There was no indication of a mutagenic effect on any of the tester strains employed.

[*Comments:* Only bacteria in the high-dose group and the two controls were tested in the absence of the S-9 mix which was incompatible with OECD guidelines. Observations were not confirmed in an independent experiment as suggested by OECD guidelines. The stability of the test compound in DMSO at 37°C over 48 h was not evaluated. The phenotype of the strains was not validated (Histidine dependence, crystal violet and UV sensitivity. No assay acceptance criteria were specified. No statistical analyses were performed.]

Evenchik Z (1987) Cotnion-M Assessment of mutagenic potential in histidine auxotrophs of salmonella typhimurium (The Ames Test). Report no. MAK/141/AZN. Lab: Life Sciences Research Israel Ltd., Ness Ziona, Israel and Israel Institute for Biological Research, Ziona, Israel. Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel. Study duration: 31 March – 5 May 1987. Report date: 6 May 1987.

Guidelines & GLP: The test method employed was according to Ames *et al* (1975) and was reported to comply with OECD guideline 471 (adopted May 1983). This study was not quality assured.

Materials & Methods

Cotnion-M (azinphos-methyl) (purity and batch no. unspecified; Makhteshim Chemical Works Ltd., Israel) was diluted in DMSO and tested on duplicate culture plates of *Salmonella typhimurium* strains TA1535, TA100, TA1538, TA98 and TA1537 (derived from cultures provided by Dr Bruce Ames, University of California, CA, USA) in the presence and absence of S-9 mix (4%). S-9 mix was derived from the post-mitochondrial fraction of liver homogenates from 2-month old CD rats (Charles River Breeding Laboratories, Margate, Kent, UK) that were induced with a series of ip injection of phenobarbital sodium and 3-methylcholanthrene over 5 days. Cultures of bacterial strains were prepared by overnight incubation in freshly inoculated broth (Oxoid Nutrient Broth no. 2). All strains were tested for their sensitivity to crystal violet or mitomycin C. Sodium azide (0.3 μg/plate), NPD (4-nitro-o-phenylenediamine; 0.2 μg/plate), ICR-191 (0.2 μg/plate) and 2-aminoanthracene (0.2 μg/plate) were prepared in DMSO and used as positive controls under the same experimental conditions as azinphos-methyl.

In a preliminary test, aliquots of an overnight culture of TA98 were overlaid onto minimal medium plates containing 0, 2.5, 25, 125, 625, 1250 or 1500 μg/plate of azinphos-methyl, DMSO (negative control) or 0.2 μg/plate NPD-20. Plates were incubated at 37°C for 48 h and examined for the number of revertants and the presence of a background lawn of non-revertant colonies. In the reverse mutation assay, overnight cultures of the tester strains (2 x 10^9/mL) were diluted into soft agar which contained azinphos-methyl at 0, 2, 10, 40, 80 or 160 μg/plate or one of the positive controls, S-9 mix or phosphate buffer, allowed to solidify then incubated at 37°C for 48 h. The number of revertants was counted per plate. To determine the viability and cell density of each strain, aliquots of a 10^{-6} culture were plated onto histidine-rich medium, incubated at 37°C for 48 h and the total number of colonies counted. Each experiment was performed twice.
Evaluation criteria: A reproducible 2-fold increase in the number of revertants over 2 consecutive dose levels, or in the last non-toxic dose level, was considered to be a mutagenic response.

Statistical analysis: Regression analysis was applied to the assumed linear part of the dose-response curve using the equation Y = a + bx where Y is the number of revertants, x is the

dose (μg/plate), a is the constant and b is the slope value. The slope was tested for a statistical difference from zero using a Student's t-test.

Results

Cytotoxicity assay: The number of revertant colonies was reduced at and above 125 μg/plate relative to the negative control (DMSO), while the background lawn was reduced at 625 μg/plate and absent at and above 1250 μg/plate. The positive control (NPD-20) produced approximately 35-fold more revertant colonies per plate than the negative control.

Reverse mutation assay: The mutagenicity of azinphos-methyl could not be determined at and above 200 μg/plate due to the turbidity of the mixture. No significant increase in revertant colonies were obtained up to 160 μg azinphos-methyl per plate, either in the presence or absence of S-9 mix.

Conclusions: Azinphos-methyl up to 160 μg/plate showed no evidence of mutagenic activity in *Salmonella typhimurium* strains TA1535, TA100, TA1538, TA98 or TA1537 in either the presence or absence of S-9 mix.

Comments: The ampicillin resistance and UV sensitivity of the strains was unconfirmed.

♦ ***Herbold, BA (1988) E1582 (common name: Azinphos-methyl) Salmonella/microsome test to evaluate for point mutagenic effects. Report no. 16689. Lab & Sponsor: Bayer AG, Toxicology Department, Wuppertal, Germany. Study duration: January 1988 – 6 May 1988. Report date: 6 May 1988.***

Guidelines and GLP: The test method employed is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983). The study is GLP compliant (formal GLP/QAU declaration of the test facility included). The study is acceptable.

Material and Methods: Azinphos-methyl (batch no. 233 796 036; purity: 92.5% [Bayer AG, Wuppertal, Germany]) was tested, first, on quadruplicate cultures of *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 at concentrations of 0, 150, 300, 600, 1200, 2400, 4800 and 9600 μg/plate, in the presence and absence of S-9 mix, and in the repeat test at concentrations of 0, 75, 150, 300, 600, 1200 and 2400 μg/plate (strains TA1535, TA100 and TA1537) and at 0, 150, 300, 600, 1200, 2400, 4800 and 9600 μg/plate (strain TA98). [All bacterial strains were obtained from Prof. Bruce Ames [University of California, Berkeley, CA, USA] and tested for crystal violet sensitivity, UV sensitivity and histidine dependence.] Positive control substances were sodium azide (SA, 10 μg/plate, TA1535) [SERVA, order no. 30175], nitrofurantoin (NF, 0.2 μg/plate, TA 100) [SERVA, order no. 30600], 4-nitro-1.2-phenylenediamine (NPDA, 0,5 μg/plate, TA98; 10 μg/plate, TA1537) [Merck, batch no. VV298757], 2-aminoanthracene (AA, 3 μg/plate, all strains) [EGA-Chemie, batch no.7413406].
The positive control substances, SA, NF and NPDA were tested without S-9 and AA with S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance. [An unspecified stability test was performed on azinphos-methyl in DMSO.]

For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats [weighing 200-300 g], induced 5 days before preparation with a single ip dose of Aroclor 1254, 500 mg/kg body weight dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.

[Bacterial suspensions were obtained from 17-h cultures which had been incubated at 37°C in nutrient broth. Bacteria were diluted into soft agar containing histidine (0.5 mM for mutant determination and 2.5 mM for titer determination). Plates were incubated at 37°C for 48 h and the number of bacterial colonies counted using an automatic counter. The toxicity of azinphos-methyl was assessed via a gross examination of the background growth on each plate, counting the number of mutants per plate and determining the titer (2 plates per concentration). In order to accept the assay, the following criteria had to be fulfilled: the negative controls had to be within the range of historical or laboratory data; the positive controls had to show a mutant level approximately double that of the control; and the titer had to be sufficient.]

Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.

Findings

[*Compound Stability*: Azinphos-methyl was stable in DMSO from 1.5-96 mg/mL at room temperature for 24 h]

Cytotoxicity test: Doses of 300 μg/plate and higher showed a bacteriotoxic effect, specific to strain. [This cytotoxic effect was detected from 600-2400 μg/plate for strain 1535, and from 300-600 μg/plate for strains TA1537, TA100 and TA98.] Therefore the cultures could only partly be used for evaluation and the experiments were repeated to get a sufficient number of evaluable plates. At 1200 [-2400] μg/plate, the substance [azinphos-methyl] started to precipitate.

Reverse mutation assay: With none of the four strains used the test resulted in a dose-dependent increase in mutant counts over the negative control, whether the test was conducted with or without S-9 mix. Conversely, the positive controls clearly increased the mutant count to well over double those of the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.

Conclusion: There was no indication of a mutagenic effect on any of the tester strains employed.

[*Comments:* The actual amount of azinphos-methyl per plate may have been lower than the nominal amount as precipitation was observed at 1200 –2400 μg/plate and above. The stability of the test compound in DMSO was only evaluated at room temperature over 24 h rather than at 37°C over 48 h. The results were not confirmed in an independent experiment. The phenotype of the strains was unconfirmed (histidine dependence, sensitivity to crystal violet, ampicillin resistance).]

Hrelia P, Morotti M, Scotti M, Vigagni F, Paolini M, Perocco P & Cantelli Forti G (1990) Genotoxic risk associated with pesticides: evidences on short-term tests. Pharmocological Research. 22: Supplement 3.

Guidelines and GLP: This is a summary publication. No test guidelines or GLP statement were provided.

Materials and Methods: Azinphos-methyl (unspecified source, batch no., solvent and concentration) was tested on *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 (unspecified source) in the presence and absence of metabolic activation. Metabolic activation was via a S9 fraction which was derived from sodium-phenobarbital plus β-naphthoflavone-induced rodent liver. No further experimental details were given.

Results and conclusions: The study authors indicated that azinphos-methyl was non-mutagenic to *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102.

Comments: This published study did not provide sufficient methodological or observational detail for regulatory purposes and is thus considered unacceptable. No data were provided.

♦ ***Lawlor TE (1987) Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) - test article Guthion. Report no. 920. Lab: Microbiological Associates Inc., Bethesda and Rockville, MD, USA. Sponsor: Mobay Corporation, Stilwell, KS, USA. Dates of experimental work: 17 June 1987 to 5 August 1987. Report date: 5 August 1987***

Guidelines and GLP: The test method employed is in compliance with the demands of OECD Guideline 471 (adopted 26 May 1983). The study is GLP compliant [21 CFR 58, 40 CFR 160 and 40 CFR 792 with the exception that the strength, purity, composition, stability or uniformity of the test or control compounds were not evaluated by the performing laboratory]. A formal QAU declaration of the test facility is included. The study is acceptable with the limitations mentioned behind.

Material and Methods: Azinphos-methyl (batch no.: 79-R-225-42; purity: 88.8%, [Mobay Corporation, Stilwell, KS, USA.]) was tested in a dose range finding test on the *Salmonella typhimurium* strain TA100 in concentrations of 0 and 10-10000 µg/plate [on selective minimal agar in both the presence and absence of S-9 mix]. The first main test on triplicate cultures of the *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 [Dr Bruce Ames, Dept Biochemistry, University of California, Berkeley, USA] included concentrations of 0, 33, 100, 333, 1000 and 2000 µg/plate, a repeat test concentrations of 100, 333, 1000, 2000, 3333 and 4000 µg/plate. The test was performed in the presence and absence of S-9 mix. [The genotype of all strains was confirmed by examining their sensitivity to crystal violet, by demonstration of ampicillin resistance and by determining their spontaneous reversion frequency.]

Positive control substances were sodium azide (SA, 1 µg/plate, TA100 and TA1535 [absence of S-9 mix]), 2-nitrofluorene (9-NF, 3 µg/plate, TA98 and TA1538 [absence of S-9 mix]), 9-aminoacridine (9-AA, 75 µg/plate, TA1537 [absence of S-9 mix]), 2-aminoanthracene (AA, 2 µg/plate, all strains [presence of S-9 mix]). The positive control substances, SA, 2-NF and 9-AA were tested without S-9 and AA with S-9 mix. The test substance was formulated in DMSO, which was also used as the negative control substance.

For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single ip dose of Aroclor 1254, 500 mg/kg body weight dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.

[The test article and S9-mix were analysed for sterility by plating an equivalent aliquot on selective agar. Following an approximate 48-h incubation period at 37 ± 2°C, revertant colonies per plate were counted either manually or by using an automated colony counter. Plates that were not counted immediately were stored at 4 ± 2°C until counting could be conducted. A gross examination of the background bacterial lawn was performed with the aid of a dissecting microscope. The following criteria were met in order to validate the assay: sensitivity to crystal violet; ampicillin resistance; suitable spontaneous reversion rates; strain titers; adequate positive control values; and a minimum of 3 non-toxic dose levels.]

Evaluation criteria: A reproducible, dose-dependent increase in the number of mutants to a level about double that of the negative control, obtained with at least one strain, is considered to be a positive result.

Findings

Cytotoxicity test: In the dose range finding test on strain TA100, doses of azinphos-methyl from 3333 μg/plate onward were bacteriotoxic [based on a slight reduction in the bacterial lawn and a reduction in the number of revertants/plate compared to the negative control. Additionally a slight precipitate was detected at 6667 and 10000 μg/plate.] In contrast, in the two main tests no bacteriotoxicity was observed in any strain up to the highest dose tested. [Actually there was evidence of bacteriotoxicity based on a reduction in the number of revertants/plate at 2000 μg/plate and above for TA1537, and at 3333 μg/plate and above for TA100, TA1535 and TA1537. In the first of the main tests a moderate precipitate was observed at the highest dose (2000 μg/plate). In the second test a slight precipitate was detected at the two highest doses (3333 and 4000 μg/plate).]

Reverse mutation assay: Azinphos-methyl did not cause a positive response in any of the tester strains with or without metabolic activation. However, in the second main test with strain TA100 the mutant counts are not assessable at the highest concentration(s) since without bacteriotoxicity and with only slight precipitation the values are far under the characteristic number of spontaneous revertants (TA100: 80-240) (see Table below). The positive controls increased the mutant count in excess of double the negative control count, and thus demonstrated the sensitivity of the test system and the efficacy of the S-9 mix.

[There was a reproducible dose-response increase in the number of TA100 revertants both in the presence and absence of metabolic activation (see Table below). As this increase was less than 2-fold above the negative control it was not considered to be significant by the study authors. Additionally, at the highest doses (2000 μg/plate for the first test or 3333 and 4000 μg/plate for the second test) there was a reduction in the number of revertants/plate due possibly to cytotoxicity.]

Effect of azinphos-methyl on the number of revertant colonies of *S. typhimurium* strain TA100

1st test Dose (μg/plate)	TA100 (- S-9 mix)	TA100 (+ S-9 mix)	2nd test Dose (μg/plate)	TA100 (- S-9 mix)	TA100 (+ S-9 mix)
0	101 [± 6]	102 [± 6]	0	138 [± 15]	135 [± 29]
33	110 [± 9]	117 [± 2]	-		
100	112 [± 4]	116 [± 16]	100	127 [± 9]	129 [± 12]
333	127 [± 10]	123 [± 5]	333	131 [± 11]	145 [± 12]
1000	169 [± 7]	198 [± 36]	1000	162 [± 11]	201 [± 18]
2000†	173 [± 7]	154 [± 18]	2000	215 [± 36]	91 [± 82]
			3333 ‡	25 [± 6]	86 [± 109]
			4000 ‡	3 [± 1]	20 [± 12]
[NaN_3	633 ± 54	4315 ± 70	NaN_3	598 ± 4	4240 ± 10]

[† slight precipitate detected; ‡ moderate precipitate detected]

Conclusion: In the assessable dose range the test material did not cause a positive response in any of the tester strains employed, neither with nor without metabolic activation.

[*Comments:* The stability of azinphos-methyl under the actual experiment conditions was undetermined. Bacteriotoxicity could have more accurately assessed by performing a bacterial count.]

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication and no guidelines or GLP statement were provided. The method employed was according to Ames *et al* (1975).

Materials and Methods: Technical grade azinphos-methyl (Chemagro Chemical Corporation, Kansas City, MO, USA, batch no. unspecified) was tested on *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 (Dr Bruce Ames, University of California, Berkeley, CA, USA) at concentrations of 1 μg-10 mg/plate in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. The solvent, media composition, assay temperatures and times, and number of test plates/sample were unspecified. Positive controls included 2-anthramine, 2-aminoacridine, 9-aminoacridine, β-propiolactone, N-methyl-N'-nitro-N-nitrosoguanidine and sodium azide (batch no's, sources, concentrations and solvents unspecified). The experiment was repeated at least once. No statistical analyses were performed.

Evaluation criteria: A dose-related increase in the number of revertants in at least one strain over 3 concentrations was considered to be a positive result.

Results and Conclusions: Azinphos-methyl had no mutagenic effect on *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538.

Comments: This summary report does not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including individual plate counts, the mean number of revertants/plate or standard deviations. The following details were unspecified: the solvent; the concentrations of positive controls; culture and assay conditions

(eg media, times, temperatures); the cytotoxicity of azinphos-methyl or whether precipitation was observed at the higher concentrations. No statistical analyses were performed. The phenotype of the test strains was unconfirmed (histidine dependence, crystal violet sensitivity and ampicillin resistance). Observations were not confirmed in an independent experiment.

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given. The method employed was according to Ames *et al* (1975).

Materials and Methods: Guthion (azinphos-methyl) (technical grade; batch no. unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) was tested on *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538 (Dr Bruce Ames, University of California, Berkeley, CA, USA) at concentrations of 0, 1, 5, 10, 50, 100, 500 and 1000 μg/plate in the presence or absence of metabolic activation. Metabolic activation was via the addition of S-9 liver homogenate which was derived from adult Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA, USA), weighing 250-300 g, that were administered a single 500 mg/kg bw ip injection of Aroclor 1254. The positive control was 4-o-tolylazo-o-toluidine (25 μg/mL, unspecified batch no. and source) while the negative control was unspecified. No further experimental details were given.

Results and Conclusions: Azinphos-methyl up to 1000 μg/plate did not cause reverse mutation in *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538 in either the presence or absence of metabolic activation.

Comments: A number of experimental details were unspecified: assay conditions (temperatures and times); solvent; the number of plates per concentration; individual plate counts; standard deviations; signs of toxicity or compound precipitation; evaluation criteria; statistical analyses. Results were not confirmed in an independent experiment. The phenotypes of the bacterial strains were unconfirmed (histidine dependence, crystal violet sensitivity and ampicillin resistance).

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The test was performed according to Ames *et al* 1975. *GLP*: No. The information of this published literature has supplementary value only since concentrations used are not clearly given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Technical grade azinphos-methyl [batch no. and source unspecified] was tested on the *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 in the

presence or absence of S-9 mix. [S-9 mix was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. The concentrations of azinphos-methyl tested were unspecified. No details were given with regard to the positive and negative controls, the number of plates utilised or whether the experiment was repeated. Following an unspecified incubation temperature and period, the number of revertant colonies on histidine-deficient agar plates, was counted.

Evaluation criteria: The presence of a dose-related increase in the number of revertants in at least 1 strain over at least 3 concentrations was considered to be a positive result.]

Findings and Conclusion: There was no mutagenic effect either in the presence or absence of S-9 mix.

[*Comments:* This published study did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. Any observations of cytotoxicity or compound precipitation were unspecified. Concentrations tested were unspecified. The stability of azinphos-methyl in the vehicle was not examined. The phenotype of the stock strains was not confirmed (requirement of histidine for growth, sensitivity to crystal violet and resistance to ampicillin). There was no indication of whether the experiment was repeated and no statistical analyses were performed.]

2.9.1.2 Escherichia coli *reverse-mutation assay*

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication and no guidelines or GLP statement were given. The method employed was according to Bridges (1972).

Materials and Methods: Technical grade azinphos-methyl (Batch no. unspecified, Chemagro Chemical Corporation, Kansas City, MO, USA) was tested on *E. coli* strain WP2 (uvrA) (unspecified source) at concentrations up to 10 mg/plate in the presence and absence of metabolic activation. A minimum of 6 unspecified concentrations were tested. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. The solvent, media composition, assay temperatures and times, and number of test plates/sample were unspecified. The negative control was the solvent while the positive controls included 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) without metabolic activation and 2-aminoanthracene with metabolic activation (batch numbers, sources, concentrations and solvents unspecified). The experiment was repeated at least once. No statistical analyses were performed.

Evaluation criteria: A dose-related increase in the number of revertants over 3 concentrations was considered to be a positive result.

Results and Conclusions: Azinphos-methyl had no mutagenic effect on *E. coli* strain WP2 (uvrA).

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including individual plate counts, the

mean number of revertants/plate or standard deviations. The following details were unspecified: the solvent; the concentrations of azinphos-methyl and positive controls; culture and assay conditions (eg media, times, temperatures); sample sizes; the cytotoxicity of azinphos-methyl or whether precipitation was observed at the higher concentrations. No statistical analyses were performed. The phenotype of the bacterial strain was unconfirmed (tryptophan dependence, mytomycin C sensitivity and ampicillin resistance).

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildamauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given.

Materials and Methods: Guthion (azinphos-methyl) (technical grade; batch no. unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) was tested on *Escherichia coli* strain WP2 (uvrA$^-$) (Dr D McCalla, unspecified location) at concentrations of 0, 1, 5, 10, 50, 100, 500 and 1000 μg/plate in the presence or absence of metabolic activation. Metabolic activation was via the addition of S-9 liver homogenate which was derived from adult Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA, USA), weighing 250-300 g, that were administered a single 500 mg/kg bw ip injection of Aroclor 1254. The negative control was the solvent (unspecified) while the positive control was 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) (0.05 μg/plate; batch no. and source unspecified). No further experimental details were given.

Results and Conclusions: Azinphos-methyl up to 1000 μg/plate did not cause reverse mutation in *Escherichia coli* strain WP2 (uvrA$^-$) in either the presence or absence of metabolic activation.

Comments: A number of experimental details were unspecified: assay conditions (temperatures and times); solvent; negative control; the number of plates per concentration; individual plate counts; standard deviations; signs of toxicity or compound precipitation; evaluation criteria; statistical analyses. Observations were not confirmed in an independent experiment. The phenotype of the bacterial strain was unconfirmed (tryptophan dependence, mytomycin C sensitivity and ampicillin resistance).

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The test was performed according to the method of Bridges, 1972. GLP: No. The information of this published literature has supplementary value only since concentrations used are not clearly given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Azinphos-methyl [technical grade, batch no. and source unspecified] was tested on the *Escherichia coli* strain WP2 [source unspecified] in the presence or absence of S-9 mix. [S-9 mix was derived from adult male Sprague-Dawley rats weighing 250–300 g that had been induced with Aroclor-1254. The concentrations of azinphos-methyl tested were unspecified although the study authors indicated that a minimum of 6 concentrations were investigated. No details were given with regard to the number of plates utilised or whether the experiment was repeated. Positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and 2-aminoanthracene (unspecified concentrations) in the presence and absence of metabolic activation. No further experimental details were given.

Evaluation criteria: The presence of a dose-related increase in the number of revertants in at least 1 strain over at least 3 concentrations was considered to be a positive result.]

Findings and Conclusions: Azinphos-methyl had no mutagenic effect [on E. coli strain WP2 ($uvrA^-$)] either in the presence or absence of S-9 mix.

[*Comments:* This published report did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: any observations of cytotoxicity or compound precipitation; test concentrations; the stability of azinphos-methyl in the vehicle. The phenotype of WP2 was not confirmed (requirement of tryptophan for growth, Mitomycin C sensitivity and resistance to ampicillin). There was no indication of whether the experiment was repeated. No statistical analyses were performed.]

2.9.1.3 Saccharomyces cerevisiae *reverse-mutation assay*

♦ ***Hoorn AJW (1983) Mutagenic evaluation of R1582 (common name: azinphos-methyl) in the reverse mutation induction assay with Saccharomyces cerevisiae strains S138 and S211α. Report no. R2503. Lab: Litton Bionetics, Veenendal, The Netherlands. Sponsor: Bayer AG, Wuppertal, Germany. Study duration: 9 May 1983 - 2 June 1983. Report date: 16 June 1983***

Guidelines and GLP: The method used was according to Pittman and Brusick, 1971. The study can be considered to be in compliance with the requirements of OECD Guideline 480 (adopted 23 October 1983). The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.

Material and Methods: Suspension cultures of *Saccharomyces cerevisiae* strains S138 and S211α [Dr RK Mortimer, University of California, Berkeley, USA] were exposed to Azinphos-methyl (batch no. 230205060; purity: 91.1% [Bayer AG, Wuppertal, Germany]) for 3 hours in the presence or absence of S-9 mix. The doses employed were 0, 33.3, 100, 333.3, 1000, 3333.3 and 10000 μg/mL. [Aliquots of the test suspensions were plated onto both methionine deficient and complete media, and incubated at an unspecified temperature for approximately 3 days for determination of the total cell number, and 5–7 day for determination of the number of revertants.] The test material and the positive control materials, ethylmethanesulphonate (EMS, 1%, S138, [source unspecified]) or quinacrine mustard (QM, 10 μg/mL, S211α, [source unspecified]) and sterigmatocystine (SC, 5 μg/mL, both strains, [source unspecified]), were formulated in DMSO, which also served as vehicle control. EMS and QM were used without S-9 mix and SC with S-9 mix.

The cytotoxicity test was performed on strain S211α using 14 concentrations of test material in the range of 1.22 to 10000 μg/mL.

The metabolic activation system was a commercially available supernatant prepared from adult Sprague-Dawley rat liver pre-treated with Aroclor 1254 according to established technique.

Evaluation criteria: If the solvent control value is within the normal range, a test material that produces a positive dose-response over 3 concentrations, with the highest increase equal to twice the solvent control value, is considered to be a mutagenic agent.

Findings

Cytotoxicity test with strain S211α: The test revealed no cytotoxicity at any of the dose levels employed. [In contrast, during the main test there was an approximately 50% decrease in the number of methionine revertants, and the number of revertants per 10^6 survivors, in strain S211α at 1000 μg/mL and above in the absence of activation. Although no similar effect was detected in the presence of metabolic activation, these result were suggestive of a cytotoxic effect at the highest treatment level.]

Reverse mutation assay with strain S138 and S211α: The results of the tests conducted both with and without S-9 mix revealed no increase in the number of revertants at any of the concentrations of azinphos-methyl used. Revertant frequencies were all comparable to the solvent controls. A marked increase in revertants/survivors was noted with the control substances, demonstrating the sensitivity of the system. [Sterigmatocystine, which was used as the positive control in the presence of metabolic activation did not cause a 2-fold increase in the number of methionine revertants on strain S211α compared to the solvent control.]

Conclusion: The test material did not exhibit mutagenic activity in *S. cerevisiae* strains S138 and S211α under the test conditions employed.

[*Comments:* The phenotype of the strains S138 and S211α was unconfirmed (requirement of methionine for growth). One of the positive controls (sterigmatocystine) used on strain S211α, was inactive. The cytotoxicity of azinphos-methyl was evaluated in only 1 of the 2 strains (S211α).]

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication and no guidelines or GLP statement were given. The method employed was according to Zimmermann (1975).

Materials and Methods: Technical grade azinphos-methyl (Chemagro Chemical Corporation, Kansas City, MO, USA, batch no. unspecified) was tested on *S. cerevisiae* strain D7 (Dr FK Zimmermann, Fachbereich Biologie Technische Hochschule, Darmstadt, Germany) at 5 unspecified concentrations ranging from 10-50 mg/plate in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250–300 g that had been induced with Aroclor-1254. The solvent, media composition, assay temperatures and times, or number of test

plates/sample were unspecified. The negative control was the solvent and the positive control was 1, 2, 3, 4-diepoxybutane (source, batch no., concentration and solvent unspecified). The experiment was repeated at least once. No statistical analyses were performed.

Evaluation criteria: A dose-related increase in the number of revertants over 3 concentrations was considered to be a positive result.

Results and Conclusions: Azinphos-methyl had no mutagenic effect on *S. cerevisiae* strain D7.

Comments: This summary report does not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including individual plate counts, the mean number of revertants/plate or standard deviations. The following details were unspecified: the solvent; the concentrations of azinphos-methyl and positive controls; culture and assay conditions (eg media, times, temperatures); the cytotoxicity of azinphos-methyl or whether precipitation was observed at the higher concentrations. No statistical analyses were performed.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was according to that published by Zimmermann, 1975. *GLP*: No. The information of this published literature has supplementary value only since concentrations used are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Azinphos-methyl [technical grade; batch no. and source unspecified] was tested on the yeast strain *S. cerevisiae* D7 [Dr FK Zimmermann, Fachbereich Biologie Technische Hochschule, Darmstadt, Germany] which requires isoleucine for growth. Five concentrations of azinphos-methyl were assayed both with and without metabolic activation [using S-9 mix, which was derived from adult male Sprague-Dawley rats weighing 250–300 g that had been induced with Aroclor-1254. No details were given with regard to the number of plates utilised or whether the experiment was repeated. Solvent was used as the negative control and 1, 2, 3, 4-di-epoxybutane as the positive control (unspecified concentration). Following an unspecified incubation temperature and period, the number of revertant colonies on isoleucine-deficient agar plates, was counted.

Evaluation criteria: The presence of a reproducible, dose-related increase in the number of revertants over at least 3 concentrations was considered to be a positive result.]

Findings and Conclusion: No mutagenic effect was found either in the presence or absence of S-9 mix.

[*Comments:* This published report did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. Any observations of cytotoxicity or compound precipitation were unspecified. Concentrations tested were unspecified. The stability of azinphos-methyl in the vehicle was not examined. The phenotype of *S. cerevisiae*

D7 was not confirmed (requirement of isoleucine for growth). There was no indication as to whether of the experiment was repeated.]

2.9.1.4 Schizosaccharomyces pombe *forward mutation assay*

Gilot-Delhalle J, Moutschen CJ & Moutschen-Dahmen M (1983) Mutagenicity of some organophosphorus compounds at the ade6 locus of Schizosaccharomyces pombe. Mutation Research. 117: 139 – 148.

Guidelines and GLP: This is a published study and no GLP or test guidelines were provided.

Materials and Methods: Azinphos-methyl (minimum 99% purity, batch no. unspecified, Riedel-de Haen AG, Seelze, Germany) was dissolved in 5% DMSO and incubated with 10^8/mL stationary-phase *Schizosaccharomyces pombe SP-198 ade 6-60/rad 10-198/h*$^-$ (unspecified source) at concentrations of 3-95 mM for 1 h at 37°C in the presence and absence of metabolic activation. Metabolic activation was via the addition of a S9 liver fraction which was derived from 3 phenobarbital-induced 3 month-old strain Q male mice (source and body weight unspecified). Test mixtures were plated onto complete agar and incubated for 4-5 days at 27°C. Positive controls included 0.2-5 mM methyl methanesulfonate (MMS) and 5-25 mM ethyl methanesulfonate (EMS) (batch no's and sources unspecified). The number of white colonies or sectors was counted and these were differentiated from the original purple phenotype. Survivorship was also scored using the same plating media. No further experimental details were given.

Results

Toxicity: Measurement of survivorship (no. of colonies/plate) indicated that the LD_{50} of azinphos-methyl was 80 mM while the positive controls, MMS and EMS, had LD_{50} values of 0.5 and 6 mM respectively.

Mutagenicity: Graphical representation of the data illustrated a linear dose-response relationship between the concentration of azinphos-methyl and the percentage of mutants scored, with the effect observed in the absence of metabolic activation approximately 2-fold greater than the effect observed in the presence of metabolic activation.

Conclusions: Azinphos-methyl up to a concentration of 95 mM was mutagenic to *Schizosaccharomyces pombe* strain SP-198.

Comments: This published study did not contain sufficient methodological or observational data for regulatory purposes. The following information was unspecified: raw or individual plate data; standard deviations or errors; details of the negative/solvent control (including any results); assay acceptance and evaluation criteria. The data were not subjected to statistical analyses.

2.9.1.5 Mouse lymphoma cell forward-mutation assay.

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication and no guidelines or GLP statement were provided. The method employed was according to Clive *et al* (1979).

Materials and Methods: Technical grade azinphos-methyl (Batch no. unspecified, Chemagro Chemical Corporation, Kansas City, MO, USA) was tested on the mouse lymphoma cell line L5178Y (unspecified source) at unspecified concentrations in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. Following exposure to azinphos-methyl, cells were cloned in the presence of trifluorothymidine (unspecified source and concentration). The solvent, media composition, assay temperatures and times, and number of test plates/sample were unspecified. Solvent was used as the negative control while 500 μg/mL ethyl methanesulfonate and 5 μg/mL 3-methylcholanthrene were the positive controls in the absence and presence of metabolic activation respectively. The experiment was repeated at least once. No statistical analyses were performed. No assay acceptance criteria were given.

Evaluation criteria: A positive result was considered if the mutation frequency of at least one azinphos-methyl concentration was a minimum of twice the background mutation frequency, or if there was a dose-related increase in the mutation frequency.]

Results and Conclusions: Dose-response curves were constructed by the study authors and illustrated that there was a dose-response relationship with regard to the mutation frequency in treated L5178Y cells in the presence and absence of metabolic activation. The effect in the presence of activation was greater than the effect in the absence of activation.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including individual colony counts for treated and control groups, survival and cloning efficiencies, and mutant frequencies, including standard deviations. The following details were unspecified: the solvent; the concentrations of azinphos-methyl and positive controls; culture and assay conditions (eg media, times, temperatures); the cytotoxicity of azinphos-methyl or whether precipitation was observed at the higher concentrations; methods used to enumerate numbers of viable and mutant cells. No statistical analyses were performed.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was according to that published by Clive *et al* 1979. *GLP*: No. The information of this published literature has supplementary value only since concentrations used are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: L5178 mouse lymphoma cells [source unspecified] were used to detect if azinphos-methyl [technical grade; unspecified batch no. and source] produced a mutation from cells heterozygous for thymidine kinase ($TK^{+/-}$) to cells homozygous for this enzyme ($TK^{-/-}$). The test was performed with or without a metabolic system [using S-9 mix, which was derived from adult male Sprague-Dawley rats weighing 250–300 g that had been induced with Aroclor-1254. No details were given with regard to the number of plates utilised

or whether the experiment was repeated. Cells were incubated with azinphos-methyl (unspecified concentrations, time, temperature, media) and cloned in the presence of 5-trifluorothymidine (TFT) (unspecified concentration). No details were given with regard to the positive and negative controls.

Evaluation criteria: A positive result was considered if the mutation frequency of at least one concentration was minimum of twice the background mutation frequency, if there was a concentration-related increase in mutation frequency, and if these findings were repeatable.]

Findings and Conclusion: Without further information, a positive response with metabolic activation and a negative response without activation was declared in a tabulated summary.

[*Comments:* This published report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: observations of cytotoxicity or compound precipitation; concentrations tested; assay conditions (culture/media conditions, incubation times and temperatures); survival and cloning efficiencies; methods used to enumerate viable and mutant cells. The stability of azinphos-methyl in the vehicle was not examined. There was no indication of whether the experiment was repeated. No statistical analyses were performed.]

2.9.2 DNA Damage and Repair

2.9.2.1 ^{32}P-postlabeling studies

Shah RG, Lagueux J, Kapur S, Levallois P, Ayotte P, Tremblay M, Zee J & Poirier GG (1997) Determination of genotoxicity of the metabolites of the pesticides Guthion, Sencor, Lorox, Reglone, Daconil and Admire by ^{32}P-postlabeling. Molecular and Cellular Biochemistry. 169: 177 – 184.

Guidelines and GLP: No test guidelines or GLP statement were given. ^{32}P-postlabelling studies were conducted based on the method of Randerath *et al* (1981)

Materials and Methods: Guthion (azinphos-methyl) (Unspecified batch no., Miles Canada Inc., Ontario, Canada) at a concentration of 1 mM was reacted with rat liver S9 homogenate (2 mg/mL) (Microbial Associated, Rockville, USA) at for 1 h at 37°C. Metabolites were extracted with diethyl ether, dried, redissolved and incubated with calf thymus DNA for 3.5 h at 37°C. The positive control was 100 μM benxo(α)pyrene (Sigma, USA) and the negative control was an ether extract of the S9 homogenate. DNA was extracted, precipitated, dried and resuspended in water and the resulting 260/280 and 230/260 ratios were 1.7-1.9 and 0.4 respectively.

DNA (10 μg) was digested overnight and subsequently enriched for adducts by either nuclease P1 or butanol extraction. In both methods adducts were labelled with 100 μCi γ-^{32}P-ATP in kinase buffer for 45 min at 37°C. Samples were analysed by thin layer chromatography on PEI-cellulose plates. The adducts obtained with nuclease P1 enrichment were scraped from TLC plates and quantified by scintillation counting. Two independent experiments were performed.

Results and Conclusions: Using nuclease P1 enrichment, 7 adducts were detected in azinphos-methyl-treated DNA and totalled 69.7 adducts/10^9 nucleotides. In contrast, 2

adducts were detected in the negative control and totalled 5.6 adducts/10^9 nucleotides. Two adducts were common to both treated and negative control DNA, however the levels in treated DNA were 5.2 and 22-fold greater than the negative control. For the positive control [benxo(α)pyrene], a total of 6 adducts were detected totalling 1893 adducts/10^9 nucleotides. No adducts were detected in azinphos-methyl-treated DNA using butanol extraction as the enrichment method. These results indicated that 1 mM Guthion (azinphos-methyl) induced adduct formation in calf thymus DNA.

Comments: Sample sizes were unspecified. No raw data or standard deviations/errors were given.

2.9.2.2 Differential toxicity assays

Herbold BA (1984) R1582 (common name: azinphos-methyl): Pol-test on E. coli to evaluate for potential DNA damage. Report no. 12478. Lab & Sponsor: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study duration: February 1984. Report date: 22 February 1984.

Guidelines and GLP: The method employed was that of Rosenkranz and Leifer, 1980. For this method no OECD Guideline exists. When the study was performed GLP was not compulsory.

Material and Methods: Azinphos-methyl (batch no. 230 205 060; purity: 91.1%, [Bayer AG, Wuppertal-Elberfeld, Germany]) was administered, in quadruplicate cultures, to *E. coli*, strain (K12)p3478 (repair deficient) and strain W3110 (repair proficient) [sources unspecified], at dose levels of 625, 1250, 2500, 5000 and 10000 μg/plate. [Bacteria were maintained in warm full media at 37°C prior to plating on nutrient broth plates. Azinphos-methyl was placed on small round filter papers and then onto these nutrient broth plates.] The cultures were incubated for 24 hours [at 37°C] and then the inhibition zone diameters were measured. Azinphos-methyl and the positive control material, methylmethanesulphonate (MMS, 10μl/plate [amount/plate unspecified]) [Merck, Darmstadt, batch no. 1171879], were formulated in DMSO, which also served as the solvent control. [An unspecified stability test revealed no change to azinphos-methyl in DMSO.] The negative control substance was chloramphenicol (CAP, 30μg/plate, vehicle not given) [(Boehringer Mannheim, batch no. 2123391080). The vehicle for CAP was actually DMSO and the vehicle for MMS was unspecified.] The test was done in the presence and absence of S-9 mix.

For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single ip dose of Aroclor 1254, 500 mg/kg bw dissolved in peanut oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.

Evaluation criteria: A reproducible increase in the difference in inhibition zone diameter between the two strains of more than 2 mm was considered positive.
Findings: None of the concentrations of azinphos-methyl produced an inhibition zone, either with or without metabolic activation. The positive control substance, MMS, clearly increased the difference in inhibition zone diameters [by 21.9 and 23.4 mm in the absence and presence of metabolic activation respectively].

Conclusion: There was no evidence of DNA damage produced by azinphos-methyl up to and including a concentration of 10000 μg/plate.

[*Comments:* Although the experiment was apparently performed in quadruplicate, raw data and standard deviations were not provided. No statistical analyses were performed. There was no indication of whether the experiment was repeated.]

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given.

Materials and Methods: Sterile filter discs impregnated with 1 mg of azinphos-methyl (technical grade; batch no. and solvent unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) were placed on the centre of agar plates which contained *E. coli* strains W3110 or p3478 (*polA*$^-$) (Dr H Rosenkranz, unspecified location). Plates were incubated for 16 h at 37°C and the width of the zone of toxicity or inhibition of growth was measured. Positive controls were 1-phenyl-3,3-dimethyl-triazene (1 mL) and chloramphenicol (30 μg) (batch no's and sources unspecified). The assay was performed at least 3 times. No further experimental details were given.

Results and Conclusions: There was no difference in the zone of inhibition between *E. coli* strains W3110 and p3478, which indicated that azinphos-methyl was not mutagenic.

Comments: Sample sizes and the use of a negative control were unspecified. Results for the positive control, and any indication of the variability of the data were not given (eg standard deviations of errors). There was no indication of whether the experiment was performed in the absence or presence of metabolic activation. No statistical analyses were performed.

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given.

Materials and Methods: Sterile filter discs impregnated with 1 mg of azinphos-methyl (technical grade; batch no. and solvent unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) were placed on the centre of agar plates which contained *Bacillus subtillus* strains H17(rec$^+$) or M45(rec$^-$) (Dr Kada, unspecified location). Plates were incubated for 16 h at 37°C and the width of the zone of toxicity or inhibition of growth was measured. Positive controls were 1-phenyl-3,3-dimethyl-triazene (1 mL) and chloramphenicol (30 μg) (batch

no's and sources unspecified). The assay was performed at least 3 times. No further experimental details were given.

Results and Conclusions: There was no difference in the zone of inhibition between *Bacillus subtillus* strains H17 and M45 which indicated that azinphos-methyl was non-mutagenic.

Comments: Sample sizes and the use of a negative control were unspecified. Results for the positive control, and any indication of the variability of the data were not given (eg standard deviations of errors). There was no indication of whether the experiment was performed in the absence or presence of metabolic activation. No statistical analyses were performed.

2.9.2.3 Unscheduled DNA synthesis assays

Hrelia P, Morotti M, Scotti M, Vigagni F, Paolini M, Perocco P & Cantelli Forti G (1990) Genotoxic risk associated with pesticides: evidences on short-term tests. Pharmocological Research. 22: Supplement 3.

Guidelines and GLP: This is a summary publication. No test guidelines or GLP statement were provided.

Materials and Methods: Azinphos-methyl (unspecified source, batch no., solvent and concentration) was tested in an unscheduled DNA synthesis assay in the presence and absence of metabolic activation. Metabolic activation was via a S9 fraction which was derived from sodium-phenobarbital plus β-naphthoflavone-induced rodent liver. No further experimental details were given.

Results and Conclusions: The study authors indicated that azinphos-methyl did not cause unscheduled DNA synthesis.

Comments: This published study did not provide sufficient methodological or observational detail for regulatory purposes and is thus considered unacceptable. No data were provided.

♦ ***Myhr BC (1983) Evaluation of R1582 in the primary rat hepatocyte unscheduled DNA synthesis assay. Report no. R2686. Lab: Litton Bionetics Inc., Kensington, MD, USA. Sponsor: Bayer AG, Wuppertal, Germany. Study duration: 14 June 1983-19 September 1983. Report date: November 1983.***

[*Guidelines and GLP*:] The method employed is based on that described by Williams, 1977 and according to OECD Guideline 482 (adopted 26 May 1983). The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.

Material and Methods: Primary rat hepatocytes from an adult male Fischer 344 rat [obtained from Charles River Breeding Laboratories, Inc. (unspecified location) and weighing 150-300 g] were exposed to azinphos-methyl (batch no. 230 205 060; purity: 91.1%, [Bayer AG, Wuppertal, Germany]) in 3 replicate cultures for 18-19 hours [presumably at 37 °C]. Azinphos-methyl was formulated in DMSO, which also served as the negative control. The concentrations of azinphos-methyl tested for nuclear labelling were: 0.25, 0.5, 1.0, 2.5, 5.0, 10.1, 25.1 and 50.3 μg/mL. This dose range was based on a preliminary cytotoxicity study

performed at 15 concentrations of azinphos-methyl from 0.025 to 1005 μg/mL. The positive control substance was 2-acetyl aminofluorene (AAF, 0.05 μg/mL, [source unspecified]).

[Monolayer cultures were established on coverslips and maintained at 37 ± 2°C in a humidified atmosphere of 5% CO_2 and in complete Williams Medium E (WME). The assay was initiated within 3 h of establishment by replacing the medium with WME containing azinphos-methyl at the nominal concentrations. Each treatment was performed on 5 cultures with 3 of these used for the assay itself and 2 for the cytotoxicity measurement.] Each culture received 1 μCi/mL ^{3}H-thymidine at the time of exposure to test and control substances. [At 20-24 h after initiation of the treatment the number of viable cells was counted. The nuclei of labelled cells were swollen, fixed and dried.] Following exposure, slides were prepared for autoradiography.

[Unscheduled DNA synthesis (UDS) was microscopically evaluated by counting nuclear grains using a video screen and automatic counter. The net nuclear grain count was determined for 50 randomly selected cells on each coverslip. Only nuclei with normal morphologies were scored and any nuclei blackened by grains too numerous to count were excluded.]

Assay acceptance criteria: The assay was considered acceptable when the following criteria were met: the viability of the hepatocytes when first isolated, and of the resultant monolayer cultures was greater than 70%; the number of viable cells in the solvent control was relatively stable over the course of the experiment; labelling of the solvent control did not exceed an average of 2 net grains /nucleus; the positive control was responsive (for AAF the typical mean net nuclear grain count was 16 ± 7; the net grain count was obtained from at least 2 replicate cultures and at least 50 cells/culture; a minimum of 6 dose levels were analysed; and the highest analysed dose approached an excessive toxicity.

Evaluation criteria: The test substance is considered active in the UDS assay at applied concentrations that cause:

1. An increase in the mean net nuclear grain count to [exceed] at least 6[.43] grains per nucleus, after subtraction of the concurrent negative control value, and/or

2. An increase in the percentage of nuclei having 6 or more net grains to at least 10% of the analysed population, after subtraction of the concurrent negative control value, and/or

3. The percentage of nuclei with 20 or more grains to reach or exceed 2% of the analysed population.

Findings

Cytotoxicity test: Concentrations in the range of 100.5-1005 μg/mL were lethal, and at 50.3 μg/mL only 21.5% of the cells survived. [At 25.1 μg/mL the viability was 59.4% while lower concentrations of azinphos-methyl had no effect on cell survival or morphology. The viability and morphology of the negative/solvent control cultures were stable over the course of the experiment.]

UDS assay: The nuclear labelling of the azinphos-methyl treated cultures was equivalent to the negative control level and did not meet the criteria for a positive test result. The positive

control material, AAF, induced massive UDS [with 17.79 grains/ nucleus counted compared to 0.43 for the solvent control].

Conclusion: The test material was evaluated as inactive in the rat primary hepatocyte UDS assay.

[*Comments:* No raw data was provided. No indication of the variability of the data was provided (standard deviations or errors).]

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication and no guidelines or GLP statement were specified. The method employed was according to Waters *et al* (1982).

Materials and Methods: Technical grade azinphos-methyl (batch no. unspecified, Chemagro Chemical Corporation, Kansas City, MO, USA) was incubated with WI-38 human lung fibroblast cells (unspecified source) at 5 unspecified concentrations in the presence and absence of metabolic activation. The concentrations were based on an undisclosed preliminary study. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. The media composition, assay temperatures and times, concentration of ^{3}H-thymidine, number of tests/sample or numbers of cells examined were unspecified. DMSO was used as the negative control while the positive controls were 4-nitroquinoline-N-oxide and dimethylnitrosamine in the presence and absence of metabolic activation respectively (sources, concentrations and solvents unspecified). The experiment was repeated at least once. No statistical analyses were performed. No assay acceptance criteria were given.

Evaluation criteria: A positive result was considered if there was a significant concentration-related increase in thymidine incorporation in treated cells compared with the negative control.

Results and Conclusions: Azinphos-methyl did not cause unscheduled DNA synthesis in WI-38 human lung fibroblast cells in either the presence or absence of metabolic activation.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following details were unspecified: the concentrations of azinphos-methyl and positive controls; culture and assay conditions (eg media, concentration of ^{3}H-thymidine, times, temperatures, sample sizes, number of cells examined); the toxicity of azinphos-methyl or whether precipitation was observed at the higher concentrations. No statistical analyses were performed.

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given.

Materials and Methods: Guthion (azinphos-methyl) (technical grade; batch no. unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) was incubated with WI-38 cells (unspecified source) at concentrations of 0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M in the presence and absence of metabolic activation. Metabolic activation was via the addition of mouse S-9 liver homogenate plus unspecified cofactors. The positive controls were 10 μM 4-nitroquinoline-N-oxide (4NQO) and 10 mM dimethylnitrosamine (DMN) (batch no. and sources unspecified) in the absence and presence of metabolic activation respectively. Cells were cultured in complete Eagle's basal medium for 1-2 weeks prior to commencement of the assay. Cells were also pre-incubated with 10^{-2} M hydroxyurea which was also added at each step to eliminate s-phase cells.

Contact-inhibited WI-38 cells were incubated with azinphos-methyl and 1 μCi/mL ^{3}H-TdR at 37°C, for 3 h in the absence, and 1 h in the presence, of S-9 liver homogenate. Cells incubated with S-9 liver homogenate were incubated with ^{3}H-TdR for an additional 4 h. Cells were then incubated with labelled thymidine (unspecified concentration, incubation time and temperature, source). DNA was extracted from each sample by the PCA-hydrolysis procedure. The total DNA content was determined after reaction with diphenylamine, and the level of incorporation of ^{3}H-TdR measured by scintillation counting. Results were compared with the background rate of incorporation. No further experimental details were given.

Evaluation criteria: A positive response in the absence of metabolic activation was considered when the mean negative control value was ≤ 100 dpm/μg DNA, the mean value for the positive control was at least 8 times that of the negative control and the mean value for azinphos-methyl was at least twice that of the negative control. A positive response in the presence of metabolic activation was considered when the mean negative control value was ≤ 70 dpm/μg DNA, the mean value for the positive control was at least 4 times that of the negative control and the mean value for azinphos-methyl was at least 1.5 times that of the negative control.

Results and Conclusions: In the absence of metabolic activation, azinphos-methyl did not induce unscheduled DNA synthesis (UDS) in WI-38 cells. In contrast, azinphos-methyl caused an increase in UDS in the presence of metabolic activation, at 10^{-5} and 10^{-4} M, although no dose-response effect was evident (see Table below). The highest concentration of azinphos-methyl failed to induce UDS in accordance with the evaluation criteria which the study author interpreted as a cytotoxic effect, or due to the inhibition of DNA repair.

Effect of azinphos-methyl on unscheduled DNA synthesis (dpm/μg DNA) in WI-38 cells.

Absence of metabolic activation (n = 5)							
	0 M	**10^{-7} M**	**10^{-6} M**	**10^{-5} M**	**10^{-4} M**	**10^{-3} M**	**4NQO**
Mean	95	108	89	79	63	49	822
SE	5	10	7	7	6	10	36
Fold incr.	-	1.14	0.94	0.83	0.67	0.51	8.66
Presence of metabolic activation (n = 3)							
Mean	35	-	-	71	60	50	353
SE	5	-	-	3	10	4	31
Fold incr.	-	-	-	2.03*	1.71*	1.43	10.10

* positive result

Comments: The same concentrations of azinphos-methyl were not examined in the presence and absence of metabolic activation. The concentration and incubation time with unlabelled thymidine were unspecified.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was a variation of the technique reported by Simmon, 1978a. *GLP*: No. The information of this published literature has supplementary value only since methodical details (e. g. concentrations used, exposure time) are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Human lung fibroblasts [strain WI-38, unspecified source] were incubated with the test chemical [technical grade; unspecified batch no. and source] and tritiated-thymidine [unspecified concentration], with or without metabolic activation, for a few hours. [The assay was conducted on synchronised, non-s-phase cells. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254.] The DNA was extracted and the tritiated-thymidine content measured by liquid scintillation counting. Testing was performed at 5 concentrations of test substance [based on a preliminary range-finding study] and 6 replicates of each concentration to facilitate statistical evaluation. [Positive controls included 4-nitroquinoline-N-oxide in the absence of S-9 mix and dimethylnitrosamine in the presence of S-9 mix (concentrations, batch no.s and sources unspecified. DMSO was used as the negative control. No further experimental details were given.

Evaluation criteria: A significant dose-related increase in thymidine incorporation was considered to be a positive result.]

Findings and Conclusion: Azinphos-methyl was found not to induce unscheduled DNA synthesis.

[*Comments:* This published report was a summary only and did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: concentrations of azinphos-methyl and positive controls; assay temperatures and times; cell culture conditions; statistical analyses. The stability of azinphos-methyl in the vehicle/media under the experimental conditions was unspecified. There was no indication of whether the experiment was repeated.]

2.9.2.4 DNA recombination assays

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines, GLP statement or literature citations were given.

Materials and Methods: Technical grade azinphos-methyl (batch no. unspecified, Chemagro Chemical Corporation, Kansas City, MO, USA) was tested on *Salmonella typhimurium* strains SL400 (rec^-/rfa^-) and SL4525 (rec^+/rfa^-) (Dr Bruce Stocker, Stanford University, Stanford, CA, USA), and strains TA1978 (rfa^-) and TA1538 ($uvrB^-/rfa^-$) (Dr Bruce Ames, University of California, CA, USA). Disks of filter paper, containing azinphos-methyl at a minimum of 2 unspecified concentrations, were placed on the surface of 2 agar plates which contained nutrient broth and one bacterial strain. Plates were incubated for 1 day at an unspecified temperature and the zone of inhibition of bacterial growth measured. The positive control was methyl methanesulfonate and the negative controls were ampicillin, kanamycin and chloramphenicol (unspecified sources, batch no., solvents and concentrations). The number of plates/sample was unspecified. The experiment was repeated.

Evaluation criteria: A positive response was indicated by a larger zone of inhibition for the recombination-deficient strain than for the normal strain.

Results and Conclusions: Azinphos-methyl did not cause recombination in the *Salmonella typhimurium* strains SL400, SL4525, TA1978 and TA1538.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: concentrations of azinphos-methyl, positive and negative controls; solvents; sample sizes; incubation temperature. The toxicity of azinphos-methyl or observations of precipitation were not given. No statistical analyses were performed.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was similar to that published by Slater *et al* 1971.
GLP: No. The information of this published literature has supplementary value only since concentrations used are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Azinphos-methyl [technical grade; batch no. and source unspecified] was tested in two [unspecified] concentrations on two pairs of *S. typhimurium* strains, SL4700 and SL4525 [Dr Bruce Stocker, Stanford University, USA], and TA1978 and TA1538 (strains with an rfa^- mutation) [(Dr Bruce Ames, University of California, USA). No further details were given with regard to the maintenance and growth of the bacterial strains, the assay conditions or the evaluation criteria.]

Findings and Conclusion: [The summary data provided indicated that] azinphos-methyl was found not to induce DNA damage in this test system.

[*Comments:* This published report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following details were unspecified: observations of cytotoxicity or compound precipitation;

concentrations tested, the stability of azinphos-methyl in the vehicle. There was no indication of whether the experiment was repeated. No statistical analyses were performed.]

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method employed was according to Brusick and Mayer (1973).

Materials and Methods: Technical grade azinphos-methyl (batch no. unspecified; Chemagro Chemical Corporation, Kansas City, MO, USA) was incubated with stationary-phase *Saccharomyces cerevisiae* strain D3 (unspecified source) at 5 unspecified concentrations up to 50 mg/mL in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. The positive control was 1, 2, 3, 4-diepoxybutane (unspecified source, batch no., solvent and concentration). The experiment was repeated at least once. No further experimental details were given.

Evaluation criteria: A dose-related increase of more than 3-fold in the numbers of mitotic recombinants per mL, and per 10^5 surviving cells, was considered a positive response.

Results and Conclusions: The summary table and figure presented by the study authors indicated that 50 mg/mL azinphos-methyl induced mitotic recombination in *Saccharomyces cerevisiae* strain D3 in the presence and absence of metabolic activation. Additionally, the effect in the absence of metabolic activation was approximately 2-fold greater than that observed in the presence of metabolic activation.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: concentrations of azinphos-methyl, positive and negative controls; solvents; sample sizes; incubation times and temperature. The toxicity of azinphos-methyl or observations of precipitation were not given, considering that 50 mg/mL is a relatively high concentration. No statistical analyses were performed.

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP statement or test guidelines were given.

Materials and Methods: Guthion (azinphos-methyl) (technical grade; batch no. unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) was incubated with *Saccharomyces cerevisiae* strain D3 (unspecified source) at concentrations of 4.5 and 5% in the presence and absence of metabolic activation for 4 h at 30°C. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced

with Aroclor-1254. To determine the number of mitotic recombinants, samples were serially diluted to 10^{-3} and plated on tryptone-yeast-agar. Plates were incubated for 2 days at 30 °C, followed by an additional 2 days at 4°C. Plates were examined microscopically and the number of red colonies or sectors scored. To determine the number of survivors, samples were serially diluted to 10^{-5} and plated on tryptone-yeast-agar and incubated for 2 days at 30°C. The negative control was unspecified while the positive control was 0.1% 1,2,3,4-diepoxybutane (batch no. and source unspecified) both with and without metabolic activation. No further experimental details were given.

Evaluation criteria: A positive response was indicated by a greater-than 3-fold increase in the number of mitotic recombinants per mL and in the number of mitotic recombinants per 10^5 survivors.

Results and Conclusions: There was no treatment-related effect on the number of survivors/mL which indicated that azinphos-methyl was not toxic at the concentrations tested (see Table below). Azinphos-methyl at concentrations of 4.5 and 5% caused a greater than 3-fold increase in mitotic recombinants per mL and per 10^5 survivors, relative to the control, both in the presence and absence of metabolic activation (see Table below). The effect observed in the presence and absence of metabolic activation was equivalent. These observations indicated that azinphos-methyl induced recombination in *Saccharomyces cerevisiae* strain D3.

The effect of azinphos-methyl on mitotic recombination in *Saccharomyces cerevisiae* strain D3.

	-ve control	**+ ve control (0.1%) 1,2,3,4-diepoxybutane**	**4.5% azinphos-methyl**	**5% azinphos-methyl‡**
Survivors/mL (x 10^{-7})				
- S9	5.7, 9.1‡	5.8	5.3	5.7
+ S9	5.8, 8.6‡	4.6	5.8	6.2
Mitotic recombinants/mL (x 10^{-3})				
- S9	4.5, 8‡	1650	15	68
+ S9	4.5, 9.5‡	1435	15	80
Increase in mitotic Recombinants relative to the control †				
- S9	-	367	3.3	8.5
+ S9	-	319	3.3	8.4
Mitotic recombinants/10^5 survivors				
- S9	7.9, 8.8‡	2845	28.3	119.3
+ S9	7.8, 11.0‡	3122	25.9	129
Increase in mitotic recombinants/10^5 survivors relative to the control †				
- S9				
+ S9	-	360	3.6	14
	-	400	3.3	11.7

† calculated by the reviewing toxicologist; ‡ results of a 2nd experiment

Comments: Sample sizes, solvent, negative control and standard deviations/errors were unspecified.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R. and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was according to that published by Brusick and Mayer, 1973. *GLP*: No. The information of this published literature has supplementary value only since concentrations used are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: The yeast strain *S. cerevisiae* D3 [unspecified source] was used. Azinphos-methyl [technical grade; batch no. and source unspecified] was tested in five [unspecified] concentrations both with and without metabolic activation. [Activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. No details were given with regard to the assay conditions, or the growth and maintenance of D3, except that cells were grown on adenine-containing media. The number of red colonies per plate were counted and used to determine the recombinogenic activity of azinphos-methyl.

Evaluation criteria: A positive response was indicated by a greater-than 3-fold dose-response increase in the absolute number of recombinants/mL and in the relative number of mitotic recombinants/10^5 survivors.]

Findings and Conclusion: Without further information, a positive response was declared in a tabulated summary, either with and without metabolic activation.

[*Comments:* This published report did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: observations of cytotoxicity or compound precipitation; concentrations; stability of azinphos-methyl in the vehicle/media; assay conditions (media and growth conditions, incubation times and temperatures). There was no indication of whether the experiment was repeated.]

2.9.2.5 Reversion, gene conversion and crossing over assays.

Bianchi L, Zannoli A, Pizzala R, Stivala LA & Chiesara E (1994) Genotoxicity of five pesticides and their mixtures in *Saccharomyces cerevisiae* D7. Mutation Research. 321: 203 – 211.

Guidelines and GLP: No guidelines or GLP statement were provided.

Materials and Methods: Saccharomyces cerevisiae strain D7 (FK Zimmermann, Darmstad, Germany) was incubated at 10^7 cells/mL with technical grade azinphos-methyl (Bayer, unspecified location, CAS no. 86-50-0) at concentrations of 500, 1000, 5000, 10000 and 25000 μg/mL for 5 h at 28°C in the presence and absence of metabolic activation. Metabolic activation was via a rodent liver S9 fraction which was derived from sodium-phenobarbital plus β-naphthoflavone-induced Sprague-Dawley rats (unspecified weight and source). The dose selection was based on a preliminary cytotoxicity assay. The solvent was DMSO which was also used as the negative control. Ethyl methanesufonate (1%) and cyclophosphamide (100 μg/mL) were used as the positive controls in the absence and presence of metabolic

activation respectively (unspecified sources and batch no.). Cells were plated onto isoleucine-free medium, tryptophan-free medium and complete medium, and incubated for 5 h at 28°C then score for survivors and convertants. Isoleucine revertants were scored after 8 days incubation. The experiment was performed in triplicate and data were analysed using a Student's t-test. No evaluation criteria were given.

Results: At 25000 μg/mL, azinphos-methyl had a slightly toxic effect as shown by an approximately 10% reduction in survivors compared to the negative control, both in the presence and absence of metabolic activation (see Table below). In the absence of metabolic activation, there was a statistically significant increase ($p < 0.05$) in the number of convertants per 10^5 survivors at 25000 μg/mL (see Table below). At 10000 and 25000 μg/mL, in the absence of metabolic activation, a statistically significant increase ($p < 0.05$) in the number of revertants per 10^6 survivors was detected (see Table below). There was no treatment-related effect observed in the presence of metabolic activation. Both positive controls were observed to induce a significant increase ($p < 0.01$) in the numbers of convertants and revertants (see Table below).

Effect of azinphos-methyl on gene reversion and conversion in Saccharomyces cerevisiae strain D7.

Treatment	Survivors (%)	Convertants/10^5 survivors	Revertants/10^6 survivors
Without S9			
Negative control	100	0.85 ± 0.32	0.72 ± 0.23
1% EMS	52 ± 13.1	247.55 ± 12.62**	707.67 ± 45.93**
25000 μg/mL	87 ± 2.2	2.23 ± 0.68*	4.94 ± 2.54*
10000 μg/mL	94 ± 5.2	1.74 ± 0.58	4.11 ± 2.53*
5000 μg/mL	97 ± 6.5	1.25 ± 0.51	1.9 ± 0.2
1000 μg/mL	100 ± 0.9	1.08 ± 0.36	0.98 ± 0.04
500 μg/mL	99 ± 3.4	0.66 ± 0.36	0.56 ± 0.28
With S9			
Negative control	100	0.98 ± 0.70	0.56 ± 0.20
1% CPA	74 ± 8.1	31.68 ± 8.63**	39.92 ± 10.02**
25000 μg/mL	88 ± 5.8	1.55 ± 1.09	1.79 ± 1.08
10000 μg/mL	101 ± 0.3	1.15 ± 0.87	1.28 ± 1.11
5000 μg/mL	99 ± 1.3	1.11 ± 1.13	0.95 ± 0.59
1000 μg/mL	98 ± 2.5	1.11 ± 1.39	0.68 ± 0.49
500 μg/mL	101 ± 0.2	0.87 ± 0.95	0.63 ± 0.11

All results are means ± 1 SEM; EMS = ethyl methanesufonate; CPA = cyclophosphamide; * statistically different to the control at $p < 0.05$; ** statistically different to the control at $p < 0.01$

Conclusions: At 10000 μg/mL and above, in the absence of metabolic activation, azinphos-methyl induced reversion and gene conversion in *Saccharomyces cerevisiae* strain D7.

Comments: No evaluation criteria were given. There was no indication whether the experiment was repeated.

Hrelia P, Morotti M, Scotti M, Vigagni F, Paolini M, Perocco P & Cantelli Forti G (1990) Genotoxic risk associated with pesticides: evidences on short-term tests. Pharmocological Research. 22: Supplement 3.

Guidelines and GLP: This is a summary publication. No test guidelines or GLP statement were provided.

Materials and Methods: Saccharomyces cerevisiae strain D7 was incubated with azinphos-methyl (unspecified source, batch no., solvent and concentration) for 2 h in the presence and absence of metabolic activation. Metabolic activation was via a S9 fraction which was derived from sodium-phenobarbital plus β-naphthoflavone-induced rodent liver. No further experimental details were given.

Results and Conclusions: The study authors indicated that azinphos-methyl caused an increase in mitotic crossing-over.

Comments: This published study did not provide sufficient methodological or observational detail for regulatory purposes and is thus considered unacceptable. No data were provided.

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method employed was according to Zimmermann *et al* (1975).

Materials and Methods: Technical grade azinphos-methyl (batch no. unspecified; Chemagro Chemical Corporation, Kansas City, MO, USA) was tested on stationary-phase *Saccharomyces cerevisiae* strain D7 (unspecified source) in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. Following exposure to an unspecified concentration of azinphos-methyl, *S. cerevisiae* D7 was grown on adenine-containing media. The experiment was repeated at least once. No further experimental details were given.

The number of mitotic recombinants was determined based on the numbers of twin-sectored colonies of the genotype ade2-40/ade2-40 (deep red) and ade2-119/ade2-119 (pink) which were derived from the original heteroallelic condition ade2-40/ade2-119 (white). The number of pink, red, white and pink, and red and white colonies indicated the total number of aberrants. The numbers of mitotic recombinants and total aberrants per 10^5 survivors, and the number of gene convertants per 10^6 survivors were calculated by the study authors.

Evaluation criteria: A dose-related increase in the numbers of aberrant or convertant colonies indicated that mitotic crossing over and mitotic gene conversion had occurred.

Results and Conclusions: The summary table presented by the study authors indicated that azinphos-methyl did not cause mitotic recombination or gene conversion in *Saccharomyces cerevisiae* strain D7 in the presence and absence of metabolic activation.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: concentrations of azinphos-methyl, positive and negative controls; solvents; sample sizes; incubation times and temperature; culture conditions and media for D7. The toxicity of azinphos-methyl or observations of

precipitation were not given. No statistical analyses were performed. Results were not confirmed in an independent experiment.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was according to that published by Zimmermann, 1975. *GLP*: No. The information of this published literature has supplementary value only since concentrations used are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: The yeast strain S. cerevisiae D7 [unspecified source] was used and the frequency of mitotic recombinants, gene convertants and total aberrants was determined, both in the presence and absence of metabolic activation. [Activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. Following exposure to an unspecified concentration of azinphos-methyl (technical grade; batch no., source, incubation time and temperature unspecified) *S. cerevisiae* D7 was grown on adenine-containing media. No further details were given with regard to the assay conditions.

The number of mitotic recombinants was determined based on the numbers of twin-sectored colonies of the genotype ade2-40/ade2-40 (deep red) and ade2-119/ade2-119 (pink) which were derived from the original heteroallelic condition ade2-40/ade2-119 (white). The number of pink, red, white and pink, and red and white colonies indicated the total number of aberrants. The number of total aberrants per 10^5 survivors and the number of gene convertants per 10^6 survivors was calculated.

Evaluation criteria: A dose-related increase in the numbers of aberrant or convertant colonies indicated a positive response for crossing over and mitotic gene conversion.]
Findings and Conclusion: Azinphos-methyl was not mutagenic in this test system.

[*Comments:* This published report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following details were unspecified: concentrations; the stability of azinphos-methyl in the vehicle/media under the experimental conditions; assay conditions (incubation time and temperature); positive and negative controls. There was no indication of whether the experiment was repeated. The data were not subjected to statistical analyses.]

2.9.3 *In vitro* chromosomal effect assays

2.9.3.1 Sister chromatid exchange

♦ ***Chen HH, Sirianni SR & Huang CC (1982a) Sister chromatid exchange in chinese hamster cells treated with seventeen organophosphorus compounds in the presence of a metabolic activation system. Environmental Mutagenesis. 4: 621 - 624.***

♦ ***Chen HH, Sirianni SR & Huang CC (1982b) Sister-chromatid exchanges and cell-cycle delay in chinese hamster V79 cells treated with 9 organophosphorus compounds (8 pesticides and 1 defoliant). Mutation Research. 103: 307 - 313.***

Guidelines and GLP: GLP: No. The information of this published literature has supplementary value only since not all methodical details are given in these references.

Material and Methods: V79 cells [unspecified source] were treated with azinphos-methyl [98.7% purity, batch no. unspecified, Mobay Chemical Corp., Kansas City, MO, USA] in concentrations of 0 (DMSO), 2.5, 5.0, 10.0, 20.0 μg/mL (without S-9 mix) or 0 (DMSO), 5.0, 10.0, 20.0, 25.0 μg/mL (with S-9 mix). [Rapidly growing cells were treated with azinphos-methyl in the presence and absence of S-9 mix in complete RPMI1640 medium. 5-bromo-2'deoxyuridine (BUdR) solution (10 μg/mL) (Sigma Chemical Co., St Louis, MO, USA) was immediately added and incubated for 32 h at an unspecified temperature in the dark. Colcemide (0.04 μg/mL) was added 2 h prior to harvesting, followed by preparation of slides and SCE staining. Cyclophosphamide (5 μg/mL) dissolved in sterile water or DMSO was used as the positive control both in the presence and absence of S-9 mix. The negative/solvent control in the presence and absence of S-9 mix was DMSO. The frequencies of first (darkly stained chromatids), second (differential staining of chromatids), third and further mitoses (< ¼ darkly stained chromatids) in each sample were determined by microscopic analysis of staining patterns.

Evaluation criteria: No evaluation criteria were specified.]

Findings and Conclusion: No sister chromatid exchanges were found in these investigations. [The study authors indicated that azinphos-methyl was highly toxic to V79 cells at doses of 40 and 80 μg/mL. Additionally, all dose levels caused a large reduction in mitoses and resulted in no differentiation of metaphases. Graphically presented results illustrated that azinphos-methyl caused substantial cell-cycle delay.]

[*Comments:* These published reports lacked methodological or observational detail. No raw data were provided. Statistical analyses were not performed on the data. The stability of azinphos-methyl in the vehicle/media under the experimental conditions was unspecified. There was no indication of whether the experiment was repeated.]

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method employed was according to Perry and Evans (1975) and Stetka and Wolff (1976).

Materials and Methods: Technical grade azinphos-methyl (batch no. unspecified; Chemagro Chemical Corporation, Kansas City, MO, USA) was incubated with Chinese hamster ovary (CHO) cells (American Type Culture Collection, Rockville, Maryland, USA) in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. Details of the solvent and negative control were unspecified. The positive

controls were ethyl methanesulfonate and dimethylnitrosamine (source, batch no., solvents and concentrations unspecified) in the absence and presence of metabolic activation respectively. Five unspecified concentrations of azinphos-methyl, and positive and negative controls were tested. Duplicate coded samples were analysed by 2 cytogeneticists for at least 3 concentrations of azinphos-methyl and the controls. Each cytogeneticist analysed 25 cells per sample and scored the total number of sister chromatid exchanges (SCEs), and number of chromosomes per cell. No further experimental details were provided.

Evaluation criteria: A positive result was considered if both cytogeneticists agreed that: azinphos-methyl induced SCE at a minimum of twice the background frequency; that a dose-related increase in SCE frequency was detected over a minimum of 3 concentrations; that at least 1 value was significantly greater ($p < 0.001$) than the background frequency.

Results and Conclusions: Azinphos-methyl did not cause SCE in CHO cells either in the presence and absence of metabolic activation.

Comments: This summary report did not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: concentrations of azinphos-methyl, positive and negative controls; solvents; sample sizes; incubation times and temperature; cell culture conditions; the toxicity of azinphos-methyl. No statistical analyses appear to have been performed as specified in the evaluation criteria.

Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.

Guidelines and GLP: No GLP or test guidelines were provided. Data were subsequently analysed and published by Garrett *et al* (1986).

Materials and Methods: Chinese hamster ovary (CHO) cells (American Type Culture Collection, Rockville, Maryland, USA) were treated with 5 unspecified concentrations of technical grade azinphos-methyl (batch no. and source unspecified) in the presence and absence of metabolic activation. Metabolic activation was via S-9 mix which was derived from adult male Sprague-Dawley rats weighing 250-300 g that had been induced with Aroclor-1254. Positive controls were ethylmethanesulfonate (unspecified concentration and source) in the absence of S-9 mix, and dimethyl-nitrosamine (unspecified concentration and source) in the presence of S-9 mix. The solvent used for azinphos-methyl and positive controls was unspecified. Duplicate coded samples of at least 3 concentrations of azinphos-methyl and the controls, were analysed by two cytogeneticists. Twenty-five cells per sample were analysed for the total number of sister chromatid exchanges (SCE) per cell and the number of chromosomes per cell. No further experimental details were given including incubation times and temperatures, or statistical analyses.

Evaluation criteria: A positive result was concluded if both cytogeneticists agreed that azinphos-methyl induced at least twice the baseline SCE frequencies, or that a dose-response relationship was detected over at least 3 concentrations with at least one value statistically higher than the control at $p < 0.001$.

Results and Conclusions: The summary data provided in this published report indicated that azinphos-methyl did not cause SCE in CHO cells

Comments: This published report is a summary only and did not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: concentrations of azinphos-methyl and positive controls; assay times and temperatures; details of statistical analyses; stability of azinphos-methyl in the vehicle/media under the experimental conditions. There was no indication of whether the experiment was repeated. The toxicity of azinphos-methyl to CHO cells was not investigated.

Gomez-Arroyo S, Noriega-Aldana N, Juarez-Rodriguez D & Villalobos-Pietrini R (1987) Sister chromatid exchange induced by the organophosphorus insecticides methyl parathion, dimethoate, phoxim and methyl azinphos in cultured human lymphocytes. Contam. Ambient. 3: 63 – 70.

Guidelines and GLP: No GLP or test guidelines were provided.

Materials and Methods: Peripheral blood (5 mL) was taken from an unspecified number of healthy volunteers and established in McCoys 5A complete media containing phytohaemagglutinin for 72 h at 37°C. Peripheral blood cultures were incubated with 5 µg/mL 5-bromodeoxyuridine (Sigma; unspecified batch no.) and azinphos-methyl (Bayer AG, Wuppertal-Elberfeld, Germany; unspecified purity and batch no.) at concentrations of 0, 2, 4, 8, 10, 20, and 30 ppm for 70 h followed by the addition of colchicine (Merck; unspecified batch no. and concentration) for 2 h. These concentrations were based on an undisclosed preliminary experiment. Cells were harvested, fixed, stained and microscopically examined, and 50 second-division metaphases analysed for each duplicate sample. A terminal SCE was recorded as 1 exchange and an interstitial SCE as 2 exchanges. Data were analysed using a student's t-test with a significant result concluded when $p < 0.001$. No further experimental details were given.

Results and Conclusions: Azinphos-methyl up to 30 ppm did not induce SCE in human lymphocytes. The study authors indicated that the solubility limit for azinphos-methyl was 30 ppm.

Comments: This published study lacked some methodological and observational detail. The stability of azinphos-methyl, under the experimental conditions tested, was not evaluated. No positive control was utilised to validate the assay. The toxicity of azinphos-methyl was unspecified. The experiment appeared to be performed in the absence of metabolic activation only.

Hrelia P, Morotti M, Scotti M, Vigagni F, Paolini M, Perocco P & Cantelli Forti G (1990) Genotoxic risk associated with pesticides: evidences on short-term tests. Pharmocological Research. 22: Supplement 3.

Guidelines and GLP: This is a summary publication. No test guidelines or GLP statement were provided.

Materials and Methods: Azinphos-methyl (unspecified source, batch no., solvent and concentration) was tested on human peripheral blood lymphocytes (unspecified source) for it

ability to cause SCE in the presence and absence of metabolic activation. Metabolic activation was via a S9 fraction which was derived from sodium-phenobarbital plus β-naphthoflavone-induced rodent liver. No further experimental details were given.

Results and Conclusions: The study authors indicated that azinphos-methyl did not cause SCE in human lymphocytes.

Comments: This published study did not provide sufficient methodological or observational detail for regulatory purposes and is thus considered unacceptable. No data were provided.

2.9.3.2 Clastogenicity

♦ ***Alam MT, Corbeil M, Chagnon A & Kasatiya SS (1974) Chromosomal anomalies induced by the organic phosphate pesticide Guthion in chinese hamster cells. Chromosoma (Ber.). 49: 77-86, 1974.***

Guidelines and GLP: No reference or guideline was mentioned for the method employed. *GLP*: No. The information of this published literature has supplementary value only since not all procedural details are specified.

Material and Methods: Triplicate cultures of CHO-Kl cells [American Type Culture Collection, Rockville, Maryland, USA] were treated with azinphos-methyl (batch no. not given, purity: ca. 90%, [Chemagro Chemical Corporation, Kansas City, MO, USA]) in concentrations of 60, 80, 100 and 120 μg/mL for 18 hours and the chromosomal changes determined. [Cells were maintained throughout the experiment in complete Medium 199 at 37°C.

Cytoxicity study: Asynchronous cells were pulsed with 0.5 μCi/mL ^{3}H-TdR for 15 min prior to the addition of azinphos-methyl. Cells were harvested at 2 h intervals and the number of s-phase cells progressing into mitosis measured by autoradiography. The mitotic index was also calculated for each sample.
Chromosomal study: Six hours after seeding, triplicate flasks of cells were treated with fresh media which contained azinphos-methyl. No positive control was included in the assay. Details of the negative control were unspecified. Cells were incubated for 18 h, washed and allowed to recover in fresh medium for 12-24 h. Following treatment with colchicine (0.05 μg/mL) (unspecified source and batch no.) for 3 h, cells were harvested, stained and microscopically examined for chromosomal aberrations with 500-2542 metaphases examined for each treatment. Chromatid and isochromatid breaks were scored as a single break, a chromosomal exchange was scored as a single break in each of the chromosomes involved. Terminal chromatid deletions were scored when determining the mean breaks/cell. Regional sensitivity to azinphos-methyl-induced breakage was evaluated by dividing chromosomes 1 and 2 into segments. During microscopic evaluation any metaphases that exhibited breakage in either chromosome 1 or 2 were photographed, enlarged and the specific segments where the breaks occurred identified.]

Findings and Conclusion: At clear cytotoxic concentrations, chromosomal anomalies were observed. [Results from cytotoxic experiments were undisclosed although the study authors concluded that 80-120 μg/mL azinphos-methyl caused cell cycle arrest, while 60 μg/mL caused a delay in cell-cycle progression. There was a clear dose-response trend with regard to

the incidence of abnormal metaphases, chromatid breaks, chromatid exchanges and the mean number of breaks/cell (see Table below). However, statistical analysis of the later data and revealed a highly significant difference (p = 0.005) to the control at every concentration (see Table below).

Summary of results showing the effect of azinphos-methyl on the incidence of chromosomal aberrations in CH-K1 cells. (Data shown are following a recovery period of 24 h)

Azinphos-methyl conc. (μg/mL)	N	Abnormal Metaphases (%)	Chromatid breaks (%)	Isochromatid breaks (%)	Chromatid exchanges (%)	Mean no. breaks/cell
0	1000	0.6	0.6	0.0	0.0	0.006
60	1000	2.20	2.6	0.1	1.2	0.051*
80	1000	7.80	8.3	0.2	8.7	0.259*
100	500	11.60	10.8	0.6	24.2	0.598*
120	1000	18.70	23.10	0.3	27.6	0.786*

* significantly different to the control at p = 0.005

A χ^2 test revealed no difference in the incidence of breaks between chromosome 1 and 2 (χ^2 = 0.22; p > 0.50). There was a non-random distribution of breaks in both chromosomes, with the highest incidence of breaks on chromosome 1 detected in regions 3, 4, 7 and 8, and in regions 3 and 4 on chromosome 2.

Comments: This published report lacked certain methodological or observational detail. No raw data was given. An indication of the variability of the data was not provided (standard deviations or errors). Details of all statistical analyses were unspecified. Details of the control were not given. No positive control was tested. The experiment appears to have been performed in the absence of metabolic activation. Although the study authors indicated that a cytotoxicity experiment was performed, no supporting data were provided (eg mitotic indices).]

Alam MT & Kasatiya SS (1976) Cytological effects of an organic phosphate pesticide on human cells in vitro. Can. J. Genet. Cytol. 18: 665 – 671.

Alam MT & Kasatiya SS (1975) Chromosome damage induced by an organic phosphate pesticide in human cells. Can. J. Genet. Cytol. 17(3): 544.

Guidelines and GLP: No test guidelines or GLP statement was given.

Materials and Methods: The human cell lines WI-38 (diploid) and HEp-2 (heterodiploid) (unspecified sources) were treated with technical grade azinphos-methyl (90% ai content, batch no. unspecified, Chemagro Chemical Corporation, Kansas City, MO, USA) in a similar manner to that described by Alam *et al* (1974). Cells were cultured in complete Eagle's minimal essential medium throughout the experiment. To determine the effect of azinphos-methyl on mitosis, log-phase WI-38 cells were treated with 120 μg/mL azinphos-methyl and samples analysed, with and without colcemid treatment [unspecified concentration], at 30 min, 60 min and then hourly. To examine the chromosomal effects of azinphos-methyl, WI-38 cells were treated at concentrations of 0, 120, 140 and 160 μg/mL, and HEp-2 cells at concentrations of 0, 140 and 160 μg/mL. After 18 h, fresh media was added and HEp-2 and

WI-38 cells allowed to recover for 4-24 h and 24-72 h respectively. Colcemid (0.05 μg/mL) (unspecified batch no. and source) was added for 2-4 h and cells harvested, fixed, stained and microscopically examined. No further experimental details were given.

Evaluation criteria: Chromatid and isochromatid breaks were counted as single breaks, while a chromosomal exchange and dicentric were scored as 2 breaks. Although discontinuities of chromosomal regions without distal nonalignment were scored as gaps, they were not used to determine the mean number of breaks per cell.

Statistical analysis: The Kruskal-Wallis 1-way ANOVA by ranks was used to analyse the difference in chromosome breaks between treated and control cells.

Results: Azinphos-methyl was graphically illustrated to inhibit entry of WI-38 cells into mitosis at 120 μg/mL. The study authors indicated that no accumulation of metaphases was evident in treated cells while 8.5% of metaphases in control cells were arrested within 3 h.

There was a clear, statistically significant ($p < 0.05$), dose-response effect with regard to the mean number of breaks per cell per treatment in both WI-38 and HEp-2 cells (see Table below). Generally, the incidence of abnormal metaphases, gaps, chromatid breaks, chromatid exchanges in treated cells was markedly higher than control cells at all azinphos-methyl concentrations and at each recovery time (see Table below).

Although actual incidences were unspecified, photographic evidence illustrated that azinphos-methyl caused: a failure of chromosomal condensation; chromosomal constriction; chromosome despiralisation; pulverisation of chromosomes, chromosomal breaks and exchanges.

Summary of results showing the incidence of chromosomal aberrations in WI-38 and HEp-2 cells following exposure to azinphos-methyl for 18 h.

Conc. (μg/mL)	Recovery Time (h)	N	Abnormal Metaphases (%)	Gaps (%)	Chromatid Breaks (%)	Chromatid Exchanges (%)	Mean no. breaks/ cell/treatment
WI-38							
0	-	1000	0.9	0.9	1.1	0.0	NS
120	24	1000	3.4	1.0	2.1	1.4	0.042*
	32	500	3.8	2.2	3.2	1.4	
	40	500	2.6	1.0	2.0	1.0	
	48	500	1.0	0.6	0.8	0.2	
140	24	500	2.6	0.8	2.0	1.0	0.058*
	32	500	3.4	1.0	2.6	1.4	
	40	500	4.6	1.8	4.2	3.4	
	48	500	1.4	1.0	0.6	1.0	
160	40	1000	5.9	2.3	7.6	2.67	0.165*
	48	600	12.8	1.6	11.7	7.2	
	56	500	9.8	3.2	10.2	3.2	
	72	500	6.2	2.0	4.6	3.8	
HEp-2							
0	-	600	2.8	1.0	0.6	2.8	NS
140	4	600	11.3	13.3	14.2	3.7	0.228*
	8	500	16.4	4.8	21.2	4.2	
	12	200	11.0	7.5	17.5	11.5	
	16	500	6.8	2.4	2.6	5.0	

Conc. (μg/mL)	Recovery Time (h)	N	Abnormal Metaphases (%)	Gaps (%)	Chromatid Breaks (%)	Chromatid Exchanges (%)	Mean no. breaks/ cell/treatment
	24	300	8.3	2.6	5.7	6.3	
160	4	150	38.0	31.3	83.3	3.4	0.304*
	12	500	13.2	8.2	20.2	1.8	
	16	500	9.6	3.6	6.6	7.8	
	24	500	13.8	3.0	12.8	7.2	

* significantly different to the control at $p < 0.05$; NS = not specified

Conclusion: Azinphos-methyl (120 μg/mL) inhibited WI-38 cells from entering mitosis. Up to 160 μg/mL azinphos-methyl caused an increase in the incidence chromosomal aberrations (mainly chromatid breaks and exchanges) in WI-38 and HEp-2 cells.

Comments: This published study lacked certain methodological and observational details. No rationale was given for the narrow dose selection. Sample sizes and solvent details were unspecified. A positive control was not used for comparison to validate the assay. Mitotic indices were not calculated. The effect of azinphos-methyl on mitosis was performed only on WI-38 cells. Individual culture data were not provided. The number of cells analysed at each concentration varied. The experiment did not appear to be performed in the presence of metabolic activation.

♦ ***Herbold BA (1986) E1582 (common name: azinphos-methyl): cytogenetic study with human lymphocyte cultures in vitro to evaluate for harmful effect on chromosomes. Report no. 15145. Lab & Sponsor: Institute of Toxicology, Industrial Chemicals Division, Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: 11 February 1986 - 24 June 1986. Report date: 20 October 1986.***

Guidelines and GLP: In general, the study can be considered to be in compliance with the requirements of OECD Guideline 473 (adopted 26 May 1983). Deviation: Only one preparation time was used after a cultivation time of 72 hours. The study is GLP compliant. A formal QAU declaration of the test facility is included. The study is acceptable.

Material and Methods: Human lymphocytes [derived from 1 healthy male and female] were exposed in 2 separate cultures to azinphos-methyl (batch no. 230505073 = 233596230; purity: 91.9% [Bayer AG, Wuppertal-Elberfeld, Germany]) at concentrations of 0, 1, 10 and 100 μg/mL in the absence of S-9 mix, and at 0, 5, 50 and 500 μg/mL in the presence of S-9 mix. [The experiment was performed in at least quadruplicate.] Azinphos-methyl and the positive control substances cyclophosphamide (CP, 10 μg/mL, [Roth, batch no. 1148865] with S-9 mix) and mitomycin C (MMC, 0.1 μg/mL, [Asta, batch no. 0044438] without S-9 mix) were formulated in DMSO, which also served as the negative control. [The study author indicated that azinphos-methyl was stable in DMSO.] After cultivation of the cells for 48 hours [at 37°C], in the non-activated cultures the cells were exposed to azinphos-methyl for 24 hours and in the activated cultures for 2.5 hours [at 37°C. Colcemid (0.4 μg/mL, unspecified source) was added to all cultures after 21 hours exposure to azinphos-methyl]. All cells were prepared after a total of 72 hours. [Cells were swollen in hypotonic solution, fixed and dropped onto slides (2-3 slides/sample), and stained.] Approximately 200 metaphases (100 per donor) per concentration, with or without S-9 mix, were [microscopically] examined for structural chromosomal changes. [The mitotic index and chromosomal aberration rate were determined for each control and test sample.]

For the activation experiments, S-9 mix was derived from adult male Sprague-Dawley rats, induced 5 days before preparation with a single ip dose of Aroclor 1254, 500 mg/kg bw dissolved in corn oil. The liver supernatant fluid was prepared and combined with an appropriate co-factor solution according to established technique.

Evaluation criteria: The test is considered positive if there is a dose dependent and statistically significant increase in the chromosome aberration rate over the negative control. A dose-dependent increase lacking statistical significance or a significantly elevated aberration frequency which is not concentration related would be assessed equivocal.

*Statistic*s: [A 1-sided corrected] Chi^2 test. [A statistical difference in the mitotic index and aberration rate between control and treated cultures was determined when $p < 0.01$ and $p < 0.05$ respectively.]

Findings

Cytotoxicity test with S-9 mix: A significant reduction in mitotic index was noted at 50 µg/mL to 86.9% [$p \leq 0.05$] and at 500 µg/mL to 43.2% [$p \leq 0.01$] of the solvent control. The positive control, MMC, reduced the mitotic index to 86.6% [$p \leq 0.05$]. CP was without effect on mitotic frequency. [MMC was actually not used as the positive control with the S-9 mix. Only the effect of azinphos-methyl on the mitotic index at the highest dose (500 µg/mL) was a significant effect as it complied with the study authors evaluation criteria that $p \leq 0.01$.]

Cytotoxicity test without S-9 mix: The mitotic index fell significantly [$p < 0.01$] at 100 µg/mL azinphos-methyl to 28.0% of the solvent control value. [The mitotic index of MMC-treated cultures was significantly lower ($p < 0.01$; 86.6%) than the solvent control].

Chromosome aberration assay without S-9 mix: There were no significant inter-group differences [in the incidence of chromosomal aberrations] between the solvent control and the cultures treated with azinphos-methyl at any concentration [(see Table below).

Summary of results showing the effect of azinphos-methyl on the incidence of chromosomal aberrations in human lymphocytes without metabolic activation

Treatment	Metaphases with aberrations incl. gaps		Metaphases with aberrations excl. gaps		Metaphases with exchanges		Polyploidies n/evaluated meta-phases (x)	
(µg/mL)	n	%	n	%	n	%	n/x	%
DMSO (0)	6	3.0	5	2.5			0/400	0
(1)	2	1.0	1	0.5			2/400	0.5
(10)	8	4.0	5	2.5			0/400	0
(100)	11	5.5	10	5.0	1	0.5	1/400	0.3
MMC (0.1)	44**	23.0	41**	21.5	7*	3.5	1/400	0.3

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

In contrast the positive control (MMC) had a significantly higher incidence of metaphases with aberrations including ($p \leq 0.01$; 23.0%) and excluding gaps ($p < 0.01$; 21.5%), and metaphases with exchanges ($p \leq 0.05$; 3.5%), compared to the solvent control (see Table above).]

Chromosome aberration assay with S-9 mix: Statistically significant, treatment-related variations in all parameters were noted at the highest concentration of 500 μg/mL. [At 500 μg/mL the incidence of metaphases with aberrations including/excluding gaps was 17.0% ($p < 0.05$), metaphases with exchanges was 10.0% ($p < 0.05$) and polyploidies was 3.3% (see Table below).] This concentration [500 μg/mL] was clearly cytotoxic, inducing a reduction in mitotic index to 43.2% relative to the solvent control. There were no effects at concentrations up to 50 μg/mL. The positive controls, MMC and CP, both exhibited a clear clastogenic effect, thus demonstrating the sensitivity of the system [(see Table below). MMC was not used as the positive control in the presence of S-9 mix. The incidence of metaphases with aberrations including (17.5%) and excluding (16.5%) gaps was significantly higher in CP-treated cultures compared to the solvent control ($p < 0.05$).]

Summary of results showing the effect of azinphos-methyl + metabolic activation on the incidence of chromosomal aberrations in human lymphocytes

Treatment	Metaphases with aberrations incl. gaps		Metaphases with aberrations excl. gaps		Metaphases with exchanges		Polyploidies n/evaluated metaphases (x)	
(μg/ml)	n	%	n	%	n	%	n/x	%
DMS0 (0)	5	2.5	5	2.5	1	0.5	0/400	0
(5)	8	4.0	5	2.5			0/400	0
(50)	11	5.5	9	4.5	1	0.5	2/400	0.5
(500)	34*	[17.0]	34*	[17.0]	20*	10.0	10/300	3.3
CP (10)	35*	17.5	33*	16.5	1	0.5	1/300	0.3

* $p < 0.05$

Conclusion: A clastogenic effect of azinphos-methyl was indicated only at the cytotoxic concentration of 500 μg/mL [in the presence of metabolic activation with S-9 mix]. Azinphos-methyl did not show any harmful effects on the chromosomes of human lymphocytes at concentrations up to 100 μg/mL without S-9 mix and at concentrations up to 50 μg/mL with S-9 mix.

[*Comments:* Although a statement was made pertaining to the stability of azinphos-methyl in DMSO, the stability under the experimental conditions was not investigated. Cells were washed 2.5 h after the addition of the S-9 mix to reduce its toxic side-effects, which implied that these cells were exposed to azinphos-methyl for a much shorter period of time than cells not exposed to metabolic activation. Individual culture data were not given. No indication of the variability of the data was provided (standard deviations or errors).]

2.9.3.3 Micronucleus test (Cytokinesis-blocked human lymphocytes)

Bianchi-Santamaria A, Gobbi M, Cembran M & Arnaboldi A (1997) Human lymphocyte micronucleus genotoxicity test with mixtures of phytochemicals in environmental concentrations. Mutation Research. 388: 27 – 32.

Guidelines and GLP: No test guidelines or GLP statement were provided. The micronucleus test using human peripheral blood lymphocytes was performed according to Fenech and Morley (1985).

Materials and Methods: Azinphos-methyl (CAS no. 642-71-9, unspecified purity and source) was dissolved in DMSO and tested at concentrations of 0.06, 0.6 and 6 μg/mL on peripheral

blood lymphocytes that were taken from 4 healthy male and females volunteers. Concentrations were selected based on the calculation of azinphos-methyl in foodstuffs by Camoni *et al* (1990). Bleomycin (50 μg/mL, Phone-Poulenc Pharma, France, unspecified batch no.) was used as the positive control while DMSO was used as the negative control. Test and control samples were incubated with heparinised whole blood under dim light for 2 h at 37°C. Cells were washed then incubated for 44 h in complete RPMI1640 medium containing phytohaemagglutinin (2%, Difco, unspecified batch no. and location). Cytochalasin B (3 μg/mL, Sigma, unspecified batch no.) was added for 28 h then the cells were harvested, fixed, stained and microscopically examined for micronuclei. For each sample 1000 cells were scored and the incidence of micronuclei recorded. Four independent experiments were performed. Cell proliferation was measured by calculating the nuclear division index (NDI) [NDI = M1 + 2M2 + 3M3 + 4M4/n where M1-M4 are the number of nuclei/cell (from experiments 1-4) and n is the total number of cells scored. A number of other pesticides (benomyl, diazinon, dimethoate and pirimiphos-methyl) were also tested concurrently, both individually and in mixtures of 2 or more.

Statistical analysis: Data were analysed using the Poisson regression test.

Results: The study authors indicated that a 10-fold increase in the number of micronucleated cells was observed in lymphocytes treated with the positive control (bleomycin), although no supporting data were provided. The frequency of micronuclei in treated lymphocytes was approximately 1.8-fold greater than the frequency detected in control cells, however, no dose-response effect was evident (see Table below). This effect was unlikely to be biologically significant as the data fell within the control range of 0.475 to 1.100 micronuclei/100 cytokinesis-blocked cells, which was determined from concurrent controls for the other pesticides. There was no treatment-related effect on the NDI (see Table below). The study authors concluded that azinphos-methyl caused a weak yet statistically significant elevation in the frequency of micronuclei, however no specific details of statistical analyses or p values were given.

Incidence of micronuclei in cytokinesis-blocked human lymphocytes that had been treated with azinphos-methyl for 2 h. (NDI = nuclear division index)

Observation	Control	0.06 μg/mL	0.6 μg/mL	6 μg/mL
Mean frequency of micronuclei/100 cells ± 1 SD*	0.53 ± 0.13	0.93 ± 0.29	0.93 ± 0.26	0.95 ± 0.10
Mean* NDI ± 1 SD*	0.16 ± 0.05	0.15 ± 0.04	0.13 ± 0.05	0.13 + 0.04

* calculated by reviewing toxicologist

Conclusions: The study authors concluded that azinphos-methyl showed weak genotoxic activity in human peripheral blood lymphocytes as shown by an approximately 1.8-fold increase in micronuclei at 0.06-6 μg/mL relative to the control. Furthermore, the study authors suggested that this weak genotoxic effect was possibly due to the absence of metabolic activation. This result was not considered to be toxicologically relevant as no dose-response effect was evident and due to the high variability of the negative control data.

Comments: Data for the positive control (bleomycin) and sample sizes for each concentration were unspecified. The robustness of the assay is questionable due to the high variability of the negative control data. Justification for the use of the Poisson regression test to analyse the data was not given.

2.9.4 *In vivo* chromosomal effect assays

2.9.4.1 Micronucleus test

♦ ***Herbold BA (1979a) Micronucleus test on mouse to evaluate R 1582 for potential mutagenic effects. Report no. 8521. Lab & Sponsor: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study duration: March - May 1979. Report date: 19 July 1979.***

Guidelines and GLP: The method employed was based on that of Schmid [*et al*] (1975), the principle of whose method was taken over into OECD Guideline 474 (adopted 26 May 1983). When the study was performed, GLP was not compulsory. The study is acceptable.

Material and Methods: Groups of 5 male and 5 female, [8-12-week old] NMRI mice (source: S. Ivanovas GmbH, Kisslegg/Allgäu, Germany, body weight range 22-32 g) received azinphos-methyl (batch no. 230 705 148/201-300; purity 92.3% [Bayer AG, Wuppertal-Elberfeld, Germany]) as 2 PO administrations at an interval of 24 hours. [Mice were randomly assigned to the treatment groups and housed in Type I Makrolon cages (unspecified source) in groups of no more than 3 with pelleted ®Atromin chow (unspecified source) and tap water available *ad libitum*. Room temperature and humidity were maintained at 20-26°C and approximately 60% respectively. A 12 h light/dark cycle was maintained throughout the study.] The dose levels employed were 2 x 2.5 and 2 x 5.0 mg/kg bw.

The test material was suspended in 0.5% Cremophor/water, which was the negative control material. [The test solution was administered orally using a stomach tube.] Trenimon® [unspecified source], the positive control material, was administered intraperitoneally as a solution in water, at a dose level of 2 x 0.125 mg/kg bw. [The dose volume for each treatment and control group was equivalent.] Six hours after the second dose, the animals were killed [by decapitation] and femoral bone marrow smears were prepared. Azinphos-methyl dose selection was based on a preliminary test at dose levels of up to 2 x 7.5 mg/kg bw, at which dose one mouse died.

Evaluation: 1000 polychromatic erythrocytes (PCE)/animal were examined by microscope for the presence of micronuclei [and the ratio of PCE to normchromatic erythrocytes (NCE) determined.] The number of normochromatic erythrocytes (NCE)/1000 PCE was determined at the same time in order to detect non-test substance-related bone marrow depression or general effects of the test substance on bone marrow erythropoiesis.

Statistics: Non-parametric ranking test of Wilcoxon. [A difference was considered to be statistically significant when $p < 0.05$.]

Findings

[*Clinical observations*: Treated mice exhibited no abnormal clinical signs (appetite, physical appearance and motor activity did not differ to the negative control). A single female treated with 5 mg/kg bw died, however the study authors stated that its death was not due to the effect of the test substance.]

There were no effects of azinphos-methyl on the incidence of micronuclei at PO dose levels up to and including 2 x 5 mg/kg bw. There was no effect on erythrocyte production, since

there was no change in the ratio of normochromatic to polychromatic erythrocytes. [Bone marrow depression was detected in 1 female negative control and 1 female from the 2.5 mg/kg bw group. The study authors concluded that this depression was not due to azinphos-methyl and these animals were consequently excluded form the analyses.] By contrast, Trenimon® inhibited erythropoiesis and significantly [$p < 0.01$] increased the incidence of polychromatic erythrocytes with micronuclei [mean of 23.9/1000 PCE (range 1-85) compared to a mean of 1.6/1000 PCE (range 0-3) for the negative control. Inhibition of erythropoiesis was indicated by an elevated level of NCE/1000 PCE compared to the control (mean of 4153.0 (range 2480-6247) compared to a mean of 705.6 (range 418-929) for the control.]

Conclusion: Azinphos-methyl was not mutagenic in the micronucleus test on the bone marrow of mice at PO dose levels of up to 2 x 5 mg/kg bw.

[*Comments:* No individual animal data were provided for the highest dose level (5 mg/kg bw). No rationale was provided for the use of the repeated treatment schedule (eg pharmacokinetic considerations). The use of a single sampling time, 6 h after the second PO dose was unjustified - OECD guidelines recommend that if a repeated treatment schedule is utilised then at least 3 samples should be taken starting not earlier than 12 h after the second treatment.]

♦ ***Herbold BA (1995) E1582 - Micronucleus test on the mouse. Report no. 24015. Lab & Sponsor: Bayer AG, Institute of Carcinogenecity and Genotoxicity, Wuppertal-Elberfeld, Germany. Study duration: 17 January – 6 March 1995. Report date: 24 May 1995.***

Guidelines and GLP: The study was run according to OECD Guideline 474 (adopted 26 May 1983). *GLP*: Yes. [This study conforms to OECD principles of GLP according to Annex 1 ChemG (Bundesanzeiger Nr. 42a of the 2nd of March 1983 and Bundesgesetzblatt, Part I, of 29th July 1994. This study was quality assured.]

Material and Methods: Azinphos-methyl (batch no. 230405033; purity 92.2%, [Bayer AG, Wuppertal-Elberfeld, Germany]) was intraperitoneally administered to [6-12-wk old] male and female mice (Hsd/Win: NMRI; source: Harlan Winkelmann Borchen, Germany) [weighing 27-46 g] at a dose level of 5 mg/kg bw. [The study authors indicated that batch analysis revealed that azinphos-methyl was stable in the test vehicle at room temperature at 0.1-10 mg/mL for at least 24 h.] Each group comprised 10 [randomly assigned] mice, 5 males and 5 females. The test material was suspended in 0.5% Cremophor/water, which was the negative control material. The positive control material cyclophosphamide (CP, 20 mg/kg bw, [ASTA Medica AG, batch no. 043530]) was administered in water. The selection of dose was based on a pilot test (5 animals/group, 5, 10, 20 and 100 mg/kg bw ip) in which all animals died from the dose of 10 mg/kg bw onward. [The dose-volume administered to each group was 10 mL/kg.]

[Mice were acclimatised for at least 1 week prior to commencement of the study and housed individually in type I cages (unspecified source). Mice were exposed to a 12h light/dark cycle with room temperature and humidity maintained at 22 $\pm$ 1.5°C and 40–70% respectively throughout the study. There were approximately 10 air changes/h. Tap water and feed (Altromin 1324 Standard Diet, unspecified source) were available *ad libitum*.] Sixteen, 24 or 48 hours after the dosage, the animals were killed [unspecified means] and femoral bone marrow smears were prepared (control groups after 24 hours only). [At least 1 intact femur was prepared from each animal.]

Evaluation: 1000 polychromatic erythrocytes (PCE)/animal were examined by microscope for the presence of micronuclei. The number of normochromatic erythrocytes (NCE)/1000 PCE was determined at the same time in order to detect non-test substance-related bone marrow depression or general effects of the test substance on bone marrow erythropoiesis. [The number of NCE showing micronuclei were also scored.]

Statistics: Non-parametric ranking test of Wilcoxon [was used to analyse differences in the number of PCE with micronuclei and the number of NCE, between treatment and negative control groups. A statistical difference was concluded when $p < 0.05$).]

[*Assessment and assay criteria*: A positive test was considered when, at any of the intervals, there was a statistically significant increase in the number of PCE with micronuclei in comparison to the negative control. The assay was considered acceptable if the positive and negative controls were within the range of historical data generated in the performing laboratory or that were available in the literature.]

Findings

General tolerance: [Four treatment-related deaths were recorded.] Signs like apathy, spasm, difficulty in breathing, and lethality occurred in the substance treated animals [up to 6 h after a single ip dose of 5 mg/kg bw. Thereafter there were no abnormal clinical signs exhibited by treated mice (feeding behaviour, external appearance and physical activity were the same as the negative control group).]

Assessment of clastogenic potential: [As there were no apparent difference in results between males and females they were evaluated together.] No effect of azinphos-methyl on the incidence of micronuclei and on the erythrocyte production was noted in the substance treated animals. By contrast, CP significantly increased [$p < 0.01$] the incidence of polychromatic erythrocytes with micronuclei [(14.3 $\pm$ 6.5 micronucleated cells/1000 PCE) compared to the negative control (1.1 + 1.4 micronucleated cells/1000 PCE). All negative and positive control values were within the historical ranges generated by the performing laboratory.]

Conclusion: Azinphos-methyl showed no clastogenic effect in this micronucleus test on the mouse.

[*Comments:* Sampling of bone marrow from a single femur is not recommended by OECD guidelines, which suggest that marrow should be taken from both femurs.

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method used was according to Schmid (1976).

Materials and Methods: Technical grade azinphos-methyl (Chemagro Chemical Corporation, Kansas City, MO, USA, batch no. unspecified) was dissolved in an unspecified solvent and administered by PO gavage or ip injection to an unspecified number of male Swiss-Webster mice (unspecified source, age and weight) at an unspecified concentration. The negative and

positive controls were solvent and trimethylphosphate (unspecified source, batch no., solvent and concentration) respectively. Details of housing and feeding conditions, and incubation times were unspecified. Eight randomly-selected mice from each treatment group were sacrificed, cardiac blood and bone marrow extracted, and slides prepared (the method of sacrifice, blood/marrow collection and slide preparation were unspecified). The number of polychromatophilic cells was scored for each slide and the results statistically analysed according to Mackay and MacGregor (1979). No further experimental details were given.

Results and Conclusions: The study authors concluded that azinphos-methyl had no mutagenic effects on bone marrow and blood cells from male Swiss-Webster mice.

Comments: This summary report does not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: concentrations of azinphos-methyl, positive and negative controls; solvents; sample sizes; incubation times and temperature; housing and feeding conditions; administration route; sampling schedule; the toxicity of azinphos-methyl. No criteria were given for identification of micronucleated cells.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: The method employed was that of Schmid, 1976. *GLP*: No. The information of this published literature has supplementary value only since methodical details (eg used concentrations, solvent) are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Test substance [batch no., purity and source unspecified] was administered by PO gavage or ip injection to male Swiss-Webster mice [source, age, weight, acclimatisation period, housing conditions (eg cage type, no. mice/cage, room temperature and humidity, food and water type/availability) unspecified. The dose level of azinphos-methyl tested, along with the vehicle used was unspecified. Trimethylphosphate (unspecified source, batch no. and concentration) was used as the positive control.] Eight [randomly selected] mice per group were used. [Mice were sacrificed (unspecified means) and] 500 polychromatic erythrocytes from cardiac blood and 500 from bone marrow were examined. The number of micronucleated cells per 500 polychromatophylic cells was recorded.]

Findings and Conclusion: Azinphos-methyl was not mutagenic in that micronucleus test in mice.

[*Comments:* This summary report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The dose level/s and exposure times of azinphos-methyl and trimethylphosphate were unspecified. The stability of azinphos-methyl in the vehicle was unspecified. No evaluation criteria were given.]

2.9.4.2 Mammalian bone marrow cytogenetic test

Hrelia P, Morotti M, Scotti M, Vigagni F, Paolini M, Perocco P & Cantelli Forti G (1990) Genotoxic risk associated with pesticides: evidences on short-term tests. Pharmocological Research. 22: Supplement 3.

Guidelines and GLP: This is a summary publication. No test guidelines or GLP statement were provided.

Materials and Methods: Azinphos-methyl (unspecified source, batch no., solvent and concentration) was administered as an ip injection to rats (unspecified strain, source, age and bw) at doses corresponding to 80, 50 and 25% of the LD_{50}. No further experimental details were given.

Results and Conclusions: The study authors indicated that azinphos-methyl tested negative in the rat cytogenetic test.

Comments: This published study does not provide sufficient methodological or observational detail for regulatory purposes and is thus considered unacceptable. No data were provided.

Henderson LM, Proudlock RJ & Gray VM (1988) Analysis of metaphase chromosomes obtained from bone marrow of rats treated with azinphos-methyl. Report no. MBS 23/871337. Lab: Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England. Sponsor: Makhteshim-Agan (America) Inc., New York, NY, USA. Study duration: 9 June 1987 – 12 October 1987. Report date: 28 January 1988.

Guidelines & GLP: A formal statement indicated that the study complied with the following GLP standards: US FDA, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978 and subsequent amendments; Japanese Ministry of Health and Welfare, Notification no. 313 Pharmaceuticals Affairs Bureau, 31 March 1982; OECD, ISBN 92064-12367-9, Paris 1982; US EPA Toxic Substances control, GLP regulations 40 CFR Part 792, Federal Register Vol 48 no. 230 pp 53922 1983; US EPA FIFRA, GLP Title 40 CFR Part 160 Federal Register 29 November 1983. A formal quality assurance statement was provided.

Materials & Methods:

Azinphos-methyl (92% TGAC; batch no. P-44; Makhteshim Chemical Works Ltd., Israel) in 1% methylcellulose was administered by gastric intubation to 15 SPF CD albino rats/group (45-50 g, age unspecified; Charles River UK Ltd., Kent, England) at 0 (control) or 6.28 mg/kg bw in a dose volume of 20 mL/kg bw. The dose selection was based on a preliminary toxicity test in which 2-5 rats/sex/group received 5, 20, 80, 320 and 1280 or 8, 12, 18 and 27 mg/kg bw azinphos-methyl as a single dose. The LD_{10}/48 h was estimated to be 6.28 mg/kg bw based on the MTD in this preliminary study.

Rats were randomly assigned to each group and acclimatised for 10 days. An unspecified number of rats were housed in plastic disposable cages (unspecified source). The temperature was maintained at 16-28°C, air was ventilated at 30 changes/h and a 12 h light cycle was maintained throughout the study. Food (Scientific Feeds rodent breeding diet LAD 1; unspecified source) and tap water were available *ad libitum*, except in animals dosed by intubation who were deprived of food overnight prior to and for 2 h after dose administration.

Rats were examined daily for clinical signs and all animals which died were subjected to a thorough macroscopic post mortem examination. Two hours prior to sacrifice, 0.4 mg/mL colchicine in 0.9% saline (Sigma, London, England) was administered as an ip injection to all rats at a dose volume of 10 mL/kg bw yielding a final dose of 4 mg/kg bw. At 6, 24 and 48 h, 5 rats/sex/group were sacrificed by cervical dislocation. A concurrent positive control group (5 rats/sex) received an ip injection of 40 mg/kg bw cyclophosphamide (Sigma, London, England) and were sacrificed after 24 hours. Both femurs were dissected from each animal and the bone marrow removed, exposed to a hypotonic solution, fixed and stained. A total of 50 diploid metaphases were microscopically examined from each animal. Chromosomal aberrations were classified as gaps or breaks (chromatid or isochromatid breaks), translocations, exchanges, acentric chromosome fragments, minute chromosomal fragments, chromosome rings, complete metaphase pulverisation and polycentric chromosomes.

Evaluation criteria: No evaluation criteria were provided.

Statistical analysis: The incidence of aberrant cells was analysed using a Wilcoxon's sum of ranks test.

Results

Two males and 2 females died within an hour of dosing and these were replaced with additional animals from a satellite group. Clinical signs were detected in the majority of rats but were generally only slight to moderate in nature. The most common clinical signs that persisted over the study period included pilo-erection and hunched posture, while lethargy, decreased respiratory rate, pallor of extremities and body tremors were observed in some animals within 3 hours of dosing.

There was no increase in the incidence of bone marrow cells with chromosomal aberrations in rats treated with azinphos-methyl, while the incidence of aberrant bone marrow cells in animals treated with cyclophosphamide was approximately 10%, a result which was highly significant ($p < 0.001$).

Conclusion: Azinphos-methyl showed no evidence of clastogenicity in rat bone marrow cells at a dose which was clearly toxic.

Comments: The main criticisms of this study were the use of only a single dose level, the lack of specific evaluation criteria and the absence of results for the post-mortem examination of dead animals.

2.9.4.3 Dominant lethal

Arnold D (1971) Mutagenic study with Guthion in albino mice. Report no. E8921. Lab: Industrial Bio-Test Laboratories Inc., Northbrook, IL, USA. Sponsor: Chemagro Corp., Kansas City, MO, USA. Study duration: unspecified. Report date: 10 May 1971.

Guidelines and GLP: No GLP statement or test guidelines were provided.

Materials and Methods: Groups of 12, 70-80 day-old old male Charles River albino mice (unspecified source and body weight) were administered a single ip injection of Guthion [azinphos-methyl (technical, batch no. 0050092, source unspecified)] in 0.0025% corn oil at

dose levels of 125 or 250 μg/kg bw. The dose levels were selected based on a maximum tolerated dose (MTD) which was derived from a previous acute toxicity study. Control mice received an equivalent volume of corn oil. Methyl methanesulfonate was used as the reference mutagen. No details were given with regard to housing and feeding conditions. Immediately following dose administration, each male was placed in a cage with 3 untreated virgin females, and every week for 6 consecutive weeks, female mice were replaced with 3 virgins. Males were sacrificed by CO_2 asphyxiation after the sixth week of mating. Females were sacrificed approximately 1 week after removal from the breeding cage.

The numbers of implantation sites, embryos and corpora lutea were recorded, the later used to determine whether females were pregnant. The number of resorption sites were also determined with these scored as early (deciduomata) or late deaths. The mutagenicity of azinphos-methyl was determined, based on the proportion of implants that were classed as early deaths, or on the number of viable embryos. Mating indices were calculated by the study authors. No statistical analyses were performed.

Results

Acute toxicity study: Severe tremors and salivation occurred within 20 min of administration of 0.7-7 mg/kg bw azinphos-methyl, with deaths observed within 30-40 min (2/2, 1/2, 2/2 and 1/2 for 7, 1, 0.7 and 0.5 mg/kg bw respectively). Mild tremors were observed at 0.5 mg/kg bw with 1/2 mice dying after 2 days. At 0.1 mg/kg bw there was no observable effect.

Mutagenic study: There were no deaths or clinical signs observed during the study. Although no statistical analyses were performed on any data, there appeared to be no difference in the fertility index, number of corpora lutea, implantation sites, early and late resorption sites, or viable embryos at 125 and 250 μg/kg bw compared to the control. There appeared to be no treatment-related effect on mutation rates or the number of pre-implantation losses.

Conclusions: It appeared that azinphos-methyl did not induce a dominant lethal response when administered to male mice up to 250 μg/kg bw.

Comments: Statistical analyses were not performed on any data. No means or standard deviations/errors were provided by the study author. No evaluation criteria were specified. No data or comment was given regarding the reference mutagen. Two dose levels were tested while OECD guidelines suggest that 3 dose-levels should be examined. The fact that females were not checked following mating and that they were sacrificed 1 week after removal from the mating cage, suggests that they may not have been examined during the second half of pregnancy as recommended by OECD guidelines.

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method used was according to Simmon (1978).

Materials and Methods: Technical grade azinphos-methyl (Chemagro Chemical Corporation, Kansas City, MO, USA, batch no., concentration unspecified) was administered to male mice (unspecified strain, source, body weight and age) in the diet for 7 weeks. Control animals

received the same diet without azinphos-methyl. Each male was allowed to mate with 2 virgin females over 7 days, with the females replaced weekly for 8 weeks. Details of housing and feeding conditions were unspecified. All females were sacrificed at mid-term of pregnancy by an unspecified means and a complete autopsy performed. The numbers of early and late foetal deaths, viable foetuses, and copora lutea were scored for each female. A t-test was performed on angular transformed data to analyse the index of dead implants per total implants. No further experimental details were given.

Evaluation criteria: A statistically significant increase in the mortality of implants was considered a positive result.

Results and Conclusions: The study authors concluded that azinphos-methyl did not induce a dominant lethal response when administered to male mice.

Comments: This summary report does not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: strain, source, age and weight of mice; dose level/s of azinphos-methyl; number of animals per group; housing and feeding conditions; clinical signs; number of pregnant and non-pregnant females. No positive control was used, including any reference mutagen or historical control data. Females did not appear to have been checked after mating. The stability of azinphos-methyl in the diet was not evaluated.

♦ ***Herbold BA (1979b) R1582 - Dominant lethal study on the male mouse to test for mutagenic effects. Report no. 8425. Lab & Sponsor: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Study duration: unspecified. Report date: 7 June 1979.***

Guidelines and GLP: When the study was performed, no specific method was compulsory. In principle, the method used has been taken over into OECD Guideline 478 (adopted 4 April 1984). Deviations from this guideline: No information on a positive control group or historical data were given, the females were not inspected to check mating (see below), only one dose was used. When the study was performed, GLP was not compulsory. The study is acceptable.

Material and Methods: Azinphos-methyl (batch no. 230705148/201-300; purity 92.3%, [Bayer AG, Wuppertal-Elberfeld, Germany]) was administered as a single PO dose of 4 mg/kg bw, in Cremophor/water, to a group of 50 male NMRI mice (source: S. Ivanovas GmbH, Kisslegg/Allgäu, Germany, body weight range 31-43 g). A similar group of control mice received an equivalent volume of vehicle alone. [No positive control was tested.] The dose level of azinphos-methyl was selected on the basis of a preliminary experiment in which groups of 5 female mice received single PO doses of azinphos-methyl at levels of 2.5, 5.0 and 10.0 mg/kg bw. A dose of 2.5 mg/kg bw was tolerated without inducing signs of intoxication. The number of untreated female mice used was 598 per experimental group (ie 50 females/mating).

Starting on the day of test substance administration, a series of 12 matings was performed, each mating interval lasting for 4 days. Each male was caged for 4 days with an untreated virgin female [weighing 28-33 g] after which time the female was removed and replaced by another female. During the period of 12 matings, all germ cell stages present in the testes at the time of test substance administration were theoretically capable of effecting insemination

and fertilising eggs. [Mice were housed in Makrolon cages (unspecified source) with females caged singularly after mating. Room temperature and humidity were maintained at 23 $\pm$ 1°C and 60% respectively. A 12 h light/dark cycle was maintained throughout the study, and pelleted ®Altromin chow (unspecified source) and tap water were available *ad libitum*.] The females were not inspected for the presence of a vaginal plug. In place of this, an interval of about 14 days from the mid-point of the mating period to the inspection of the female was established.

Evaluation: The dominant lethal test is capable of detecting artificially induced mutations (lethal factors) in the male germ cell by determining the early death of affected embryos. Thus, the uterus of each female was examined to determine pre-implantation and post-implantation losses, the criteria for assessment. The total implants, viable and dead implants (sum of the deciduomata, resorptions and dead embryos) and the corpora lutea were counted.

Statistics: [2-way] Analysis of variance [dead implants, all implants (square-root transformed), ratio of dead implants to total implants (angular transformed)], Dunnett test [if the ANOVA showed a significance at $p < 0.05$], Kolmogorov-Smirnov test [frequency distribution of viable, dead and total implants, and pre-implantation loss.]

Findings

General tolerance of the test substance: The male mice showed no signs of damage. Their food consumption, physical appearance and motor activity were unaffected and did not differ from the controls. There were no deaths. [Two females from the control group and two from the azinphos-methyl group died during the study. The 2 dead females from the treatment group were recorded as having pneumonitis.]

Fertility: The fertility of the test group was unaffected by test substance administration. [There was no treatment-related effect on the fertilisation quota.]
Pre-implantation loss: There were no statistically significant differences in this parameter between the control and test groups [based on the total implant and Corpora lutea counts per female. An ANOVA of the implant numbers and a Kolmogorov-Smirnov test on total implants, and preimplantation losses based on the Corpora lutea, revealed no deviations from control values.]

Post-implantation loss: There were no statistically significant differences in this parameter between the control and test groups, either in terms of absolute numbers of dead implants or the ratio of dead implants to total implants [as determined by an ANOVA. Comparison of the frequency distributions of dead and viable implants, using a Kolmogorov-Smirnov test, revealed no significant differences in the individual mating periods or during the entire study between control and treatment groups.]

Conclusion: The dominant lethal test on male mice provided no evidence of a mutagenic effect of azinphos-methyl on any of the various stages of male germ cell development.

[*Comments:* This study had a number of flaws including the absence of a positive control or historical control data from the performing laboratory, and only a single dose of azinphos-methyl was tested. The preliminary dose range-finding study was performed on females while the subsequent dominant lethal test used treated males.]

Simmon VF (1978) In vivo and in vitro mutagenicity assays of selected pesticides. In: A rational evaluation of pesticidal vs. mutagenic/carcinogenic action. Hart RW, Kraybill HF and De Serres FJ (eds.). US Department of Health, Education, and Welfare. DHEW Publication no. (NIH) 78-1306. USA.

Data contained in this report were also published in Waters *et al* (1981 and 1983) and Wildemauwe *et al* (1983).

Guidelines and GLP: No GLP or test guidelines were given.

Materials and Methods: Guthion® (azinphos-methyl) (technical grade; batch no. unspecified; Mobay Chemical Corporation, Kansas City, MO, USA) was dissolved/suspended in 3% corn oil and incorporated into a finely ground commercial diet (unspecified source) at concentrations of 0, 20, 40 and 80 mg/kg. These concentrations were selected based on the MTD. The diet was not analysed for ai content, homogeneity or stability. Groups of twenty, 3-4 mo-old adult male ICR/SIM mice (Simonsen Laboratories, Gilroy, CA, USA) were fed the test diet for 7 weeks. Untreated control mice were fed a diet containing 3% corn oil. Positive controls were given a single ip injection of 0.2 mg/kg bw of triethylenemelamine (TEM) 2 h before mating. No details were given regarding housing and feeding conditions. Each male was allowed to mate with two, 10-12 week-old virgin females for 7 days, with these females replaced weekly for 8 weeks. Females were sacrificed at midterm of pregnancy and a complete autopsy performed. Each female was scored for early foetal deaths, late foetal deaths and viable foetuses.

Statistical analyses: The index of dead implants per total implants, and fertility and death indices were analysed using a t-test on Arcsine- or angular-transformed data.

Results and Conclusions: The study author indicated that azinphos-methyl was non-mutagenic, although no supporting data were provided.
Comments: This published report did not include raw or individual animal data. The absence/presence of clinical signs in both males and females was unreported. The number of copora lutea, methods used to determine that mating had occurred, and criteria for scoring dominant lethals were unspecified. The concentration, stability and homogeneity of azinphos-methyl in the diet were not evaluated.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.***

Guidelines and GLP: *GLP*: No. The information of this published literature has supplementary value only since methodical details (e. g. doses used) are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: Test [unspecified purity, batch no. and source] and vehicle control [corn oil] groups of 20 adult male ICR/SIM mice were used. [Mice were supplied by Simonsen Laboratories (Gilroy, CA, USA), were 3-4 months old and their body weight unspecified. Housing and feeding conditions were unspecified.] The test substance was administered at three [unspecified] dose levels, the MTD and one half and one quarter of the MTD. [These dose levels were selected based on a previous acute toxicity study.] The test

substance was dissolved in [3%] corn oil, added to ground diet and administered for a period of 7 weeks. [Control males were administered a diet containing 3% corn oil. Positive controls were given a single ip injection of triethylenemelamine (TEM) at an unspecified dose, 2 h before the first mating.] At the end of the treatment period, each male was caged with 2 virgin females [10-12-week old] for a period of 7 days. Females were replaced weekly for a total of 8 weeks. Females were sacrificed mid-term of gestation [by an unspecified means] and scored for early and late foetal deaths, and living foetuses. [A full autopsy was performed on each female.]

[*Statistical analysis*: The index of dead implants per total implants was analysed using a t-test following angular transformation of the data.]

Findings and Conclusion: [No details of clinical signs were provided.] This dominant lethal test on male mice provided no evidence of a mutagenic effect of azinphos-methyl.
[*Comments:* This summary report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. The following information was unspecified: details of housing and feeding conditions; dose-levels of azinphos-methyl and TEM; clinical signs; results for the positive control. The number of pregnant vs non-pregnant females, and the number of corpora lutea (for determination of pre-implantation loss) were not scored. Females did not appear to have been checked after mating. Additionally the ai, stability and homogeneity of the diet were not evaluated.]

2.9.4.4 Sex-linked recessive lethal test in Drosophila melanogaster

Sandhu SS, Waters MD, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson T, Jones DCL, Valencia R & Stack F (1985) Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci. 34: 185 – 219.

Guidelines and GLP: This is a summary publication. No guidelines or GLP statement were given. The method used was according to Waters *et al* (1980).

Materials and Methods: Technical grade azinphos-methyl (unspecified batch no.; Chemagro Chemical Corporation, Kansas City, MO, USA) was administered in the diet to *Drosophila melanogaster* at concentrations ranging from 0.25-1.0 ppm for 72 h. No further experimental details were given.

Results and Conclusions: The study authors concluded that azinphos-methyl had no mutagenic effect on the germ line of *Drosophila melanogaster*.

Comments: This summary report does not provide sufficient methodological or observational data for regulatory purposes. No raw data were provided including means or standard deviations. The following information was unspecified: strain, age and source of *Drosophila melanogaster*; number of males treated; number of sterile males; number of F_2 cultures established; number of F_2 cultures without progeny; number of chromosomes tested; number of chromosomes carrying a lethal mutation detected at each germ cell stage; treatment and sampling schedule; toxicity data; .solvent; evaluation criteria; statistical evaluation.

♦ ***Waters MD, Sandhu SS, Simmon VF, Mortelmans KE, Mitchell AD, Jorgenson TA, Jones DCL, Valencia R & Garrett NE (1982) Study of pesticide genotoxicity. In Fleck R***

and Hollaender A (eds.). Genetic Toxicology; an agricultural perspective. Plenum Press New York and London. p. 275-326.

Guidelines and GLP: *GLP*: No. The information of this published literature has supplementary value only since methodical details (eg doses used) are not given. [Data were subsequently analysed and published by Garrett *et al* (1986).]

Material and Methods: The sex-linked recessive lethal test can detect lethal point mutations and small deletions on the X-chromosomes which constitute about 20% of the Drosophila genome. Male fruit flies [carrying the genes BAR (B) and yellow (y) on the X chromosome] were exposed to azinphos-methyl [unspecified purity, batch no., source, dose-level, route/method of administration, vehicle and exposure time] and crossed to untreated females [carrying the Inscy X-chromosome in homozygous condition. Details regarding the source, strain, age, and number of flies used, were unspecified. No information on the use of positive or negative controls was provided.] The male and female progeny of this mating were in turn mated with each other and the following progeny (F_2 generation and, if required, F_3 generation) was examined for mutation defects [using a dissecting microscope.

Evaluation criteria: If at least 2 Bar-eyed males were present in the F_2 generation then the culture was scored as non-lethal. If there were at least 20 progeny and no Bar-eyed males detected, or if there were less than 20 progeny, then the cultures were retested. In the retest, 3 yellow-Bar F_2 females were mated with their brothers. If no Bar-eyed F_3 males were detected than the culture was scored as lethal.
Statistical analysis: The overall mutation frequency was determined by analysing tabulated results with an unspecified statistical test. A 0.2% increase in the background mutation rate at $p < 0.05$ was considered to be a positive effect.]

Findings and Conclusion: This sex-linked recessive lethal test on *Drosophila melanogaster* provided no evidence of a mutagenic effect of azinphos-methyl.

[*Comments:* This summary report does not provide sufficient methodological or observational detail for regulatory purposes. No raw data were provided. Test results were not confirmed in a separate experiment. The following details were unspecified: the actual statistical test used; stock/strains; age of insects, number of males treated; number of sterile males; number of F_2 cultures established; number of F_2 cultures without progeny; number of chromosomes tested; number of chromosomes carrying a lethal mutation detected at each germ cell stage; test conditions (treatment description and sampling schedule, dose levels of azinphos-methyl, negative/solvent and positive controls).]

2.10 SPECIAL STUDIES

2.10.1 Neurotoxicity

In vivo studies

Rats

Sheets LP & Hamilton BF (1994) An acute oral neurotoxicity screening study with technical grade azinphos-methyl (Guthion) in Fischer 344 rats. Project no: 93-412-UM, Lab: Miles Inc, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104, USA, Sponsor: Bayer AG, Germany. Study duration: June 28 – August 29, 1994. Report no. 7377. Report date: August 29, 1994.

Quality assured study. Conducted in compliance with the FIFRA GLP standards of 40 CFR part 160 and the OECD Principles of GLP, C(81)30 (Final) Annex 2 (Paris, May 1981) and in accordance with US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS, 1991, EPA 540/0991-123, PB 91-154617.

Study: To investigate neurotoxicity after a single dose, technical grade azinphos-methyl (Bayer, purity: 92.8% Batch no: 3030050) in 0.5% (w/v) methylcellulose with 0.4% (w/v) Tween 80 in deionised water was administered by PO gavage to overnight fasted Fischer 344 CDF (F-344)/BR rats (SASCO Inc., Madison, WI, USA, 18 rats/sex/dose group, 9 week old, body weight 195-202 g for males, and 133-137 g for females) at 0 (vehicle), 2, 6 and 12 mg/kg bw for males, and 0, 1, 3 and 6 mg/kg bw for females. Twelve rats/sex/dose group were used for neurobehavioural testing, and the remaining animals (6/sex/dose) were used in satellite groups for ChE determinations. The dosing volume was 5 mL/kg bw. A further 6 rats/sex were assessed using functional observational battery (FOB) and motor activity tests during the pre-treatment week and reserved as replacement animals for the main study. Animals in the main study were sacrificed (method unspecified) at 15 days post treatment after the final FOB and motor activity tests. There was no positive control group in the study.

Dose selection was based on the results of a preliminary dose range-finding study in which doses of the test substance were administered to rats at 1, 2, 4, 9 and 12 mg/kg bw, and 0.6 mg/kg bw for females (4 rats/sex/dose). The NOELs for clinical signs which generally appeared about 30 minutes after treatment were 4 and 2 mg/kg bw for males and females respectively. Therefore, at the high dose in the main study, clear signs of toxicity (without causing mortality) were anticipated. At the mid dose, an intermediate level of cholinergic toxicity was expected whereas the low dose was chosen to produce no evidence of such toxicity. Testing for FOB was done at 30 minutes to approximately 1 h post treatment, and motor activity assessments at about 1.5 to 3 h post treatment.

The test animals were acclimatised to the laboratory conditions for at least 6 days prior to placement on the study. They were housed individually and provided with Purina Mills Rodent Lab Chow 5001-4 and tap water *ad libitum* during acclimatisation and post dosing. The stability of the test chemical was determined using LC procedures. Homogeneity of the dosing solutions were verified by analysing three samples each of the nominal 0.1 and 4.0 mg/mL (ie 0.5 and 20 mg/kg bw respectively) dosing suspensions.

Observations: Cage side observations for mortality, moribundity, and clinical signs of cholinergic toxicity were made at least once daily. Individual body weights were determined pre-treatment, on the day of dosing, and thereafter weekly for 2 weeks.

Neurological tests, namely FOB that closely followed the battery of tests described by Moser (1989) were conducted on each test animals. Landing foot splay, grip strength, motor activity, locomotor activity and habituation were assessed in a FOB. Motor activity was measured by testing individual animals for 90 minutes in one of eight figure-eight mazes (as measured by light beam interruption) in the presence of "white noise". All animals were tested on four occasions, ie one week prior to treatment, about 30 minutes after dosing on day 0, and weekly thereafter for 2 weeks. The following parameters were monitored.

Home cage observations: posture, piloerection, gait abnormalities, involuntary motor movements, vocalisations and others such as decreased activity, repetitive head hobbing (mutation) and increased reactivity.

Observations during handling; ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, pupil size, pupil response, lacrimation salivation, stains and others such as alopecia, bite marks, broken teeth/malocclusion, dehydration, emaciation, exophthalmia, missing toe nail(s).

Open field observations: piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behaviour, gait abnormalities, vocalisations, arousal, rearing, defecation and urination.

Reflex/physiologic observations and measurements; approach, touch, auditory and tail pinch responses, righting reflex, grip strength, landing foot splay, body weight and body temperature.

Plasma and red blood cell (RBC) ChE activities were determined (method unspecified) using blood samples collected from the orbital plexus of animals approximately 2 weeks before dosing, and again about 90 minutes after dosing on day 0 (satellite group). Brain ChE activity was assessed immediately after collection of blood samples for plasma and RBC ChE analysis.

Except for the satellite animals, all rats that survived to study termination were subjected to complete gross necropsy. Based on randomisation for FOB and motor activity testing on day 14 post treatment, the first 6 rats/sex/dose from the main study were anaesthetised by ip administration of pentobarbital (50 mg/kg bw), and sacrificed by whole body perfusion with sodium nitrite in phosphate buffer. This was followed by in situ fixation of tissues using 4% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer. The entire brain and spinal cord, both eyes with optic nerve, selected bilateral peripheral nerves (sciatic, tibial and sural), the Gasserian ganglion and gastrocnemius muscle were dissected from each animal and fixed in 10% buffered formalin. The brain was weighed upon removal and the brain:body weight ratio was determined. The following tissues from the perfused control and high-dose groups, and mid-dose females were further processed for histological examination: coronal sections from six levels of the brain (olfactory region, forebrain, midbrain, pons, medulla oblongata and cerebellum), cross and longitudinal sections from three levels of the spinal cord (cervical, thoracic and lumbar), dorsal root ganglia including dorsal and ventral root fibres, cauda equina, Gasserian ganglion, eyes, optic nerves, gastrocnemius muscle and additional

tissues from hippocampus and cerebellar cortex. Statistical tests used to compare the group differences included ANOVA, Dunnett's test, Bartlett's test, general linear and categorical modelling, and analysis of contrasts procedures.

Results: No substantial decrease in concentration of azinphos-methyl in the dosing solution was noted after 8-9 days of storage. The coefficient of variation of the test substance in the vehicle ranged from 1.9-2.0% (ie from 91.5% to 105% of the nominal dose levels). Consequently, the analytically confirmed doses were 0, 2, 6 and 13 mg/kg bw for males and 0, 1, 3 and 6 mg/kg bw for females.

Five males (1 in the main study and 4 in the satellite group) and 15 females (9 in the main study and all in the satellite group) died after receiving the highest dose. These deaths were attributed to treatment. No further mortalities occurred prior to terminal sacrifice. There were no inter-group differences in group mean body weights of the surviving animals. In relation to pre-treatment data, the body weights on treatment day were lower in all groups (about 5% and 10% in males and females respectively). Statistical significance of this difference, however, was unclear from the data provided. Further, the change was attributed to overnight fasting by the study authors.

In males, clinical signs were observed predominantly during the first week after dosing. Treatment-related clinical signs were muscle fasciculations (2/12 and 8/12 at 6 and 12 mg/kg bw respectively), tremors (1/12 at 12 mg/kg bw), incoordinated gait (1/12 at 12 mg/kg bw), oral stain (3/12 and 10/12 at 6 and 12 mg/kg bw respectively), and urine stain (1/12, 2/12 and 10/12 at 2, 6 and 12 mg/kg bw respectively) and red nasal stain (2/12, 2/12 and 7/12 at 2, 6 and 12 mg/kg bw respectively). One surviving female at 6 mg/kg bw, had oral and urine staining for about 2 days after treatment.

Treatment-related FOB findings in treated rats were recorded at the time of maximal ChE inhibition (ie about 30 minutes after dosing) at doses ≥ 1.0 mg/kg bw on day 0, but not at any dose group on day 7 or 14 post treatment. A summary of FOB findings is presented in the following Table. In addition, males at 6 and 12 mg/kg bw and females at 6 mg/kg bw had significantly lower ($p \leq 0.05$) body temperature (by about 3.4% and 3.9%, and 2.8% for males and females respectively) following treatment on day 0, and appeared treatment-related. Both males and females at the high-dose had significantly reduced forelimb grip performance (by about 31% and 27% respectively). Similarly, significant reductions ($p \leq 0.05$) in hind limb grip performance were noted only in males at 6 and 12 mg/kg bw (reduced by 18% and 30% respectively). These manifestations were attributed to acute cholinergic effects of the test substance. Foot splay was unaffected by treatment.

FOB findings noted after dosing (day 0) in rats treated with azinphos-methyl

FOB test parameter	Males			Females		
	Dose (mg/kg bw)					
	2	6	12	1	3	6
Number of animals tested	12	12	12	12	12	3
Autonomic effects						
Lacrimation	0	3	1	0	0	1*
Salivation	0	4	4	0	0	1*
Neuromuscular effects						
Incoordination (home cage)	0	1	3	0	0	1*
Incoordination (open field)	0	6*	7*	0	0	1*
Repetitive chewing (home cage)	0	3	7*	0	0	1*

FOB test parameter	Males			Females		
	Dose (mg/kg bw)					
	2	6	12	1	3	6
Repetitive chewing (open field)	0	8*	10*	0	0	1*
Posture	0	3*	6*	0	1	1*
Minimal movement	1	3	6	0	0	1
CNS effects						
Muscle fasciculations (home cage)	0	8*	12*	0	0	1*
Muscle fasciculations (open field)	0	12*	12*	0	0	1*
Tremors (home cage)	0	3	9*	0	0	1*
Tremors (open field)	0	6*	9*	0	0	1*

*Significantly different from corresponding controls ($p \leq 0.05$).

In males, the response profile of remaining reflex/physiological observations such as touch (5/12 and 6/12), righting (slightly uncoordinated, 6/12 and 3/12) and righting (land on side, 2/12 and 5/12) for the 6 and 12 mg/kg bw groups respectively, was significantly different ($p \leq 0.05$) from the controls and appeared treatment-related, although a dose-response relationship was not always observed.

Maze activity in treated rats was lower on day 0 (by about 64%, 60%, 77% and 79% for males at 0, 2, 6 and 12 mg/kg and 71%, 78%, 79% and 85% for females at 0, 1, 3 and 6 mg/kg bw respectively) compared to the corresponding values reported at pre-treatment and appeared treatment-related. In comparison to the concurrent controls, dose related reductions in motor activity was seen in males at 6 and 12 mg/kg bw (about 36% and 43% respectively), and in females (about 19% for the 1 amg 3 mg/kg bw groups and about 44% at 6 mg/kg bw). The reduced motor activity in control males and females noted on day 0 was attributed to overnight fasting. In males, the motor activity was depressed by about 14% at 12 mg/kg bw compared to the controls, with partial recovery on day 7, and complete recovery of activity was seen by day 14. In females, the motor activity appeared slower to recover showing deficits of about 20%, 10% and 8% at 1, 3 and 6 mg/kg bw respectively compared to the corresponding controls on day 14.

Dose related changes in locomotor activity were also noted in males on day 0, with about 47% and 77% depressions at 6 and 12 mg/kg bw respectively, compared to the corresponding controls. In females, the reductions in locomotor activity were about 26%, 15% and 63% at 1, 3 and 6 mg/kg bw respectively, in comparison to the parallel controls. Complete recovery of locomotor activity was seen in all rats of both sexes, except for males at 12 mg/kg bw that showed a deficit of 20% compared to the controls by day 7. Complete recovery was seen in all animals by day 14. Treatment-related reductions were also seen in interval motor and locomotor activities on day 0 after treatment but recovered completely by day 7 on study. Habituation was not affected by treatment.

Data on ChE activities are presented in the following Table.

Percent inhibition of ChE activity (relative to the corresponding controls) in rats treated with azinphos-methyl

Dose (mg/kg bw)	Plasma ChE	RBC ChE	Brain ChE
Males			
2	32*	33*	15
6	57*	67*	74*
12[a]	50*	63*	88*
Females			

1	11	17	5
3	36*	65*	51*
6[b]	-	-	-

*Significantly different from the corresponding controls ($p \leq 0.05$); [a]Four animals died after treatment on day 0; [b]All six animals in the group died after treatment on day 0.

As shown in the Table, plasma and RBC ChE activities at the time of maximal inhibition (ie about 90 minutes after dosing) on day 0 were significantly lower ($p \leq 0.05$) in males at all dose levels. In females, this was seen only at doses ≥ 1.0 mg/kg bw. The brain ChE activity was significantly ($p \leq 0.05$) lower in males at doses ≥ 2.0 mg/kg bw, and at doses ≥ 1.0 mg/kg bw in females.

No inter-group differences were noted in the remaining test parameters including terminal body weights, brain weights and gross pathology of treated rats of either sex. In histopathology, incidences of minimal (grade 1 on a scale of 1 to 5) degeneration of spinal cord nerve fibres (lumbar, 1/6), and fibres in spinal nerve roots (1/6) together with cyst and chromatolysis (1/6) were evident in 3 different rats at the high dose group. However, these histological changes were not observed in the high dose females (6 mg/kg bw), and therefore, not considered to be toxicologically significant.

Conclusions: Administration of azinphos-methyl by PO gavage as a single dose at 2, 6 and 12 mg/kg bw to male rats or at 1, 3 and 6 mg/kg bw to female rats resulted in mortalities in high-dose animals only, namely five males (1 in the main study and 4 in the satellite group), and 15 females (9 in the main study and all in the satellite group). Treatment-related clinical signs observed were muscle fasciculations, tremors, incoordinated gait, and oral, urine and red nasal stain in males, and oral and urine stain in females. FOB findings on days 0, 7 and 14 post treatment revealed significant effects on day 0, when the acute effects of the test chemical were maximal. No histopathological changes attributable to treatment were observed. Significant plasma and RBC ChE inhibition was observed about 90 minutes after dosing in males at 2 mg/kg bw and above and in females at 3 mg/kg bw. Brain ChE activity was significantly lower in males at doses 6.0 mg/kg bw and above, and in females at 3 mg/kg bw.

♦♦ ***Sheets LP & Hamilton BF (1995) A subchronic dietary neurotoxicity screening study with technical grade azinphos-methyl (Guthion®) in Fischer 344 rats. Project no: 93-472-VJ, Lab: Miles Inc, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104, USA, Sponsor: Miles Inc, Kansas City, Missouri, USA. Study duration: September 6 – December 9, 1993. Study completion date: February 14, 1995.***

The study was conducted in accordance with US-EPA-FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity; NTIS, 1991, EPA 540/09-91-123, PB 91-154617 and in compliance with the FIFRA GLP Standards and the OECD Principles of GLP.

Study and observations: In a subchronic neurotoxicity study, groups of 18 male and 18 female Fischer 344 rats were administered the technical grade of azinphos-methyl (purity: 92.2%) in the diet for 13 weeks at nominal doses of 0, 15, 45, or 120 ppm for males (0, 0.91, 2.81, and 7.87 mg/kg bw/d mean intake) and 0, 15, 45, or 90 ppm for females (0, 1.05, 3.23, and 6.99 mg/kg bw/d mean intake). Twelve rats per sex per dose were used for neurobehavioral evaluation, with half used for neuropathology. The remaining six per sex per dose were used for cholinesterase determinations. The following observations and measurements were included in the study: clinical observations, mortality, body weight, food consumption,

automated measurements of activity (figure-eight maze), a functional observational battery (FOB), ChE activity (plasma, erythrocytes and brain), brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system were examined microscopically for lesions. Statistical evaluations were performed using ANOVA procedures followed by a Dunnett's test. In the event of unequal variances, data were analysed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by Mann-Whitney U test for between group comparisons).

Findings: There were no deaths prior to terminal sacrifice. Treatment-related clinical signs (perianal stain, red lacrimation, increased reactivity, incoordinated gait, tremor) were evident by cage-side observations in males at 120 ppm and in females at 45 and 90 ppm. Body weight (reduced by 9-10%) and food consumption were affected by treatment at the high dose for males (120 ppm) and females (90 ppm).

The results from FOB revealed treatment-related effects in the high-dose males (perianal stain), in mid-dose females (urine stain) and in high-dose females (urine and perianal stains, increased reactivity, abnormal righting reflex, tremor, and decreased forelimb grip strength).

FOB Observations

Dose level (ppm)	0/0	15/15	45/45	120/90
Sex	**M/F**	**M/F**	**M/F**	**M/F**
HC: Increased reactivity, wk 4 #	0/0	0/0	0/0	0/6*
HC: Increased reactivity, wk 13 #	0/0	0/0	0/0	0/10*
OF: Tremors, wk 4 #	0/0	0/0	0/0	0/5*
R/P: Righting – slightly uncoord., wk 4 #	1/3	2/1	3/2	6/6*
R/P: Grip strength, forelimb, wk 4 (kg)	0.78/0.63	0.82/0.63	0.83/0.65	0.72/0.47*
R/P: Grip strength, forelimb, wk 8 (kg)	0.91/0.67	0.90/0.68	0.96/0.73	0.79/0.52*
R/P: Grip strength, forelimb, wk 13 (kg)	0.97/0.68	0.93/0.67	0.97/0.77	0.92/0.43*

* $p < 0.05$; HC: home cage observations; OF: open field observations; R/P: reflex/physiologic observations; # Incidence, based on 12 animals per group

Signs of toxicity persisted with continued exposure but there was no evidence of cumulative toxicity beyond week 4.

Treatment-related decreases in motor and locomotor activity were observed in high-dose males during weeks 4, 8, and 13 of exposure, with no evidence of cumulative toxicity beyond week 4, and in high-dose females during week 4 only.

Motor and locomotor activity

Dose level (ppm)	0/0	15/15	45/45	120/90
Sex	**M/F**	**M/F**	**M/F**	**M/F**
Motor activity, wk 4 #	482/1038	415/996	449/816	241*/460*
Motor activity, wk 8 #	387/873	449/897	403/696	228*/706
Motor activity, wk 13 #	388/732	375/764	382/724	262*/608
Locomotor activity, wk 4 @	178/384	165/375	167/335	77*/154*
Locomotor activity, wk 8 @	133/300	156/341	146/283	72*/263
Locomotor activity, wk 13 @	130/271	119/285	127/265	76*/213

* $p < 0.05$; # No. of beam interruptions (for a 90-min test session); @ No. of beam interruptions after relocation in the maze (for a 90-min test session)

A statistically significant (> 20%) inhibition of red cell cholinesterase was observed at all dose levels tested in this study, as was a statistically significant inhibition (> 20%) of plasma and brain cholinesterase at the mid and high dose.

ChE activity (% inhibition relative to control)

Dose level (ppm) Sex	15/15 M/F	45/45 M/F	120/90 M/F
Plasma ChE, wk 4	7/14*	42*/59*	75*/83*
RBC ChE, wk 4	37*/41*	88*/88*	98*/91*
Plasma ChE, wk 13	15*/13	44*/60*	69*/81*
RBC ChE, wk 13	37*/38*	84*/78*	95*/95*
Brain ChE, wk 13	8*/16*	46*/72*	82*/85*

* $p < 0.05$

Gross lesions were not evident at terminal sacrifice. Brain weight was not affected by treatment in either sex. There were no treatment-related ophthalmic findings or microscopic lesions within neural tissues or skeletal muscle.

Conclusions: In a subchronic neurotoxicity study in rats, decreased forelimb grip strength, motor activity and locomotor activity were observed in both sexes at the high-dose (M/F 120/90 ppm), but did not correlate with any pathology of the nervous system. The NOAEL for neurotoxicity (including cholinergic responses) was 45 ppm in both sexes (2.81/3.23 mg/kg bw/d). A statistically significant (> 20%) inhibition of RBC ChE was observed at all dose levels, as was statistically significant (> 20%) inhibition of plasma and brain ChE at the mid- and high-dose. The statistically significant (< 20%) inhibition of brain ChE in both sexes at the lowest dose level tested (15 ppm, about 1 mg/kg bw/d) is considered to be a marginal adverse effect. Therefore, the NOAEL for brain ChE inhibition is < 15 ppm (< 1 mg/kg bw/d).

Hens

Kimmerle G (1959) Gusathion. Study no: R1582050859, Lab: Toxicology and Industrial Hygiene Laboratory, Bayer AG, Sponsor: Bayer AG, Germany, Study duration: not stated, Report no. 1582. Report date: August 5, 1959.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Single doses of Gusathion (azinphos-methyl, purity, source unstated) in Emulgator W and water was administered as an emulsion to hens (1 bird/dose, strain, body weight, age, source not stated) by PO gavage using a stomach tube at 1.0, 2.5, 5.0, 7.5 and 10 mg/kg bw to investigate the possible demyelination effects of the chemical. Study authors stated that the signs of paralysis normally seen at 3 weeks after treatment with tri-ortho-cresyl-phosphate (TOCP) were not observed during the 8-week observation period following treatment with the test substance. Therefore, the dose levels were increased and administered similarly to hens at 15, 25, 50, 75, 100, 200 and 500 mg/kg bw. It was not clear from the report whether the investigators used the same birds in the subsequent experiment. The interval between the two experiments was not specified. Further, in an attempt to compare the toxicity of the test chemical via different routes, it was administered (vehicle unspecified) as single im injections to a different group of hens (strain, group size, body weight, age, and source unspecified) at 25 and 100 mg/kg. No negative or positive controls were used in any of these experiments. No further information on experimental methods was provided.

Findings: The bird receiving azinphos-methyl orally at 500 mg/kg bw became progressively weak, unsteady and died the following night. The study authors stated that, there was "slight loss of condition" in birds receiving azinphos-methyl orally at 15 and 100 mg/kg bw during the first 3 to 6 days after treatment. All birds in both experiments receiving the test substance orally, recovered by day 7 post treatment, except the bird receiving 200 mg/kg bw, which recovered by day 14 post treatment. The birds receiving azinphos-methyl im at 100 mg/kg bw showed some unsteadiness but recovered after 3 days post treatment.

Conclusions: Under the conditions of the study, azinphos-methyl when administered orally as a single dose at doses of up to 500 mg/kg bw did not produce any delayed neurotoxic effects. However, due to age of the study, lack of data on untreated and positive controls, and histopathology, this study was not adequate for regulatory purposes.

Kimmerle G (1964) Neurotoxicity study with Gusathion active ingredient. Study no: not stated, Lab: Institute of Toxicology, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany, Sponsor: Bayer AG, Germany, Study duration & Report no. unspecified. Report date: November 30, 1964.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: Gusathion (azinphos-methyl, purity, source unstated. batch 352) prepared as an 80% premix in Silkasil S was fed to hens (strain: HNL, 8 birds/group, age: 18-20 months old, body weight and source not stated) in the diet at 0, 75, 150, 300 and 600 ppm (equivalent to approximately 0, 9.4, 18.7, 37.5 and 75.0 mg/kg bw/d) for 30 days. The hens were offered the treated diets and water *ad libitum* and were housed individually during the study period. This was followed by a post treatment recovery period of 4 weeks during which the birds were offered untreated stock diet *ad libitum*, and housed in groups in hen stalls with runs. The treated diets were prepared weekly by mixing the premix with a complete battery hen food meal in a LOEDIGE mixer. The actual concentrations of the test substance in prepared diets were, however, were not determined.

Observations: During the 4-week post treatment observation period the birds were examined for signs of neurotoxicity (leg weakness, limping and inability to walk). Bodyweights were recorded weekly during the trial and observation periods, and the food consumption was measured weekly during the 30-day treatment period. Blood (site or method of blood collection was not specified) ChE activity was determined before the commencement of the study, after completion of the feeding trial, and at post-treatment observation periods using an unspecified method. Two birds/group were sacrificed at the conclusion of dosing and the remaining birds one day after the completion of the post treatment observation period. Nervous tissue collected for histological examination included samples of spinal cord (medulla, cervical, thoracic and lumbar regions) and sciatic nerve.

Findings: One bird at 9.4 mg/kg bw day died during the post treatment observation period due to suspected pneumonia, but no necropsy details were provided. The group mean body weight of birds at 75 mg/kg bw/d was reduced to about 13% of that of the controls by day 14 on study and remained unchanged up until conclusion of the treatment period. The hens, however, regained weight thereafter comparable to the controls during the post treatment observation period. Food consumption was unaffected by treatment. No signs of neurotoxicity or any behavioural changes were noted in any of the treated birds.

No inter-group differences in blood ChE activity was seen at pre-treatment or at day 1 post observation period. However, at day 1 post treatment, dose related depressions of about 15% and 27% in blood ChE activity were observed in birds at 37.5 and 75.0 mg/kg bw/d respectively compared to the controls.

Conclusions: Under the conditions of the study, azinphos-methyl, when administered in the diet at 9.4, 18.7, 37.5 and 75.0 mg/kg bw, did not produce any delayed neurotoxic effects in hens. However, the validity of the study findings is reduced due to age of the study, lack of a positive control group, and data limitations.

Kimmerle G (1965) Neurotoxicity study with Gusathion active ingredient. Study no: not stated, Lab: Institute of Toxicology, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany, Sponsor: Bayer AG, Germany, Study duration & Report no. unspecified. Report date: May 20, 1965.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: The test animals, experimental methods, parameters observed and the frequency of observations in this study were essentially similar to those described in the previous study (Kimmerle, 1964). However, the dose levels of azinphos-methyl (source and purity unspecified) administered in the diet were higher, ie 900, 1200, 1500 and 1800 ppm (equivalent to approximately 112.5, 150.0, 187.0 and 225.0 mg/kg bw/d).

Findings: One bird at 150 mg/kg bw day died during the last week of the 4-week treatment period. The cause of the death was not specified and necropsy details were not provided. Food consumption in treatment groups was reduced by about 27%, 43%, 40% and 44% (for 112.5, 150.0, 187.0 and 225.0 mg/kg bw/d respectively) compared to the controls during the treatment period. All birds including the controls lost body weight during the treatment period. The controls and hens at 112.5 and 150.0, mg/kg bw/d were about 15%, 23% and 21% respectively lighter when compared with corresponding pre-treatment body weights at the conclusion of the 30-day treatment period. The groups receiving the test substance at 187.0 and 225.0 mg/kg bw/d showed about 27% of loss of body weight at the same observation time. The body weight data appeared consistent with the observations on food consumption. The birds had not achieved their pre-treatment body weights by the completion of the recovery period, though there was some recovery of body weights in all groups. Blood ChE activity was unaffected by treatment. No signs of neurotoxicity or any behavioural changes related to treatment were noted. Histopathological findings were reported in the following study (Grundman, 1965).

Conclusions: Under the conditions of the study, azinphos-methyl when administered in the diet at 112.5, 150.0, 187.0 and 225.0 mg/kg bw, did not produce any delayed neurotoxic effects in hens. However, the validity of the study findings is reduced due to age of the study, lack of a positive control group, and data limitations.

Grundman E (1965) Histological findings: Gusation. Study no: not stated, Lab: Institute of Experimental Pathology, Wuppertal-Elberfeld, Bayer AG, Germany. Sponsor: Bayer AG, Germany. Study duration and report no. unspecified. Report date: November 12, 1965.

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: In this study, samples of sciatic nerve and spinal medulla (cervical, thoracic and lumbar regions) of hens (strain, body weight, age and source unspecified) which had received azinphos-methyl (purity, source unspecified) in the diet at 0 (control, 6 birds), 1500 (4 birds) and 1800 (6 birds) ppm (equivalent to approximately 187.0 and 225.0 mg/kg bw/d respectively), in the previous study (Kimmerle, 1965) were histologically examined. One bird from each group was sacrificed (method unspecified) at the end of the 4-week exposure period and the remainder after a 4-week post treatment observation period. Samples of the sciatic nerve and myelin sheath were stained using hematoxylin and eosin, and van Gieson stain. From the 3 stages of the spinal medulla, hematoxylin and eosin, lipid and myelin sheath stains were prepared. Ganglion cells were stained using cresyl violet. No further information on experimental methods was provided.

Findings: Slight to clear interstitial infiltration was found in the sciatic nerve and spinal medulla in both test and control birds and severe interstitial infiltration was found in the sciatic nerve in one hen receiving the test substance at 187.0 mg/kg bw/d. However, no clear dose response relationship was observed. The study authors stated that, "these infiltrations were related to non-specific accompanying infections" but no post mortem findings or further supporting data were provided. No treatment-related changes in the ganglion cells, glia or nerve fibres were noted.

Conclusions: Under the conditions of the study, no histopathological evidence of neurotoxicity was observed in hens treated with azinphos-methyl at 187.0 and 225.0 mg/kg bw/d for 4 weeks. However, the validity of the study findings is reduced due to age of the study, lack of a positive control group, and data limitations.

Taylor RE (1965) Report on demyelination studies on hens. Project no: not stated, Lab: Harris Laboratories, 624 Peach Street, Lincoln, Nebraska 68501, USA, Sponsor: Chemagro Corporation Kansas City 64120, MS, USA. Study duration: not stated. Report no. 15949. Report date: March 12, 1965.

Pre GLP non-quality assured study. No test guidelines were cited.

Study and Observations: Groups of 6 adult, high laying strain Leghorn hens (body weight, age, source not stated) were fed with diets containing either 0, 10, 50 or 100 ppm (equivalent to approximately 0, 1.25, 6.25 or 12.5 mg/kg bw/d) of Guthion (azinphos-methyl, purity, source not stated) daily for 30 days in a study aimed to investigate the demyelination properties of the chemical. The birds were housed in broiler finisher batteries. Three birds from each group were sacrificed after the 30-day exposure period and the remaining birds were fed with untreated stock diet for an additional 30 days. After completion of the 30-day recovery period the birds were sacrificed (method unspecified), and the sciatic nerve, brain and the spinal cord (cervical, lumbar and sacral) were collected for histological examination. The tissues were fixed in Flemings solution and stained with osmic acid stain. The test diets were prepared by mixing the test substance with standard poultry feed (source unspecified) using a Day horizontal double ribbon mixer. The actual concentrations of the test substance in the diets after mixing, however, were not determined. Average daily feed consumption/bird for each group was measured during the third weeks of each of 30-day exposure and recovery periods. All hens were observed daily for evidence of leg weakness during the 60-day test period. No further information on experimental methods was provided.

Findings: No mortalities were reported and no any clinical signs related to treatment were observed. No inter-group differences in average group feed consumption were noted either during the treatment or recovery periods. Histopathological examination did not reveal any evidence of demyelination effects of the test substance.

Conclusions: Under the conditions of the study, azinphos-methyl when fed to hens at 1.25, 6.25 or 12.5 mg/kg bw/d in the diet for 30 days, did not produce delayed neurotoxic effects. However, when age of the study, lack of details on experimental methods and data limitations are considered, the findings of this study appears to be of limited regulatory value.

Roberts NL, Phillips NK, Gopinath C, Begg SE, Anderson A, Dawe IS & Kurlandski ZE (1988) Acute delayed neurotoxicity study with azinphos-methyl in the domestic hen. Study no: not stated, Lab: Huntington research Center Ltd, Huntington, Cambridge PE18 6ES, England. Sponsor: Makhteshim Chemical Works Ltd, PO Box 60, Beer-Sheva 84100, Israel. Study duration: November 9, 1987 – January 8, 1988. Report no. MBS 22/871705. Report date: July 14, 1988.

Quality assured GLP study. The protocol followed in this study was based on the US EPA FIFRA Guidelines of 1984.

Study: A group of 10 adult ISA strain hens (bw: 2.1 to 2.4 kg, about 12 months old, Elemby Farm Eggs, Peterborough, England) were treated with a single dose of azinphos-methyl (purity: unspecified, Makhteshim Chemical Works Ltd) in corn oil by PO gavage at 455 mg/kg bw to investigate the delayed neurotoxic properties of the chemical. The dose level used in the study was based on the results of a preliminary LD_{50} and a dose range finding study (data provided). The positive control group (10 birds) received a single dose of tri-o-cresyl phosphate (TOCP) similarly at 500 mg/kg bw. A concurrent vehicle control group of 10 birds received corn oil only. A further group of 12 birds were treated with azinphos-methyl and maintained for use as replacements. The birds were starved overnight before dosing. Prior to dosing, the birds were treated intramuscularly with atropine sulphate and 2-PAM. The test solutions were prepared on the day of dosing, and dosing was followed by a 21-day observation period. All birds in the test substance and negative control groups were then re-dosed with either azinphos-methyl or corn oil at the same dose levels as administered previously, which was followed by a further 21-day observation period. The birds were housed according to treatment group in floor pens under standard laboratory conditions and provided with pelleted standard HRC layer ration (Joseph Odam Ltd, Eye Mill, UK) *ad libitum.*

Observations: The birds were examined daily for mortality, abnormal behaviour, clinical signs of toxicity and signs of delayed locomotor ataxia. The degree of ataxia was scored using a point award system (on a scale of 0-8, the highest number represented the greatest degree of ataxia) similar to that described by Cavanagh *et al* (1961). Body weights and food consumption were recorded weekly. The positive controls were sacrificed at the end of the first 21-day observation period, whereas all surviving birds in the remaining groups were sacrificed at the end of the second 21-day observation period (method unspecified), and necropsied. The following tissues were sampled for histopathology: brain (medulla/pons, cerebellar cortex, and cerebral cortex), spinal cord (multiple longitudinal and transverse sections of the cervical, thoracic and lumbar-sacral regions), and peripheral nerve (proximal and distal sciatic nerve and distal branches of the tibial nerve).

Findings: There were 16 treatment-related mortalities among groups that received azinphos-methyl, where the deaths occurred on different days after receiving either the first or second test dose. One bird in the vehicle control group was found lying on the pen floor, unable to stand on day 14 on study, and was therefore sacrificed. One bird in the positive control group was found dead on day 11, and the death was attributed to TOCP treatment. Two further deaths in the test substance groups, which occurred on day 10 after the first dose, were attributed to pecking. Clinical signs seen in birds that received azinphos-methyl included subdued appearance, unsteadiness, leg stiffness and inability to stand. The birds in the TOCP group showed no adverse effects immediately after dosing, but subsequently developed signs of delayed locomotor ataxia (score of 1-7), with the first positive signs occurring on day 11 post treatment. Three birds in this group were sacrificed at the end of day 18. Food consumption in birds treated with azinphos-methyl was variable during the first 21-day observation period, but was comparable to that of the controls after the second dosing. Food consumption in the positive controls was markedly low compared to the vehicle controls. Surviving birds that received azinphos-methyl showed bodyweight decreases (about 3-19%) during both the 7-day periods after the first and second dosing. However, the group mean bodyweight of this group was increased during days 28 and 35 on study, and was comparable to that of the vehicle controls at termination. The birds in the positive control group showed continuing weight loss during the study.

Terminal necropsy did not reveal any abnormalities in hens that received azinphos-methyl. The birds that died as a result of dosing were not necropsied. In histopathology, grade-II changes were seen in at least one level of the spinal cord, in the majority of the birds. Grade II changes were defined as disruption or fragmentation of occasional axons with rare myelin abnormalities, and in general, on any slide of the spinal cord, between one and four degenerate/altered axons detected, on a slide of a peripheral nerve, with 1 or 2 degenerated axons being included. In addition, 2 birds in this group showed grade-II changes in peripheral nerve indicating the presence of degenerating axons. In the azinphos-methyl group, all birds showed grade-II changes in at least one level of the spinal cord, with 4 hens exhibiting grade-II changes in peripheral nerve. However, the findings were indistinguishable from those of the vehicle controls, and were considered to be unrelated to treatment. The birds in the TOCP group showed grade II-IV changes in at least two levels of the spinal cord, with all birds showing similar changes in the peripheral nerve. Four birds in the TOCP group showed grade-II changes in the brain tissue.

Conclusions: Under the conditions of the study, PO administration of a single dose of azinphos-methyl at 455 mg/kg bw followed by a repeat administration after 21 days, did not produce any delayed neurotoxic effects in hens.

In vitro studies

Schmuck G (1995) In vitro effects of methylazinphos (Gusathion M) on cultured hippocampul neurons of the rat. Study no: not stated, Lab: Pharmaceutical Business Group, PH-PDT Research Toxicology, Bayer, Sponsor: Bayer, Study duration: not stated. Report no: PH 24512. Report date: November 24, 1995.

Study: In this study, the neurotoxic effects of azinphos-methyl (purity: 99.5%, Lot no: 890712, Bayer) on cultured rat hippocampal neurones were investigated. The cell cultures were treated with azinphos-methyl in DMSO (dimethyl sulfoxide) at concentrations of 0.1, 1.0, 10, 20 and 50 µg/mL. Neuronal cultures were prepared using hippocampal regions dissected out from whole brains of 16-17 days old foetuses that were obtained from pregnant

Wistar rats (source, age not specified, sacrificed by asphyxiation). The cortical tissue was pooled in sterile cultivation medium Opti-MEM (Gibco, Eggenstein) containing 5% horse serum. Individual cells were isolated by filtration of cells through two nylon meshes of different pore sizes (135 and 25 µm). The single cell suspension was then centrifuged at 500-700 g, washed twice with culture medium and resuspended in 10 mL of the culture medium. Analyses were carried out on 24 well, poly-d-lysine coated cell culture plates (Biocoat SERVA, Heidelberg) using 5 x 10^5 to 1 x 10^6 cells/well. The culture medium was changed every 2-3 days. Undesired growth of glia cells were suppressed by treatment with FUDR (deoxyfluorouridine, Sigma, Deisenhofen). No further details on experimental methods including treatment procedures of control cell cultures were provided.

Observations: The following parameters were determined at days 3, 5 and 7 post dosing: cytotoxicity as assessed by cell viability, acetyl ChE activity AChE according to Ellman, (1959), choline acetyl transferase activity (CHAT) as described by Schuberth, (1966), molecular mounts of glutamate, DNA, GABA, glutamate decarboxylase, glutamine synthetase, dopamine, tyrosine hydroxylase, neuron specific enolase (NSE), glia fibrillary acid protein (GFAP), neurofilaments and microtubuli associated protein (MAP-2) using ELISA procedures.

Findings: Results have been provided only in the form of bar graphs (change of parameter as percent of controls at different doses at days 3, 5 and 7 post dosing). Cell viability was reduced by about 10, 30 and 60% at azinphos-methyl concentrations of 1, 5 and 10 µg/mL respectively at day 3 post dosing. At day 5 post dosing, depression of cell viability was about 30% and 40%, and at day 7, the reduction was about 30% and 35% at 10 and 50 µg/mL respectively. Further, the graphs illustrated reductions in neuronal DNA of about 20% at 50 µg/mL at day 5, and about 20% and 35% at 10 and 50 µg/mL concentrations respectively, at day 7 post dosing. In general, the changes that were observed for both of the above parameters were dose and time related. No effects on DNA content were seen at day 3 post-treatment.

According to illustrations provided, the AChE and ChAT activities were inhibited at 0.1 µg/mL concentration and above in a dose and time related manner. The remaining study parameters were influenced only in the cytotoxic concentration range of the test substance. However, the changes in GFAP content, and the amount of neurofilaments in cells revealed that azinphos-methyl was toxic at concentrations of 1.0 µg/mL and above.

Conclusions: Under the conditions of the study, azinphos-methyl produced cytotoxicity in rat hippocampal cell cultures at concentrations of 5 µg/mL and above, and AChE and ChAT activities were inhibited at 0.1 µg/mL and above in a dose and time related manner. Other study parameters were influenced only in the cytotoxic concentration range of the test substance. This *in vitro* study is of limited value in establishing any regulatory endpoint.

2.10.2 Porphyrin Biosynthesis

Koeman JH, Debets FMH & Strik JJTWA (1980) The porphyrinogenic potential of pesticides with special emphasis on organophosphorus compounds. In "Field worker exposure during pesticide application" (eds: Tordoir WF and Van Heemstra EAH, Elsevier, NY) 157-162.

Study and observations: This study investigated the porphyrinogenic potential of 9 organophosphorus pesticides including azinphos-methyl (purity, source unspecified) by means of an assay procedure modified from the method of Granick (1966) using chicken embryo liver cell cultures. It was claimed that most of the porphyrinogenic chemicals known so far are halogenated hydrocarbons, including polychlorinated and polybrominated hydrocarbons (PCB's & PBB's), vinyl chloride, tetrachlorodibenzodioxin (TCDD) and hexachlorobenzene (HCB). However, the porphyrinogenic potential of chemicals *in vitro* under conditions, which induce drug metabolising enzyme systems had not been adequately studied. Therefore, this study investigated the porphyrinogenic potential of pesticides with special emphasis on organophosphorus compounds. The possible usefulness of measurement of urinary porphyrin levels in biological monitoring in relation to occupational exposure situations was discussed. Hepatocytes isolated from 15-18 days old white chicken eggs in Williams E medium supplemented with 10% foetal calf serum were used in the study. Initially, 2 mL of cell suspensions containing 1.5-2.0 x 10^6/plate were incubated in a humidified atmosphere at 37°C for 24 h in an atmosphere of 5% CO_2 in air. Some of the culture plates (number unspecified) were pre-incubated with β-naphthoflavone (3 µg/mL of medium) between the 6^{th} and 24^{th} h of the incubation period for metabolic activation. After 24 h, the medium was replaced with 2 mL of fresh medium, and azinphos-methyl in 1 µL dimethylsulfoxide (DMSO) was added to the cell culture at 10 µg/mL of medium. The cells were then incubated for another period of 24 h. Development of signs of porphyria was measured by examination of the porphyrin fluorescence using a fluorescence microscope.

Findings and conclusions: Under the conditions of the study, azinphos-methyl induced porphyria in chicken liver cell cultures following metabolic activation. Due to lack of information on the purity of the test substance, the study had limited value for regulatory purposes.

2.10.3 Haematotoxicity

Parent-Massin D & Thouvenot D (1993) In vitro study of pesticide haematotoxicity in human and rat progenitors. J Pharm Toxicol Methods 30 (4): 203-207.

Study and observations: This study evaluated the usefulness of human and mouse haematopoietic progenitor cultures in the predictive evaluation of pesticide myelotoxicity. Haematotoxicity of 9 pesticides including azinphos-methyl (purity, source unspecified) was investigated. With azinphos-methyl, only human progenitor cell cultures were used. Human bone marrow (obtained from the head femur of patients undergoing arthroplasty) cells cultured at 2 x 10^5 cells/mL in a medium containing 15% foetal calf serum and 0.3% agar were used in the study. Colony formation was stimulated by the use of 10% placenta-conditioned medium prepared in the laboratory. Cell cultures of 1 mL were incubated in the presence of azinphos-methyl (in acetone or methanol, not specified in the report) at 0.2 and 20 µg/mL (20 µL) in triplicates at 37°C in an atmosphere of 100% relative humidity with 5% CO_2 in air. Cell aggregates were counted at 7, 10 and 14 days post treatment using an inverted microscope. Aggregates of more than 50 cells were scored as colonies, whereas 20-50 cells as macro-clusters and 5-20 cells as micro-clusters. Results were expressed as percentage of growth of the standard culture incubated in the absence of azinphos-methyl. Experiments were repeated at least 3 times. The test substance was considered to be haematotoxic when the percentage of growth in aggregates was < 50%. When this percentage was > 50%, other factors such as colony distribution, macro-cluster and microcluster were taken into an account

to detect other toxicity expressions (late development or specific toxicity). Group differences were tested for statistical significance at $p < 0.05$ using one-way ANOVA procedure.

Findings and conclusions: Under the conditions of the study, azinphos-methyl did not inhibit progenitor cell development at the tested concentrations at 7 days post treatment. However, significant inhibition in progenitor cell development was seen at both concentrations (0.2 and 20 µg/mL) at 10 and 14 days post treatment. However, the validity of the findings is reduced due to lack of information on the purity of the test substance and its cytotoxicity.

2.10.4 Immunotoxicity

Vos JG, Kranjc EI, Beekhof PK & van Logten MJ (1983) Methods for testing immune effects of toxic chemicals: Evaluation of the immunotoxicity of various pesticides in the rat. In Pesticide Chemistry: Human Welfare and the Environment, IUPAC (eds: Miyamato J and Kearney PC). Vol 3. Mode of Action, Metabolism and Toxicology. Pergamon Press. 497-504.

Study and observations: In this study, 17 pesticides including azinphos-methyl (purity 85%, source unspecified) were screened for possible immunotoxic properties. Azinphos-methyl technical was administered to weaned, male Wistar rats weighing 40-60 g (6 rats/group, age, source unspecified) in the diet for 3 weeks at dietary concentrations of 5, 25 and 125 mg/kg of diet. No details were provided for animal housing and treatment procedure of the controls. The test animals were provided with the test diets (formulated using a semisynthetic diet from Trouw Ltd, The Netherlands) and water *ad libitum.* Body weight and food intake were recorded weekly. At the end of the experiment, the animals were ether anaesthetised and sacrificed by exsanguination. Weights of the following organs were recorded at termination: liver, kidney, pituitary, adrenals, thyroid, testes, thymus, spleen mesenteric and popliteal lymph nodes. Samples of these organs were also processed for histological examination. Total and differential leucocyte counts were determined using blood samples obtained at termination. Serum samples separated at termination were used for quantitative analysis of IgM and IgG using the enzyme-linked-immunosorbent assay (ELISA). Student's two-sided t-test was used to examine the group differences.

The study authors stated that in immunotoxicity assessment, the emphasis was given to the weight and histology of thymus, spleen, mesenteric and lymph nodes, peripheral lymphocyte and monocyte counts, and serum IgM and IgG levels.

Findings and conclusions: It was reported that administration of azinphos-methyl at dietary concentration of 125 mg/kg of the diet resulted in mortality, decreased terminal body weight, and decreased relative spleen, pituitary and mesenteric lymph node weights. However, no further details, actual data for any of the study parameters and food consumption were provided. Based on the above findings the study authors concluded that azinphos-methyl effected both immunological and general toxicological parameters at the same dose level. The study was not adequate for regulatory purposes.

2.11 HUMAN STUDIES

2.11.1 Toxicity Studies

Rider JA, Moeller HC & Puletti EJ (1967) Continuing studies on anticholinesterase effect of methyl parathion, initial studies with guthion, and determination of incipient toxicity level of dichlorovos in humans. Federation Proc 26 (2): Abstract no: 960.

Rider JA & Puletti EJ (1969) Studies on the anticholinesterase effects of gardona, methyl-parathion, and guthion in human subjects. Federation Proc 28(2): Abstract no: 1245.

Rider JA, Swader JI & Puletti EJ (1970) Methyl parathion and guthion anticholinesterase effects in human subjects. Federation Proc 29(2): Abstract no: 58.

Rider JA, Swader JI & Puletti EJ (1971) Anticholinesterase toxicity studies with methyl parathion, guthion and phosdrin in human subjects. Federation Proc 30(2): Abstract no: 1730.

Rider JA, Swader JI & Puletti EJ (1972) Anticholinesterase toxicity studies with guthion, phosdrin, di-syston, and trithion in human subjects. Federation Proc 30(2): Abstract no: 1382,

Rider J, Moeller HC, Puletti EJ & Swader J (undated) Studies on the anticholinesterase effects of methyl parathion, guthion, dichlorvos and gardona in human subjects. Federation Proc: Abstract no: 2144.

Study protocols, observations and findings: The data for the above studies with guthion (azinphos-methyl) have been provided in abstract form. The details appearing therein are presented in the following Table. Except for the study of Rider *et al* (1967), the authors of the remaining studies stated that plasma and erythrocyte (RBC) ChE activity was measured twice weekly during a control period and the test period each lasting approximately 4 weeks. The method of ChE determination, however, was not specified in any of the studies.

Study	Group size	Doses given (mg/day)	Duration of treatment	Findings
Rider *et al* (1967)	5/dose group	1, 1.5	Fed daily for 1 month	No significant depression of plasma or RBC ChE (measured twice weekly) was seen.
Rider & Puletti (1969)	5/dose group and 2 controls	4, 4.5 and 6		
Rider *et al* (1970)		7, 8 and 9		
Rider *et al* (1971)		18 and 20		
Rider *et al* (1972)		10, 12, 14 and 16		
Rider *et al* (undated)		2, 2.5, 3 and 3.5		

Conclusions: The abstracts provided very limited information on experimental methods, observations and findings of each study. However, according to the authors no "significant changes" in plasma or RBC ChE activities were observed in any of the human subjects in any of these studies.

Thornton JS (1971) Analysis of urine samples from human subjects treated orally with Guthion. Study no: not stated. Lab: Chemagro Corporation, Research and Development Department. Sponsor: Bayer AG, Study duration: December, 1970 to January, 1971. Report no. 30201. Report date: May 20, 1971.

Pre GLP, non quality assured study. No test guidelines were cited.

Study and observations: To develop a new urinary diagnostic method, this study investigated the correlations between the amount of azinphos-methyl ingested and the levels of anthranilic acid precursors in urine, and between the amount of anthranilic acid precursors in urine and the depression of blood ChE activity in human subjects (according to previous research by the study authors azinphos-methyl and its various metabolites and related compounds could be converted to anthranilic acid by hydrolysis with 5N KOH under reflux conditions). In this study, 5 male volunteers (age, body weight not stated) were fed with guthion (azinphos-methyl, method, purity, batch and source unspecified) at 16 mg/day for 30 days. Twenty four hour composite urine samples were collected from each volunteer on 2 consecutive days just prior to commencement of the study, on 3 consecutive days after commencement of dosing, and then at 5, 7 and 14 day intervals, and again on 3 consecutive days post treatment. Immediately after measurement of the volume, unspecified portions of urine samples were frozen. Samples from each volunteer were analysed "in a group to eliminate day to day variations due to reagents and instrumental sensitivity". Samples from 2 volunteers were analysed for anthranilic acid using a fluorometric method following ethyl acetate and chloroform extraction. Blood ChE was measured using an unspecified method. No further details on experimental methods were provided.

Findings: Average urine output was about 21% higher during the study compared to pre-treatment data. An overall increase in anthranilic acid (azinphos-methyl and/or metabolites) content in the urine was also recorded during treatment (about 1.7-fold in one sample, and about 3-fold in the other) compared to pre-treatment values. The urinary anthranilic acid content returned to near pre-treatment values following discontinuation of treatment. A correlation between the intake of azinphos-methyl and the amount of residues of azinphos-methyl and/or its metabolites was observed. According to the study author, no depression of blood ChE was detected in any of the volunteers. However, no supporting numerical data were provided. No urinary metabolites of azinphos-methyl were characterised or identified.

Conclusions: Under the conditions of the study, residues of azinphos-methyl (as anthranilic acid derivatives) were detected in urine and a correlation between the intake of the test chemical and urinary residue levels was observed. But no correlation between urinary residue levels and blood ChE depression was seen. The findings of this study are, however, of limited regulatory value due to lack of data on purity of the test chemical, blood ChE depression and urinary metabolites, experimental methods, and debatable sensitivity of the assay method used.

Franklin CA, Muir NI & Moody RP (1986) The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicol Lett 33: 127-136.

Study and observations: In this review, the data of a biological monitoring study, which was conducted to investigate the usefulness of estimation of urinary metabolites in pesticide exposure assessments was discussed. Azinphos-methyl was used as the test chemical. The results of field and laboratory experiments conducted in the present study were compared with

dermal absorption data derived from animal and human studies. Three groups of orchardists (12 from British Columbia, 6 from Ontario and 5 from Nova Scotia, body weights and ages unspecified) who sprayed their own properties with azinphos-methyl 50 WP (source, batch unspecified) at the rate of 0.8 to 2.6 kg ai/ha on one day (1 to 9.5 h) participated in the study. All except one used orchard air-blast equipment for spraying. Patches were pinned under protective clothing, and thin gloves worn under protective gloves to estimate the extent of exposure. Composite 24-h urine samples were collected one day prior to spraying, on the spray day, and at day 1or 2 post-spraying. Patches were analysed for azinphos-methyl using a standard GC or HPLC method. The levels of primary urinary alkylphosphate of azinphos-methyl, dimethylthiophosphate (DMTP), were measured using a modified Shafik (1973) method.

Findings: DMTP data in urine samples (collected for up to 72 h which included spray day plus 2 days) post exposure are presented in the following Table. Dermal exposure estimates that were calculated by extrapolating patch residue data are not included in this summary.

DMTP levels in urine of orchardists following exposure to azinphos-methyl 50 WP.

Study	Amount of ai sprayed (kg)	DMTP content[a]			
		48 h		72 h	
		µg/kg ai	Total (µg)	µg/kg ai	Total (µg)
British Columbia	2.1	112 ± 10	230 ± 33	NC	NC
Ontario	5.3	110 ± 21	539 ± 110	143 ± 23	688 ± 135
Nova Scotia	2.1	54 ± 33	105 ± 49	109 ± 86	192 ± 125

[a]Mean ± SE; ai = active ingredient; NC = Samples not collected.

The DMTP levels in 48 h urine samples (which included spray day plus 1 day post exposure) were significantly correlated with the amount of the test substance sprayed ($r = 0.81$, $p \leq 0.001$). When 48-h urine samples of each study were analysed separately to investigate the correlation between the same parameters, British Columbia and Ontario studies exhibited good correlations ($r = 0.81$ and 0.75, respectively). The Nova Scotia study, however, showed a weak association ($r = 0.23$). Two of the workers in the Nova Scotia study did not excrete any detectable amounts of DMTP in urine, even though both had unexplainably, measurable levels of azinphos-methyl residues on their patches. Extending the collection period to 72 h resulted in a 30% increase in the DMTP excreted by the workers in Ontario and a 100% increase by Nova Scotia workers.

Conclusions: The findings from this and several laboratory studies in rats suggest that urinary metabolite data provide more reliable and accurate estimate of exposure than patch data. However, in the absence of adequate knowledge on absorption and metabolism of the parent compound, urinary metabolite levels cannot be used to accurately quantify exposure.

Wester RC & Maibach HI (1985) In vivo percutaneous absorption and decontamination of pesticides in humans. J Toxicol Environ Health 16: 25-37.

Study and observations: This paper reviewed the percutaneous absorption and decontamination of a range of pesticides including guthion (azinphos-methyl, purity unspecified) in humans with reference to factors such as the applied concentration (dose), site of application (anatomical region), area of application, occlusion, skin condition (intact or abraded) and contact time (duration of exposure). Also, the data from human studies were compared with those of animal and *in vitro* studies. The methodology generally used to study

the rate of absorption was based on a mathematical comparison of the excretion of radioactivity following topical and parenteral administration of the radiolabelled pesticide. The parenteral administration assumed that 100% of the dose was systemically available when injected into human subject or an animal.

Findings and conclusions: It was reported that the proportion of the azinphos-methyl dose absorbed when applied to the intact skin was 15.9%. Factors such as the applied dose, surface area of the skin, site of application and the duration of exposure influenced the rate of absorption through the skin. Two other factors that influenced the skin absorption were occlusion of the site and application to abraded skin. These two factors increased the rate of absorption of azinphos-methyl from the skin by 3.5- and 3.8-fold respectively (from 15.9% to 56.1% to 60.5% respectively).

♦ ***McFarlane P & Freestone S (1999a) A randomised double blind ascending single oral dose study with azinphos-methyl to determine the no effect level on plasma and RBC cholinesterase activity. Report no. 17067. Lab: Inveresk Research Ltd., Edinburgh, Scotland. Sponsor: Bayer Corporation, Agricultural Division, South Metcalf, Stilwell, KS, USA. Study duration: 25 August 1998 – 21 December 1998. Report date: 19 March 1999.***

[*Guidelines and GLP*: A formal statement was provided which indicated that the study complied with the principles of Good Clinical Practice (CPMP/ICH/135/95) and OECD principles of GLP. The study was conducted in accordance with the guidelines established in the Declaration of Helsinki 1964, as amended by the 29th World Medical Assembly in Tokyo 1975, the 35th World Medical Assembly in Venice 1983, the 41st World Medical Assembly in Hong Kong 1989 and the 48th General Assembly, Somerset West, Republic of South Africa October 1996. A formal Quality Assurance statement was provided.]

Objectives: The study was performed in order to determine the safety of azinphos-methyl and to establish a NOEL for plasma and RBC ChE and compare the sensitivity with animal species at NOEL in healthy human male and female volunteers.

Material and Methods

The experiment was performed as a double blind, placebo controlled, single ascending PO dose study using 40 male and 10 female healthy volunteers. [The mean age and weight of the male subjects was 32.7 $\pm$ 9.3 years and 75.52 $\pm$ 8.36 kg respectively, and the mean female age and weight were 31.0 $\pm$ 4.6 years and 63.83 $\pm$ 6.71 kg respectively.] All subjects were selected from a panel of volunteers held at ICR and were screened within 3 weeks prior to study initiation. During that visit, the scope and intent of the study was clearly explained to the volunteers. To be entered into the study, subjects had to fulfill the following criteria: a) 18 to 50 years of age, b) no clinical important abnormal physiological findings, c) no clinically relevant abnormalities of laboratory screening (including ChE activity), d) normal ECG, e) normal arterial pressure and heart rate, f) body weight between 50 and 100 kg and within +/- 15% of ideal body weight, g) able to communicate well with the investigator and to comply with the requirements of the entire study, h) provision of written informed consent to participate. [The study protocol was reviewed and approved by an Independent Research Ethical Committee. The study authors also indicated that the study design and dose selection were discussed with the Californian EPA.

Prior to commencement of the study, a screening examination was performed which consisted of the following: medical history; complete physical examination and vital signs (pulse rate, respiratory rate and blood pressure); ECG recording; haematology, clinical chemistry, plasma and RBC ChE and urinalysis; Hepatitis B, C and HIV status; drug screening; pregnancy status (females). Subjects could be withdrawn from the study in any of the following events: serious adverse effects; major violations to the protocol; withdrawal of consent; termination of the study by the sponsor.]

On the morning of dosing, azinphos-methyl (batch no. 703-0139; purity: 91.6% [Bayer Corporation, Agricultural Division, South Metcalf, Stilwell, KS, USA]) at dose levels of 0.25, 0.5, 0.75 or 1.0 mg/kg bw, or placebo (lactose; batch no. 56GZ [Thorton and Ross, unspecified location]) were administered to the [seated] subjects in a [gelatin] capsule and with 150 mL of water. [The dose selection was based on a rat neurotoxicity study where a NOEL of 1 mg/kg bw was observed (Sheets and Hamilton 1995), and a repeat dose study in humans where the NOEL was 0.29 mg/kg bw (Rider *et al* 1971). The study was performed as a dose-escalation experiment and executed in 5 treatment blocks with only one subject in any given treatment block exposed to the next highest dose (see table below). All subjects were randomly allocated to a treatment group. In session 5, 10 females were randomised to receive the NOEL dose identified in males, or the placebo.]

[**Dosing schedule (No. of subjects**)]

Dose level (mg/kg bw)	**0**	**0.25**	**0.5**	**0.75**	**1.0**
Session 1; males	1	1			
Session 2; males	2	6	1		
Session 3; males	3		6	1	
Session 4; males	3			6	
Session 5; females	3			7	
Session 6; males	3				7

Physicians and a support staff of registered nurses and a pharmacist conducted the study. After dosing, the stay of the volunteers in the clinic was 3 days. During the conduct of the study, the attending physician/nurses explained the procedures for each phase of the study, and asked each volunteer if he/she had any questions on any procedures involved in the study, or any complaints associated with the administration of the chemical. A physical examination was performed prior to discharge from the clinical unit and all volunteers returned to the clinical unit 7 and 14 days post-dose for ChE sampling and adverse event inquiry.

Variables examined: Physical examination including vital signs [supine systolic and diastolic arterial pressure, heart and respiratory rate were measured on admission, pre-dose, 0, 2, 4, 8 and 24 h post-dose, and] PO temperature [on admission, pre-dose, 0, 2, 4, and 24 h post-dose], ECG [ventricular rate, PR interval, QRS interval, QI interval, QTc interval, QRS axis,- 30 min pre-dose and 2, 4, 8 and 24 h post-dose], continuous [single-channel] ECG monitoring [from –30 min pre-dose and 4 h post-dose], haematology [Hb, RBC, Hct, MCH, MCV, MCHC, WBC, neutrophils, lymphocytes, monocytes, eosinophils, leucocytes, platelets], clinical chemistry [urea, glucose, AST, ALT, LDH, Na, K, Cl, total protein, albumin, total bilirubin, GGT creatinine], urinalysis [pH, specific gravity, protein, glucose, ketones, bilirubin, blood, urobilinogen], plasma and RBC ChE and adverse events.

[A physical examination was performed during screening and at the end of the study, with the following parameters assessed: hands, lymph modes, chest movement, trachea, percussion, breath sounds, peripheral pulse, JVP, heart sounds, murmurs, mouth and lips, abdominal scars, abdominal tenderness, spleen, liver, kidneys, pupils, ophthalmoscopy, cranial nerves, power, sensation, reflexes, and cerebellar function.]

[Blood samples (5 mL) were collected by venipuncture at pre-dose and 24 h post-dose, and analysed for haematology and clinical chemistry parameters. Urinalysis was performed 24 h post-dose. Blood (4.5 mL) was collected at screening, day -10, -8, -4, -2, -1 and -30 min pre-dose and at 1, 2, 4, 8, 12, 24, 48 and 72 h post-dose, and day 7 and 14, for determination of plasma and RBC ChE activities. All samples collected after 4 h were stored frozen overnight prior to analysis.]

Additionally, plasma and urine were collected for analysis of concentrations of azinphos-methyl and metabolites. [Blood was collected via a cannula or by repeated venipuncture at 0, 1, 2, 4, 8, 12, 48 and 72 h post-dose. Urine was collected at -24 to 0 h pre-dose, 0 to 24, 24 to 48, and 48 to 72 h post-dose. Plasma and urine samples were stored frozen at -70°C and shipped to Bayer AG (Leverkusen, Germany) for bioanalytical analysis.]

Adverse events: All observed or reported adverse events were recorded and fully described by duration, maximum severity (mild, moderate, severe) and relationship to test compound (not related, unlikely, possible, probable or definitely related). Adverse events were coded using WHO Adverse Reaction Terminology and they were reported by primary body system and preferred term. Counting was performed by subject and not event, i.e. a subject reporting the same event more than once had that event counted only once.

Statistical methods: [Data were analysed for normality using normal probability plots and the Shapiro-Wilk test. Abnormally distributed data were ranked transformed prior to statistical analysis.] Percentage change from baseline for RBC and plasma ChE were analysed separately for male and female subjects, using a repeated measures analysis of variance with terms for dose level, timepoint and dose level by timepoint interaction. Using contrasts, the following analyses were performed at each timepoint separately: For the male data, a test for linear trend with dose was performed using linear contrast, and pairwise comparisons between placebo and each dose level were carried out. If the test for linear trend was not significant at the 5% level, then a Bonferroni adjustment was applied to the pairwise comparisons at that timepoint; otherwise no adjustment was made for multiple comparisons. For the female data, [using the error variance from the ANOVA,] a comparison between placebo and active compound was carried out [using a Student's t-distribution].

Adverse events, vital signs and other laboratory parameters were summarised descriptively by gender, dose level and timepoint (where appropriate).

[The statistical software SAS (v6.07) was used to perform all statistical analyses and generate all summary tables and data listings.]

Results

A total of 88 male volunteers were screened for this study. Of this number, 29 were rejected at the screening visit, and 18 were not entered either due to a failure to me*et al*l the inclusion/exclusion criteria or a failure to attend the clinic for admission.

A total of 21 female volunteers were screened for this study. Of this number 6, were rejected at the screening visit, and 5 were not entered either due to a failure to me*et al*l the inclusion/exclusion criteria or a failure to attend the clinic for admission.

[*Protocol deviations*: The main protocol deviations were: the study was unblinded for all subjects in dose groups 1 and 2 (subjects 001-011) due to a greater-than 25% inhibition of RBC ChE activity in subject 5, which required disclosure of the dose as it met the criteria for stopping the study; ChE samples for groups 1 and 2 were re-analysed due to an error in sample preparation; a number of baseline ChE samples (d -10, -8 and -4) were taken without the exact pre-dose day; subject 019 received an incorrect dose (1.22 times higher than required); subject 027 (placebo) did not have a blood sample taken.]

Plasma ChE: For male subjects, the test for linear trend was positive at 8 [p = 0.048] and 24 h [p = 0.038] after dosing, but the slope parameter was positive, indicating that any inhibition of ChE decreased as the dose of azinphos-methyl increased. This is probably a chance finding as it is not biologically plausible. None of the pairwise comparisons between any of the dose levels and placebo was statistically significant at any of the post-dose assessments [see Table below]. For female subjects, plasma ChE values 72 h after dosing were significant higher [p = 0.004] for the 0.75 mg/kg bw group than for placebo; this pairwise comparison was not statistically significant at any other post-dose assessment [see Table below].

[**Effect of a single PO dose of azinphos-methyl on plasma ChE activity in male and female volunteers over 72 h. Results are expressed as the mean % change from the baseline value ± SD. The baseline was the mean of all available pre-dose values at days -10, -8, -4, -2, -1 and -30 min**.]

Dose level (mg/kg bw)	**0**	**0.25**	**0.5**	**0.75**	**1.0**	**0**	**0.75**
Sex	m	m	m	m	m	f	f
No. of subjects	12	7	7	7	7	3	7
Baseline [mean iu/L]	5739	5256	6285	6402	5715	4693	5006
[SD]	[1071]	[1325]	[1199]	[1129]	[757]	[661]	[786]
1 h	-6.98	-4.63	-3.81	-7.50	-3.12	-5.92	-4.15
[SD]	[4.56]	[5.26]	[5.44]	[4.44]	[2.26]	[0.48]	[5.09]
2 h [mean]	-5.67	-5.06	-0.32	-5.06	-0.95	-1.75	-3.59
[SD]	[5.18]	[4.68]	[7.26]	[2.87]	[2.97]	[3.61]	[6.55]
4 h [mean]	-1.90	-3.62	0.23	-0.44	-2.18	-0.22	1.59
[SD]	[7.46]	[6.17]	[6.30]	[4.16]	[5.66]	[4.71]	[6.62]
8 h [mean]	-3.92	-7.19	-6.64	-2.42	0.59	-6.21	-8.17
[SD]	[5.56]	[4.35]	9.40[]	[7.09]	[4.03]	[2.13]	[5.69]
12 h [mean]	-7.36	-7.94	-6.82	-10.22	-5.68	-7.57	-9.88
[SD]	[7.28]	[3.89]	[7.98]	[2.98]	[5.59]	[3.32]	[4.99]
24 h [mean]	-2.65	-4.39	0.47	-0.68	2.72	-2.05	-1.95
[SD]	[6.97]	[7.53]	[9.98]	[6.07]	[4.57]	[0.76]	[5.11]
48 h [mean]	-6.69	-8.41	-1.80	-6.00	-6.37	-7.86	-4.17
[SD]	[5.68]	[2.84]	[7.11]	[7.73]	[3.21]	[3.39]	[4.63]
72 h [mean]	-3.65	-5.99	-3.06	1.28	-2.66	-5.56	6.71
[SD]	[7.61]	[4.72]	[6.56]	[8.74]	[3.44]	[5.56]	[11.05]
7 d [mean]	2.18	-3.19	-1.16	2.65	-1.34	-7.38	-3.37
[SD]	[10.76]	[2.78]	[4.92]	[6.99]	[4.32]	[2.70]	[5.99]
14 d [mean]	1.55	4.76	2.96	1.38	0.00	-3.52	-2.41
[SD]	[5.56]	[5.76]	[9.41]	[5.37]	[7.44]	[4.13]	[6.52]

RBC ChE: For male subjects, the test for linear trend was positive at 72 h [p = 0.009] after dosing, and the 0.25 mg/kg bw dose group showed higher values than placebo group at this time point [see Table below]. At 12 h after dosing RBC ChE was statistically significantly lower for the 0.25 mg/kg bw dose group than placebo [(see Table below]. This was not a biologically relevant change and probably due to chance as it was not observed in any of the higher dose groups.

[**Effect of a single PO dose of azinphos-methyl RBC ChE activity in male and female volunteers over 72 h. Results are expressed as the mean% change from the baseline value ± SD. The baseline was the mean of all available pre-dose values at days -10, -8, -4, -2, -1 and –30 min**.]

Dose level (mg/kg bw)	0	0.25	0.5	0.75	1.0	0	0.75
Sex No. of subjects	m 12	m 7	m 7	m 7	m 7	f 3	f 7
Baseline [mean iu/mL]	11595	12810	12712	11524	11389	10505	11239
[SD]	[1323]	[1013]	[961]	[1296]	[1280]	[281]	[795]
1 h	-0.24	2.87	-3.83	-0.86	-1.42	-0.75	5.49
[SD]	[8.79]	[6.67]	[11.49]	[4.59]	[15.19]	[12.24]	[13.95]
2 h	1.67	0.62	-6.62	5.15	-0.43	-2.41	11.93
[SD]	[7.70]	[11.21]	[12.22]	[3.29]	[14.86]	[1.22]	[13.65]
4 h	-3.12	-1.60	-6.77	2.15	0.68	0.28	2.65
[SD]	[7.46]	[5.29]	[11.44]	[6.68]	[10.12]	[7.44]	[12.50]
8 h	4.18	-0.70	-4.09	4.80	5.26	-0.58	4.46
[SD]	[8.67]	[5.58]	[14.67]	[5.36]	[6.09]	[1.82]	[11.01]
12 h	-1.69	-12.15*	4.73	3.40	-5.01	0.23	9.94
[SD]	[6.94]	[10.63]	[9.59]	[3.68]	[4.91]	[4.22]	[5.84]
24 h	-3.49	3.67	-5.88	0.29	-5.91	-4.23	4.86
[SD]	[5.90]	[4.46]	[11.23]	[7.84]	[8.67]	[5.42]	[7.90]
48 h	-2.50	1.98	-2.60	1.32	-5.89	-6.11	-2.35
[SD]	[9.12]	[6.83]	[7.85]	[5.43]	[8.53]	[3.95]	[4.77]
72 h	-3.86	6.76	-6.67	-9.55	-8.17	3.89	9.95
[SD]	[8.86]	[5.89]	[5.35]	[7.40]	[12.00]	[7.70]	[9.49]
7 d	2.66	5.46	-3.19	0.17	7.87	10.47	-1.36
[SD]	[9.25]	[4.08]	[7.72]	[7.71]	[10.06]	[7.99]	[10.92]
14 d	0.60	0.56	-9.57	1.15	3.42	-0.59	2.73
[SD]	[9.34]	[8.44]	[11.04]	[5.55]	[12.15]	[2.81]	[7.58]

[* statistically different to the control at $p < 0.05$ following pairwise comparison with the placebo after Bonferroni adjustment]

[Examination of individual subject data revealed that inhibition of RBC ChE activity was between 15–20% for 4/7 males. A different male recorded an inhibition of RBC activity of 19% at 2 h post-dose. Four males treated with 0.5 mg/kg bw azinphos-methyl exhibited a 20–28% depression in RBC activity at various times: subject 011 showed inhibition of 24 and 27% at 1 and 2 h post-dose respectively; subject 015 showed 25 and 28% inhibition at 8 and 24 h post-dose respectively; subject 017 had 17% inhibition at 4 h, and subject 018 had inhibition of 20% at 72 h. At 0.75 mg/kg bw 2/7 subjects showed a 17–22% depression in RBC activity 72 h post-dose. In the highest dose group (1mg/kg bw) only a single individual showed a 22% depression in RBC ChE activity at 72 h post-dose. In the absence of a dose-response effect with regard to an increase in RBC ChE inhibition with increasing dose, the fact that depression in RBC activity was generally not sustained over more than one sampling

point, and that subjects exhibited no cholinergic signs, these results were concluded to be not due to azinphos-methyl.] No pairwise comparisons between any of the dose levels and placebo at any of the post-dose assessments were statistically significant.

For female subjects, RBC ChE values 2 h after dosing were significantly higher [p = 0.029] for the 0.75 mg/kg bw dose group than for placebo; this pairwise comparison was not statistically significant at any other post-dose assessment [(see Table above). Examination of individual subject data revealed that only a single female treated with 0.75 mg/kg bw azinphos-methyl had a 21% depression in RBC ChE activity at 1 h post-dose and thus confirmed the absence of any treatment-related effect.]

[*Other findings*:] There were no clinically significant changes in vital signs, ECGs, haematology, clinical chemistry, urinalysis or physical examination observed for any subject receiving azinphos-methyl or placebo.

[Females (3/7) treated with 0.75 mg/kg bw azinphos-methyl had an abnormal increase in standing heart rate at 8 h post-dose compared to the placebo group (1/3) but in the absence of an effect at any other sampling times, or in males, it was not considered to be treatment-related. Two males in the 1mg/kg bw group exhibited decreased LDH activity at 24 h compared to their screen and pre-dose values and these were indicated by the study author to fall outside of the normal control range: subject 041 had LDH activities of 269, 281 and 216 iu/mL at screen, pre-dose and 24 h respectively, while for subject 049 LDH activities were 255, 248 and 226 respectively. No other perturbation in LDH activity was observed in any other individual. In the absence of a dose-response effect these observations were not concluded to be treatment-related.]

[*Adverse events*:] There was a total of 28[5] post-dose adverse events and 4 pre-dose events occurring in 23 of the subjects who entered into the study [see Table below]. None of the reported adverse events were serious and no adverse events exceeded grade 2 in severity. There was no evidence of an increase in the frequency of adverse events with an increase in dose level of azinphos-methyl. [Nine post-dose adverse events were observed in 5 females that were treated with 0.75 mg/kg bw azinphos-methyl while no post-dose adverse events were observed for the placebo group (see Table below). None of these observations were attributed to treatment for the following reasons: none were correlated with any depression in ChE activity; they could be attributed to an upper respiratory tract infection, and ward or postural conditions; no treatment-related effect was observed in males at the same or higher dose.] Although some of the adverse events were considered (when the study was blinded) to be "possibly related" to the test compound, in retrospect none of these events were considered to be compound related due to the absence of ChE inhibition at the time of the events.

[**Incidence of adverse events in male and female volunteers that were administered a single PO dose of azinphos-methyl.**]

Dose level (mg/kg bw)	**0**	**0.25**	**0.5**	**0.75**	**1.0**	**0**	**0.75**
Sex	m	m	m	m	m	f	f
No. of subjects	12	7	7	7	7	3	7
Pre-dose events	1	1	1	0	0	1	0
Post-dose events	3	3*	7	0	6	0	9

[5] Following examination of individual subject data, this number was amended due to incorrect reporting in the original study document

Subjects with adverse post-dose events	3*	3*	4*	0	4	0	5
General disorders❶	1	1	5*	0	5*	0	6*
Nervous system disorders❷	1	1	0	0	0	0	1
Respiratory system disorders❸	0	0	0	0	1	0	2*
Gastro-intestinal disorders❹	0	0*	1	0	0	0	0
Skin disorders❺	0	1	0	0	0	0	0
Vision disorders❻	1	0	1	0	0	0	0

❶ Abdominal pain, back pain, headache, leg pain, pain, pallor, rigours, skeletal pain;❷ Dizziness, headache; ❸ Pharyngitis, upper respiratory tract infection; ❹ Diarrhoea, ulcerative stomatitis; ❺ Erythematous rash; ❻ Abnormal vision, conjunctivitis; *: corrected following examination of individual subject data (incorrect data entry in the original summary table)

Conclusions: In a double blind, placebo controlled study, azinphos-methyl at single PO dose levels ranging from 0.25, 0.5, 0.75 and 1.0 mg/kg bw was found to be well tolerated when administered to healthy male subjects. A single PO dose level of 0.75 mg/kg bw was also well tolerated by healthy female subjects. No clinical relevant reductions in plasma or RBC ChE values occurred in this study. Therefore, the NOAEL for azinphos-methyl when given as a single PO dose was 1.0 mg/kg bw for males and 0.75 mg/kg bw for females.

[*Comments*: Only a single dose was tested on females. No historical control data were provided although it was indicated when results fell outside the 'normal' control range. Assay results for azinphos-methyl or metabolites of azinphos-methyl in blood and urine samples were not provided. The stability of azinphos-methyl in the vehicle was not evaluated. The sample size for the placebo was low (n = 3) and unbalanced with the test groups (n = 7).]

♦ ***McFarlane P & Freestone S (1999b) A randomised double blind placebo controlled study with azinphos-methyl to determine the no effect level on plasma and RBC cholinesterase activity after repeated doses. Report no. 17360. Lab: Inveresk Research Ltd., Edinburgh, Scotland. Sponsor: Bayer Corporation, Agricultural Division, South Metcalf, Stilwell, KS, USA. Study duration: 9 February 1999 – 15 April 1999. Report date: 3 August 1999.***

[*Guidelines and GLP*: A formal statement was provided which indicated that the study complied with the principles of Good Clinical Practice (CPMP/ICH/135/95) and OECD principles of GLP. The study was conducted in accordance with the guidelines established in the Declaration of Helsinki 1964, as amended by the 29th World Medical Assembly in Tokyo 1975, the 35th World Medical Assembly in Venice 1983, the 41st World Medical Assembly in Hong Kong 1989 and the 48th General Assembly, Somerset West, Republic of South Africa October 1996. A formal Quality Assurance statement was provided.]

Objectives: The study was performed in order to determine the no effect level for plasma and RBC cholinesterase activity after repeated dosing and to compare the human and animal sensitivities at no effect levels. [Also to establish an appropriate recommended daily intake for chronic dietary exposure, to conduct appropriate risk assessment, and to obtain information for possible biological monitoring.]

Materials and Methods

The experiment was performed as a double blind, placebo controlled study using 12 [white] male healthy volunteers. Eight of the twelve subjects received azinphos-methyl (Guthion technical; batch no. 703-0139; purity: 91.6%; [Bayer Corporation, Agricultural Division,

South Metcalf, Stilwell, KS, USA]) at a dose of 0.25 mg/kg bw/d and 4 subjects received placebo (lactose: batch no. 28811; [Inveresk Research Ltd. (IRL), Edinburgh, Scotland]) in [gelatin] capsule form once daily over a 28 day duration. [The dose was chosen following consideration by the study authors of data from previous animal and human studies. The mean age and weight of the placebo group was 35.3 $\pm$ 9.5 y and 77.7 $\pm$ 12.51 kg respectively. The mean age and weight of the group receiving azinphos-methyl was 29.3 $\pm$ 9.6 y and 69.3 $\pm$ 5.62 kg respectively. Subjects were assigned to test or placebo groups following computer-generated randomisation. Azinphos-methyl or placebo was administered to seated subjects with 150 mL of water.] All subjects were resident in the clinic from the day before the first dose until 24 h after receiving the final dose. All subjects remained under constant medical and nursing supervision and received standardised diet. [The study protocol was reviewed and approved by an Independent Research Ethics Committee. The study authors also indicated that the study design and dose selection were discussed with the Californian EPA.]

All subjects were selected from a panel of volunteers held at ICR [Inveresk Clinical Research Ltd] and were screened within 3 weeks prior to study initiation. During that visit, the scope and intent of the study was clearly explained. To be entered into the study, subjects had to fulfill the following criteria: a) 18 to 50 years of age, b) no clinical important physiological findings, c) no clinically relevant abnormalities of laboratory screening (including ChE activity), d) normal ECG, e) normal arterial pressure and heart rate, f) body weight between 50 and 100 kg and within +/- 15% of ideal body weight, g) able to communicate well with the investigator and to comply with the requirements of the entire study, h) provision of written informed consent to participate.

During the conduct of the study, the attending physician/nurses explained the procedures for each phase of the study, and asked each volunteers if he/she had any questions on any procedures involved in the study, or any complaints associated with the administration of the chemical. A physical examination was performed prior to discharge from the clinical unit and all volunteers returned to the clinical unit 7 days post final dose for adverse event inquiry.

Variable examination: Physical examination including vital signs (just before each dose and 24 h after the final dose) [supine systolic and diastolic arterial pressure, supine pulse, PO temperature, erect heart rate], ECG ([ventricular rate, PR interval, QRS interval, QI interval, QTc interval, QRS axis; immediately before dosing on] days 1, 7, 14, 21 and 28), laboratory tests (haematology, clinical chemistry, urinalysis: before dosing on days 1, 2, 14, 21 and 28), plasma and RBC ChE (before dosing on days -14, -12, -10, -8, -6, -4, -2, -1; daily before each dose and 4 h post-dose on days 1, 2, 3, 4, 5, 7, 10, 14, 17, 21, 24 and 28). [Blood (4.5 mL) was collected by venipuncture or via a cannula by registered nurses. The following haematology parameters were measured: Hb, Hct, RBC, MCH, MCV, MCHC, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, leucocytes and platelets. The following clinical chemistry parameters were measured: urea, glucose, AST, ALT, LDH, Na, K, Cl, total protein, albumin, total bilirubin, GGT and creatinine. The following urinary parameters were measured: pH, specific gravity, glucose, bilirubin, ketones, blood, protein, urobilinogen, urine volume. Urine samples showing the presence of protein or blood were centrifuged and the resulting deposit microscopically examined.]

Blood and urine samples for analysis of azinphos-methyl and metabolite concentrations were collected [before dosing] on days 1, 2, 3, 4, 5, 7, 10, 14, 17, 21, 24 and 28. [Urine was collected at -12 to 0 h before day 1, and at 0 to 4, 4 to 12 and 12 to 24 h on all other days. All

plasma and urine samples were stored at -70°C until bioanalytical analysis could be conducted by Bayer AG (Monheim, Leverkusen, Germany).]

Adverse events: All observations or reported adverse events were recorded and fully described by duration, maximum severity (mild, moderate, severe) and relationship to test compound (not related, unlikely, possible, probable or definitely related). Adverse events were coded using WHO Adverse Reaction Terminology and they were reported by primary body systems and preferred term. Counting was performed by subject and not to event, ie a subject reporting the same event more than once had that event counted only once.

Statistical methods: Percentage and absolute changes from baseline for RBC and plasma ChE were analysed using a repeated measured analysis of variance with terms for treatment, timepoint and treatment by timepoint interaction. Subject was included in the model as a random effect. Using the error variance from the ANOVA, pairwise comparisons between placebo and active were carried out at each timepoint, using Student's t-distribution.

Demographics and other baseline characteristics were summarised by treatment. Adverse events, ECG, urinalysis, vital signs and other laboratory parameters were summarised descriptively by treatment and timepoint (where appropriate).

[The statistical software SAS (v6.07) was used to perform all statistical analyses and generate all summary tables and data listings.]

Results

[The purity of azinphos-methyl was confirmed to be 89.2% in March and May 1999. A retrospective power analysis of plasma and RBC ChE activities using the software nQuery Advisor (v2.0) indicated that a sample size of 12 (8 azinphos-methyl subjects and 4 placebos) had a power of 85 $\pm$ 7.3% and 80 $\pm$ 7.8% respectively to detect a statistical difference at $p < 0.05$.]

A total of 19 male volunteers were screened for this study. Of this number, 4 were rejected at the screening visit, and 3 were not entered either due to a failure to me*et al*l the inclusion/exclusion criteria or a failure to attend the clinic for admission. Twelve healthy male subjects were entered onto this study with all 12 completing.

Plasma ChE: [There was no treatment-related effect on plasma ChE activity (see Table below). A high degree of variability in the data was also apparent (see Table below).] The analysis of the percentage changes showed that the azinphos-methyl group (0.25 mg/kg bw/d) had significantly larger increases from baseline compared with the placebo control at a number of timepoints during the course of the study. At no timepoint was there any significantly larger percentage decreases from baseline compared with placebo. None of the other pairwise comparisons was found to be statistically significant.
[**Effect of azinphos-methyl on plasma ChE activity following PO administration to male volunteers over 28 days. Results are expressed as the mean change (iu/L and %) from the baseline value $\pm$ SD. The baseline was the mean of all available pre-dose values at days -14, -12, -10, -8, -6, -4, -2 and 1**.]

Dose level (mg/kg bw)	0	0.25
No. of subjects	4	8
Baseline (iu/L)	6965 [± 1327]	5307 [± 1048]
Day 1, post-dose	-500 [± 478] (-7.0 [± 5.9]%)	-322 [± 287] (-6.0 [± 4.7]%)
Day 2, post-dose	-490 [± 486] (-6.4 [± 5.7]%)	-146 [± 318] (-2.3 [± 5.2]%)
Day 3, post-dose	-591 [± 129] (-8.5 [± 0.5]%)	23 [± 1081] (4.3 [± 30.3]%)**
Day 4, pre-dose	-296 [± 157] (-4.2 [± 2.2]%)	-89 [± 261] (-1.4 [± 4.4]%)
Day 4, post-dose	-545 [± 354] (-8.3 [± 5.8]%)	-169 [± 226] (-2.8 [± 4.6]%)
Day 7, post-dose	-464 [± 380] (-6.7 [± 5.9]%)	65 [± 317] (1.8 [± 6.6]%)
Day 14, post-dose	-379 [± 121] (-5.4 [± 1.3]%)	-185 [± 304] (-2.9 [± 5.5]%)
Day 21, post-dose	-173 [± 377] (-2.1 [± 5.7]%)	125 [± 275] (2.9 [± 6.8]%)
Day 26, pre-dose	-63 [± 615] (-1.2 [± 8.2]%)	720 [± 645] (14.5 [± 12.7]%)***
Day 27, pre-dose	102 [± 763] (0.9 [± 10.3]%)	531 [± 470] (11.1 [± 9.5]%)
Day 28, pre-dose	-146 [± 342] (-2.5 [± 4.9]%)	205† [± 317] (4.5 [± 6.9]%)
Day 28, post-dose	-213 [± 392] (-3.2 [± 6.0]%)	253 [± 388] (5.8 [± 9.0]%)*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (analysis of percentage changes) [†: changed from BgVV report; incorrect data entry]

The results obtained from the analysis of the absolute changes from baseline were similar to the analyses of the percentage changes from the baseline. The only changes were observed when all values were analysed. In this case the azinphos-methyl group (0.25 mg/kg bw/d) had significantly larger increases from baseline compared with placebo group at 4 h post-dose on day 7. There were no significantly larger decreases from the baseline compared with placebo at any timepoint.

RBC ChE: [There was no treatment-related effect on RBC ChE activity (see Table below). A high degree of variability in the data was also apparent (see Table below).] The azinphos-methyl group (0.25 mg/kg bw/d) had significantly larger percentage increases from baseline compared with placebo at pre-dose on day 4 and at 4 h post-dose on day 21. None of the other pairwise comparisons was found to be statistically significant. The results obtained from the analysis of the absolute changes from baseline were similar to the results obtained from the analyses of the percentage changes from the baseline.

[**Effect of azinphos-methyl on RBC ChE activity following PO administration to male volunteers over 28 days. Results are expressed as the mean iu/L and% change from the baseline value 1 SD). The baseline was the mean of all available pre-dose values at days –14, -12, -10, -8, -6, -4, -2 and 1**.]

Dose level (mg/kg bw)	0	0.25
No. of subjects	4	8
Baseline [iu/mL]	10933 [± 225]	11049 [+ 478]
Day 1, post-dose	748 [± 1603] (6.7 [± 14.5]%)	634 [± 747] (5.8 [± 6.9]%)
Day 2, post-dose	303 [± 344] (2.7 [± 3.2]%)	327 [± 905] (3.1 [± 8.3]%)
Day 3, post-dose	-514 [± 1212] (-4.6 [± 10.9]%)	433 [± 538] (4.0 [± 4.9]%)
Day 4, pre-dose	-1389 [± 984] (-12.7 [± 8.8]%)	208 [± 790] (2.0 [± 7.3]%)*
Day 4, post-dose	-190 [± 888] (-1.7 [± 8.0]%)	121 [± 931] (1.2 [± 8.7]%)
Day 7, post-dose	-751 [± 1306] (-6.8 [± 11.9]%)	-521 [± 798] (-4.6 [± 6.9]%)
Day 14, post-dose	-923 [± 1093] (-8.5 [± 10.0]%)	-1735 [± 799] (-15.5 [± 6.8]%)
Day 21, post-dose	-550 [± 115] (-5.0 [± 1.2]%)	503 [± 1356] (4.7 [± 12.5]%)
Day 26, pre-dose	166 [± 639] (1.6 [± 5.82]%)	-13 [± 831] (0.1 [± 7.32]%)
Day 27, pre-dose	-177 [± 979] (-1.6 [± 8.81]%)	454 [± 775] (4.2 [± 7.0]%)

Dose level (mg/kg bw)	0	0.25
Day 28, pre-dose	449 [± 1031] (4.1 [± 9.34]%)	439 [± 674] (4.1 [± 6.1]%)
Day 28, post-dose	495 [± 399] (4.5 [± 3.6]%)	277 [± 884] (2.6 [± 8.0]%)

* $p < 0.05$; ** $p < 0.01$; (analysis of percentage changes)

[*Other parameters*:] There were no clinically significant [or compound-related] changes in vital signs, ECG, haematology, [clinical chemistry,] urinalysis or physical examination observed for any subject receiving azinphos-methyl or placebo. Transient increases in ALT, AST and GGT were noted in [up to] 3 subjects [from the azinphos-methyl group and up to 2 subjects from the placebo group] during the course of the study, none of which was considered to be related to the test compound [(see Table below). An additional treatment-unrelated observation was an elevation in blood glucose in all subjects for nearly the total duration of the study.]

[**Effect of azinphos-methyl on ALT, AST and GGT activity (iu/L) following PO administration to male volunteers over 28 days. (Results are expressed as the mean ± SD, range and no. of values above the normal range).**]

Dose level (mg/kg bw)	0	0.25
No. of subjects	4	8
ALT: Baseline	29.3 [± 6.7] (20-36); 0	19.4 [± 7.1] (12-32); 0
Day 1	24.5 [± 6.6] (15-30); 0	19.8 [± 7.9] (11-34); 0
Day 7	40.3 [± 13.2] (27-57); 1	34.0 [± 27.8] (15-100); 1
Day 14	49.3 [± 19.1] (24-66); 2	53.1 [± 49.3] (13-143); 3
Day 21	44.8 [± 18.7] (27-71); 1	32.4 [± 21.6] (13-78); 1
Day 28	38.5 [± 12.4] (28-56); 1	25.8 [± 14.2] (13-58); 1
AST: Baseline	19.0 [± 5.4] (12-25); 0	20.3 [± 5.4] (11-27); 0
Day 1	15.5 [± 5.5] (9-21); 0	16.4 [± 4.4] (10-22); 0
Day 7	21.0 [± 5.8] (14-28); 0	24.0 [± 13] (11-53); 1
Day 14	22.8 [± 11.0] (11-34); 0	29.6 [± 23.6] (10-83); 1
Day 21	21.5 [± 11.1] (9-35); 0	19.0 [± 7.3] (9-33); 0
Day 28	18.0 [± 6.7] (8-22); 0	16.6 [± 6.2] (8-28); 0
GGT: Baseline	34.5 [± 18.0] (18-52); 0	27.4 [± 18.3] (7-55); 0
Day 1	30.3 [± 13.7] (16-46); 0	22.8 [± 13.9] (7-44); 0
Day 7	36.5 [± 15.8] (19-56); 0	27.9 [± 21.4] (8-72); 1
Day 14	39.8 [± 15.3] (17-50); 0	35.6 [± 31.1] (5-101); 1
Day 21	42.3 [± 17.9] (20-63); 1	32.6 [± 24.3] (7-78); 1
Day 28	40.8 [± 14.7] (22-56); 0	32.3 [± 18.6] (9-59); 0

[*Adverse events*:] There was a total of 67 adverse events which occurred following administration of azinphos-methyl or placebo over a 28 day duration [(see Table below) and all of these were transient in nature]. Seventeen of these occurred in 2 of the 4 subjects receiving the placebo [and the remaining 50 occurred in all of the azinphos-methyl subjects]. There were no adverse events which were considered to be related to the administration of azinphos-methyl or any adverse event which was related to a decrease in cholinesterase activity. [A viral infection, ward conditions and dietary factors were the probable main causes of the reported adverse events.] None of the reported adverse events were serious and no adverse events exceeded grade 2 severity [, which as defined by the WHO Body Systems and Preferred Term by Relationship, were unlikely to be treatment-related.]

[**Incidence of adverse events in male volunteers that were orally administered azinphos-methyl over 28 days.**]

Dose level (mg/kg bw)	0	0.25
No. of subjects	4	8
No. of adverse pre-dose events	0	3
No. of adverse post-dose events	17	50
No of subjects with adverse post-dose events	2	8
General disorders∂	2	6
Autonomic nervous system disorders•	1	1
Gastro-intestinal disorders÷	1	4
Liver and biliary system disorders≠	1	2
Musculo-skeletal system disorders≡	1	0
Resistance mechanism disorders≈	0	1
Respiratory system disorder…	1	6
Skin and appendages disorders |	1	2
Urinary system disorders—	0	1
White cells and RES disorders↵	0	1

∂ Abdominal pain, back pain, chest pain, headache, pain; • Mouth dry; ÷ Dyspepsia, flatulence, nausea, stomatitis ulcerative, vomiting; ≠ Gamma GT increased, hepatic enzymes increased, hepatic function abnormal; ≡ Myalgia; ≈ Abscess; … Coughing, epistaxis, rhinitis; | Pruritus, rash; — Dysuria; ↵ Lymphadenopathy

Conclusions: In a double blind, placebo controlled study, repeat PO doses of azinphos-methyl (0.25 mg/kg bw/d) were found to be safe and well tolerated when administered to healthy male subjects over a 28 day period. There were no adverse events of azinphos-methyl administration noted in any subject and no clinically relevant reductions in plasma or RBC ChE values were seen.

[*Comments*: Only one dose level was investigated. No historical control data were provided although it was indicated when results fell outside the 'normal' control range. Bioanalytical results of azinphos-methyl or metabolites of azinphos-methyl in blood and urine samples were not provided. The stability of azinphos-methyl in the vehicle was not evaluated. The ChE data displayed a high level of variability. The sample size for the placebo was low (n = 4) and unbalanced with the test groups (n = 8).]

2.11.2 Occupational Exposure

Simpson GR (1965) Exposure to guthion during formulation. Arch Environ Health 10: 53-54.

Study and observations: This published report presented the findings of a scientific officer of the Division of Occupational Health, New South Wales Department of Public Health, following a chance visit to a guthion (azinphos-methyl) and azinphos-ethyl formulation plant. It was stated that the manufacturing plant attempted to formulate these chemicals under "primitive conditions". Further, according to the study author, safety measures to prevent worker exposure "did not exist" in the plant. Whole blood ChE of 15 operators was assayed randomly over a period of 4 months using a method modified from Fleischer *et al* (1956). The data were presented in "ChE units" (in a range of 0 to 120). A reading between 78-110 units was considered normal. When the ChE level reached 60 units or less, the workers were removed from exposure. Signs of ChE toxicity were expected at 20-30 units.

Azinphos-methyl concentration in the operator's breathing zone and on drum tops about 6 meters from the process were determined (method unspecified). No further details on experimental methods were provided.

Findings: Azinphos-methyl concentration in the operator's breathing zone ranged from 0.5 to 1.0 mg/m^3. This concentration was found to be well above the tentative limit of 0.3 mg/m^3, set by the Division of Occupational Health of New South Wales Department of Public Health at that time. The level of azinphos-methyl found on drum tops was about 2.3 mg/cm^2.

During the processing season (unspecified), 13 operators (details not provided) were removed from contact as their ChE activity fell below 60 units or 60% of the pre-exposure level. Two operators showed signs of organophosphate poisoning (onset, types and duration unspecified), and one of them had to be hospitalised. The ChE levels of these two persons were 25 and 30 units respectively, and that of the remaining 13 persons ranged from 32 to 110 units. A line graph presented showed the recovery of whole blood ChE activity to about 80-90 units in about 15 days after removing these workers from the formulating plant completely. However, a comparable recovery of the enzyme activity in workers who remained in the plant doing other jobs, was noticed only after about 35 days.

Conclusions: The health effects reported in this study could be attributable to the high exposure levels of azinphos-methyl that existed in the formulating plant. However, due to data limitations, and the atmospheric levels of azinphos-ethyl were not determined, it is not possible to comment whether the effects observed were due to azinphos-methyl or azinphos-ethyl, or a combined effect of both of these chemicals.

Waggoner TB, Olson TJ & Lamb DW (1970a) Determination of the hazards to workers picking citrus treated with guthion spray concentrate formulation (3.75 lbs. AI/acre). Study no: not stated, Research Department, Chemagro Corporation. Sponsor: Bayer AG. Study duration: not stated, Report no. 28428. Report date: November 3, 1970.

[Although this report also presented data on guthion residues, only the data pertaining to plasma and red blood cell (RBC) ChE were considered in the following evaluation.]

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: In this study, plasma and erythrocyte (RBC) ChE levels in citrus pickers working in a grove of oranges sprayed with guthion SC (azinphos-methyl) in California were investigated. Nineteen workers (age: 21 years or older, 12 males and 7 females, bw: unspecified) who had no recent exposure to any ChE inhibiting agents were chosen for the study. Baseline plasma and RBC ChE activities of each of them were established using data from 3 determinations carried out at 7, 5 and 3 days before commencement of the study. It was stated that during this time they were not allowed to work in orchards, which had been sprayed with any ChE inhibiting pesticides. The orange grove used in the present study had not been sprayed with ChE inhibiting pesticides for at least 30 days prior to the test. This grove was subdivided into two blocks (sizes unspecified) and subsequently sprayed with azinphos-methyl (purity, source and batch not specified) at 4.2 kg ai/ha, one week apart.

Findings: Individual pre-exposure ChE activities appeared variable with about 10-50% variability in plasma ChE and about 10-20% variability in RBC ChE data. At the shortest re-

entry day (day 7 after spraying), 2 males/19 showed ≥20% plasma ChE inhibition compared to their respective pre-exposure averages after 3-4 h exposure. At the first re-entry on day 11 after spraying, 4/19 workers had an indication of a depression of plasma ChE levels (3 male workers, and 1 female worker) in comparison to their pre-exposure data. However, due to wide individual variability in pre-exposure plasma ChE data observed, the changes noted above could not be attributed to azinphos-methyl exposure with any certainity.

Post-exposure RBC ChE inhibition was variable (about 3-10%) and the inhibition was not significant in 17/19 individuals at any of the re-entry days. RBC ChE was depressed by about 27% in one male worker at reentry day 7, and by about 26% in a female worker at reentry day 8 after spraying, compared to their pre-exposure averages. No concurrent changes in plasma ChE was noted in either of these workers. Although it was stated that urine samples from all workers were collected, no urinary excretion data for the parent compound or its metabolites were provided.

Conclusions: Plasma and RBC ChE levels in 19 individuals who worked in a grove of orange sprayed with azinphos-methyl at 4.2 kg ai/ha were determined. Some possible inhibition of plasma and RBC ChE was seen in several individuals. However, the validity of the findings of this study is reduced due to wide variability in individual pre-exposure plasma ChE data, lack of statistical analysis, data limitations and debatable sensitivity of the ChE assay used.

Waggoner TB, Olson TJ & Lamb DW (1970b) Determination of the hazards to workers picking citrus treated with Guthion wettable powder formulation. Study no: not stated, Research Department, Chemagro Corporation. Sponsor: Bayer AG. Study duration: not stated, Report no. 28250. Report date: November 25, 1970.

[Although this report also presented data on guthion residues, only the data pertaining to plasma and red blood cell (RBC) ChE were considered in this evaluation.]

Pre GLP, non-quality assured study. No test guidelines were cited.

Study and observations: In this study, plasma and erythrocyte (RBC) ChE levels in citrus pickers working in a grove of oranges sprayed with guthion WP formulation (azinphos-methyl) were investigated. Fifteen workers (age: 21 years or older, 12 males and 3 females, body weight range not stated) who had no recent exposure to any ChE inhibiting agents were chosen for the study. Baseline plasma and RBC ChE levels of the workers were established using data from 3 determinations carried out at 7, 5 and 3 days prior to the commencement of the study. It was stated that during this time the workers were not allowed to work in orchards, which had been sprayed with any ChE inhibiting pesticides, and also the grove used had not been sprayed with such chemicals for at least 30 days prior to commencement of the study. The orange grove was subdivided into 3 blocks (unspecified sizes) and each was sprayed with azinphos-methyl WP formulation (purity, source, batch and formulation details not provided) at 4.2 kg ai/ha, on days 1, 4 and 10 days. Workers entered block 1 on the 8th day after spraying and picked fruit for 4 days. The workers spent about 6-7 h in the orchard each day, and there was no rainfall during the study. RBC and plasma ChE levels in workers were assayed using blood samples collected at 8 and 11 days after spraying. Blood samples were collected by venipuncture upon completion of a full working day. The ChE assay used was the "pH Stat assay for human blood cholinesterase" recommended by the US Public Health Service. The enzyme activity was expressed as μmoles acetylcholine/min/mL. Post-exposure

ChE activity data were compared with respective pre-exposure values to assess exposure effects.

Findings: Individual pre-exposure ChE data in this study did not show high variability as observed in the previous study by the same authors (Waggoner *et al* 1970). The variability in 11 workers was about 10% and 15% for plasma and RBC ChE, respectively. However, in 4 workers, the variability was higher, being about 25-66% for plasma ChE. The variability in RBC ChE data in these persons was about 10-30%. At the earliest re-entry day, ie at 7 days after spraying, 7/15 (4 males and all 3 females) and 5/15 workers (3 males and 2 females) exhibited depressions in plasma (about 22-65%) and RBC ChE (about 20-31%) levels, respectively compared to their pre-exposure values. On this sampling day, 4/15 (2/sex) workers showed inhibitions in both plasma and RBC ChE levels. At day 11 after spraying, 10/15 (7 males and all 3 females) and 5/15 (2 males and all 3 females) workers exhibited depressions in plasma (about 20-80%) and RBC ChE (about 24-32%) levels, respectively. Five out of 15 workers (3 males and 2 females) showed reductions in both plasma and RBC ChE activities on this sampling day. The reductions in plasma and RBC ChE activities that observed on both sampling days appear to be related to azinphos-methyl exposure.

Although urine samples from all workers were collected, no details on analyses were provided. Plasma ChE data of 2 workers at 7 days after spraying, and RBC ChE data of one worker at day 11 after spraying were not provided.

Conclusions: Plasma and RBC ChE levels in 15 workers who worked in a grove of orange sprayed with azinphos-methyl at 4.2 kg ai/ha were evaluated. Greater than 20% inhibition in plasma and/or RBC ChE activities, probably attributable to azinphos-methyl exposure, was noticed in some workers as assayed at 8 and 11 days after spraying. In comparison to the study author's previous investigation with azinphos-methyl SC, the exposure duration in the present study is longer (6-7 vs 3-4 h). The validity of the findings of this study, however, is reduced due to lack of statistical analysis, data limitations (clinical observations, urinary data) and debatable sensitivity of the ChE assay used.

Lamb DW (1980) Early studies with azinphos-methyl to determine re-entry times for citrus pickers. Mobay Chemical Corporation, Stanley Research Centre, Stilwell, Kansas, USA. In "Field worker Exposure During Pesticide Application: Studies in Environmental Science No. 7". (eds: Tordoir WF and Van Heemstra-Liquen. Elsevier Publishing Co. NY, 121-127.

[Although this report also presented data on azinphos-methyl residues, only the data pertaining to plasma and red blood cell (RBC) ChE were considered in this evaluation.]

Study and observations: This study monitored plasma and red blood cell (RBC) ChE activities in a group of citrus pickers in California, USA, together with foliar and patch residue analyses to determine a re-entry interval for azinphos-methyl. The main study consisted of 3 separate monitoring experiments. The workers involved in the study were 21 year of age or older and had no recent exposure to and ChE inhibiting pesticides. Experimental blocks were sprayed at rates of 370 mg/m^2 (azinphos-methyl WP), 250 mg/m^2 (azinphos-methyl SC), and 417 mg/m^2 (azinphos-methyl SC). Fifteen workers re-entered the blocks sprayed at 370 mg/m^2 (WP formulation), whilst 19 workers re-entered the blocks, which had azinphos-methyl SC applied at 250 and 417 mg/m^2 (sex, age and body weight range of the workers unspecified).

The workers involved in the 3 experiments entered the treated fields at 7 days post spray. Although, it was stated that the workers in blocks sprayed at 417 mg/m^2 worked only half-days due to limited availability of citrus for harvesting and cooler weather conditions. Individual plasma and RBC ChE values were determined prior to re-entry, and on various occasions. The method used for plasma and RBC ChE assay was the "pH stat assay for human blood Cholinesterase" recommended by the US Public Health Service (time and method of blood collection were not specified). Only the workers whose ChE levels were within the normal range participated in the study.

Findings: Azinphos-methyl concentration in the air surrounding the workers (60 minute sample) on day 7 post-spray at application rate of 370, 250 and 417 mg/m^2 were 0.14, 0.08 and 0.05 µg/L, respectively (time and the methods of sample collection and analysis unspecified). Plasma and RBC ChE data established for the workers in different experiments are presented in the following Table.

Greater than 20% depressions in plasma ChE activity were seen in workers exposed to 370 mg of azinphos-methyl WP/m^3 at the 2nd and 4th re-entry days. Because of this finding, further testing of workers involved in this experiment was not continued. No inhibition of plasma ChE was noted in any of the workers exposed to azinphos-methyl SC at the rate of 250 mg/m^2 (measured at the 2nd, 3rd, 5th and 6th re-entry days). However, the depressions in RBC ChE activity in workers exposed to azinphos-methyl SC at the rate of 250 mg/m^3 on the 2nd, 5th and 6th re-entry days exceeded 20%.

Mean plasma and RBC ChE values (percent of pre-exposure) in workers exposed to azinphos-methyl

Sampling day	Experiment 1		Experiment 2		Experiment 3	
	Plasma	RBC	Plasma	RBC	Plasma	RBC
8	72 (28%)	86			104	
10	60 (40%)	88				
11			119	100	101	104
14			98	78 (22%)	110	96
16					110	104
18			105	72 (28%)		
21			87	60 (40%)		

Values in parentheses represent percent ChE activity depression; Experiment 1 = Block sprayed with azinphos-methyl WP at 370 mg/m^3; Experiments 2 & 3 = Blocks sprayed with azinphos-methyl SC at 250 mg/m^3; Experiments 4 & 5 = Blocks sprayed with azinphos-methyl SC at 417 mg/m^3.

The depressions in plasma and RBC ChE activities seen above may be related to azinphos-methyl exposure and were considered to be toxicologically significant. RBC ChE activity inhibition seen in experiment 2 appeared to be slightly more pronounced (22-40%) than the RBC ChE inhibition noted in experiment 1 (12-14%). According to the data, it seems that the RBC ChE inhibition occurred more slowly and later compared to plasma ChE inhibition. The lack of ChE activity inhibition in blocks 4 and 5 workers may perhaps be due to low concentration of azinphos-methyl in the spray mix (and the shorter exposure period in block 5).

Conclusions: Under the conditions of the study, greater than 20% inhibition in plasma ChE activity was seen in workers exposed to azinphos-methyl WP at the 2nd and 4th re-entry days. Greater than 20% inhibition in RBC ChE activity was seen in workers exposed to azinphos-methyl SC at 2nd, 5th and 6th re-entry days. The depressions in plasma and RBC ChE activities

appeared to be related to azinphos-methyl exposure and were considered to be toxicologically significant. RBC ChE inhibition appeared to have occurred later compared to plasma ChE inhibition.

Faul J (1981) BBA Request-Effects on humans. In-company letter dated June 02, 1981 by the Department of DO medical, Bayer AG. Report no: not stated.

This short communication confirmed that gusathion (azinphos-methyl) was the cause of generalised dermatosis in an individual with apparently hypersensitive and dry skin. However, no effects that could be attributed to azinphos-methyl on any internal organs such as the liver were observed with adequate certainty. No further details on the chemical, affected individual or the severity of the skin reaction were provided. It is difficult to comment on this isolated observation due to data limitations.

Franklin CA, Fenske RA, Greenhalgh R, Mathieu L, Denley HV, Leffingwell JT & Spear RC (1981) Correlation of urinary pesticide metabolite excretion with estimated dermal contact in the course of occupational exposure to guthion. J Toxicol and Environ Health 7: 715-731.

[Although this report also presented data on azinphos-methyl residues, only the data pertaining to urinary metabolites, and serum and red blood cell (RBC) ChE were considered in following evaluation.]

Study and observations: In this study, exposure to and absorption of guthion 50 WP (azinphos-methyl; source, not stated) in a group of orchardists in British Columbia, Canada were estimated by air monitoring and patch techniques, and by measuring urinary alkylphosphate levels and serum and red blood cell (RBC) ChE activities. Seventeen workers (sex, body weight range not specified) who sprayed azinphos-methyl 50 WP (at 1.4 kg/ha) in their own orchards using air blast equipment participated in the study. The control group consisted of 10 residents (age, sex, body weight range unspecified) from the same area who were not involved in spraying. Composite urine samples [0-16 h and 16-24 h (morning void)] were collected from each worker prior to spray day (sampling day unspecified, as close to spray day as possible), after completion of spraying and at 1-day post spray. Each worker wore a standard set of protective clothing, which included cotton pants, a thin short sleeved cotton shirt and a thick long-sleeved coverall, and a respirator, which minimised inhalational exposure to the chemical. The workers also had the option of wearing any protective gear that they felt necessary such as gloves, boots, rubber suit and coats.

Urine collection was started in the morning of each sampling day with an empty bladder. Blood samples were collected from antecubital vein in the afternoon of an unspecified day prior to exposure, on the spray day and at day 1 post exposure (time unspecified). Urinary dimethyl phosphorothioic acid content was calculated from the sum of O, O-dimethyl phosphorothioate and O, O-dimethyl-S-ethyl phosphorothioate (DMEPT) peaks determined by a GC method. Azinphos-methyl equivalents of metabolites were calculated by multiplying the total DMEPT concentration by 1.86. Total output for the collection period was calculated by multiplying the azinphos-methyl equivalent concentration by the volume of urine collected. Total urinary metabolite concentration in a 24 h sample/individual was calculated by adding the metabolite levels in 0-16 h and 16-24 h samples. Clinical chemistry parameters were assessed using a microautoanalyser (SMA 12, Simultaneous Multiple Analysis). Serum

and RBC ChE activities were assayed by an automated method. The data were statistically analysed using the method of point biserial correlation analysis.

Findings: It was stated that clinical signs related to azinphos-methyl exposure were not observed in any of the workers and the results of the SMA 12 analyses were normal (data not provided). No depressions in serum or RBC ChE activities exceeding 15% of the pre-exposure values were seen at any of the sampling times. Recovery efficiency of dimethyl phosphorothioic acid from human urine samples fortified at 0.05 and 0.02 ppm were 98% and 88% respectively.

Urinary azinphos-methyl metabolite levels in different samples are presented in the following Table.

Excretion of azinphos-methyl metabolites in urine

Concentration of metabolites excreted (range in µg/time period)[a]					
Pre-exposure		Spray day		Post exposure	
0-16 h	16-24 h	0-16 h	16-24 h	0-16 h	16-24 h
Workers with rubberised coat and pants					
26-88	16 (N = 2)	89-1666	30-88	71-347	18-75 (N = 4)
Workers with rubberised coat					
46-54	22 (N = 2)	90-159	22-219	62-258	29-117 (N = 4)
Workers with no rubberised clothing					
91-137	- (N = 2)	93-353	32-291	69-332	23-37 (N = 6)

N = number of workers in the group; [a]Total metabolite concentration in the controls ranged from 17 to 46 µg/time period in 3 subjects; Note: Azinphos-methyl equivalents of metabolites were calculated by multiplying the total DMEPT concentration by 1.86.

The majority of workers (13/17) excreted greater than 60% of the total azinphos-methyl metabolites in 16 h on the spray day. One worker excreted about 76% of the total urinary metabolites in the 18-24 h sample (morning void). Similarly, at 1 day post exposure, the majority of the participants (13/14) excreted greater than 60% of the remaining azinphos-methyl metabolites during 0-16 h. A weak correlation was noted between the amount of active ingredient sprayed and the 24-h urinary metabolite out put ($r = 0.48$). However, the correlation between the amount of active ingredient sprayed and the 48-h urinary metabolite output appeared stronger ($r = 0.77$, $p \leq 0.01$). The correlation between the urinary metabolite output and the duration of air blast spraying showed a weaker relationship ($r = 0.43$ for both 24-h and 48-h samples). No inter-group differences in urinary metabolite excretion were seen in workers wearing different protective gear.

Conclusions: Under the conditions of the study, the majority of workers excreted greater than 60% of total azinphos-methyl metabolites in urine in 16 h on the spray day. Forty-eight hour composite urine sample appeared to be a better indicator in exposure assessment than the 24-h composite sample. Further, the total amount of azinphos-methyl sprayed was a more reliable gross index of exposure than the total time sprayed.

Miksche L (1981) Information on effects on man/occupational experience. Study number and duration: not stated. Lab: Medical Department, Bayer AG, Leverkusen, Germany. An in-company fax massage. Report no. not stated. Report date: June 12, 1981.

This short in-company communication reported that regular medical examinations of male and female employees who have been working with Gusathion MS (azinphos-methyl)

formulation using regular protective measures did not reveal any impairments to health in any of the employees. No further details on the chemical, workers examined, the types of medical tests conducted or work environment were provided.

♦♦ ***Mahler L (1991) Health effects attributed to azinphos-methyl exposure in 1991. California Department of Agriculture, pesticide illness surveillance program, worker health and safety branch.***

About ten case reports received by the California Pesticide Illness Surveillance Program in the USA, in which health effects were attributed to azinphos-methyl exposure, collected in 1991.

♦♦ ***Anonymous (1991) Pesticide residues in food - 1991. Joint FAO/WHO meeting on pesticide residues. Part II - Toxicology.***

An incident which occurred in 1987 involved 32 workers. These workers experienced symptoms including headache, nausea, weakness and vomiting upon entering a field to pick peaches three days after methomyl had been applied to the crop, and about six weeks after an application of azinphos-methyl.

McCurdy SA, Hansen ME, Weisskopf CP, Lopez RL, Schneider F, Spencer J, Sanborn JR, Krieger RI, Wilson BW, Goldsmith DF & Schenker MB (1994). Assessment of azinphosmethyl exposure in California peach harvest workers. Arch Environ Health 49(4): 289-296.

[Although this report also presented data on guthion (azinphos-methyl) residues, only the data pertaining to plasma and red blood cell (RBC) ChE and urinary excretion of azinphos-methyl and/or its metabolites were considered in this evaluation.]

Study and observations: To evaluate the usefulness in exposure assessments, this study compared measurements of urinary alkylphosphate metabolites and oxime induced reactivation of plasma and RBC ChE with measurements of foliar residues, skin and clothing contamination, and plasma and RBC ChE activities in 20 peach orchard workers exposed to guthion (azinphos-methyl). Workers (age: 18-58 years, sex, body weight range unspecified) entered orchards treated 30 days previously with azinphos-methyl (purity, source unspecified) at 1.7 kg/ha and worked for about 7.5-8 h/day in 3 blocks on 3 consecutive days, and for 18 additional days during the ensuing 6-week period. None of these individuals reported any pesticide use during the 2 weeks preceding the field entrance. Ten subjects were chosen to wear sampling garments for residue studies. Twelve persons who did not perform any agricultural work served as controls for various portions of the study.

Blood samples were collected for ChE assay (Ellman *et al* 1961) from all individuals 6 days prior to re-entry and at 3 and 44 (74 days after spraying, from 11 persons) re-entry days. It was stated that the blood samples were kept at ambient temperature for an unspecified period after blood was drawn, but the reason was not stated. Oxime reactivation of RBC ChE was performed using a method discussed in Wilson *et al* (1991). Reactivations were considered significant if they resulted in a 5% increase in activity after 2-PAMCl treatment and statistical significance at $p \leq 0.05$ (procedures given below). Plasma and RBC ChE data were presented as percentage decrease in activity in relation to pre-exposure values. Urine was collected (24 h composite) 6 days before the re-entry, and at 1, 2, 3 and 44 re-entry days. Pre-exposure samples were collected for less than a full 24 h, and the sample at 44 days consisted of a

single void. After measurement of the total volume, an unspecified portion of urine was sampled and stored frozen until assayed. The samples were analysed for creatinine using the Sigma Procedure #555 of 1983. Dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP) were analysed using a chromatographic method. Urinary metabolite levels were expressed as μmoles excreted/day. Distributions of continuous variables were described using median and percentile scores. As appropriate, the data were analysed using the following statistical procedures: Kruskal-Wallis test, Spearman rank correlation coefficient and Wilcoxon one-sample test.

Findings: Data considered in this evaluation were provided in the form of line graphs. As illustrated in these graphs, statistically significant reduction in median plasma ChE activity (9%, $p \leq 0.01$, for all workers combined) was seen on re-entry day 3 compared to the pre-exposure data. Statistically significant ($p \leq 0.001$ or $p \leq 0.01$) reductions of 7% and 19% in median RBC ChE activity were seen at re-entry days 3 and 44, respectively. According to the study authors, 7% inhibition in RBC ChE activity seen on re-entry day 3 was due to the fact that samples were not placed on ice immediately after collection. No significant oxime-induced reactivation was seen. It was stated that the workers showed steadily increasing levels of urinary azinphos-methyl metabolites throughout the exposure period, but no supporting data were provided. No significant correlation was observed between plasma ChE data and urinary metabolites ($r_s = 0.09$ and –0.39 for re-entry days 3 and 44, respectively). However, these metabolites correlated well with RBC ChE activity in blood drawn on re-entry day 3 ($r_s = 0.77$).

Conclusions: Significant reductions in median plasma and RBC ChE activities (about 9% and 7% respectively) were seen in agricultural workers exposed to azinphos-methyl on re-entry day 3. On re-entry day 44, the reductions were 12% and 19% for median plasma and RBC ChE activities, respectively with statistical significance being limited to RBC ChE activity ($p \leq 0.01$). Urinary metabolite levels of azinphos-methyl showed a better correlation ($r_s = 0.77$) with RBC ChE activity in blood drawn on re-entry day 3 than with plasma ChE activity on that sampling day ($r_s = 0.09$).

Drevenkar V, Radić Z, Vasilić Z & Reiner E (1991) Dialkylphosphorous metabolites in the urine and activities of esterases in the serum as biochemical indices for human absorption of organophosphorus pesticides. Arch Environ Contam and Toxicol 20: 417-422.

Study and observations: To compare and assess whether urinary metabolites should be used as an alternative to a ChE activity assay, orchard workers were monitored following exposure to 3 organophosphorus pesticides by measuring their urinary metabolite levels and serum ChE activity together with serum paraoxonase and arylesterase activities. One of the compounds studied was azinphos-methyl (Gusathion M, 25% powder, source, batch, formulation details not specified). The group consisted of 28 male and 8 female workers. Based on the tasks performed by the workers, there were 4 exposure levels. They were mixers (handled about 10 kg of the compound/day and wore gloves and masks, sprayers who sprayed about 10000 L of 0.1-0.3% w/v suspension/day from a cabin on a tractor and with no other personal protection), field workers who entered the orchard 1-2 weeks after spraying and others who exposed to the chemical indirectly. It was stated that the application of azinphos-methyl to the orchard took place on several occasions in two seasons in that year, one spraying session lasting for about 2-4 days and the sessions were being 2-4 weeks apart. The duration of a workday was 8 h. No further details on the orchard or worker involvements in treated areas were provided. Blood and urine samples were collected at the end of a 2-day spraying session. Because the majority

of pre-exposure samples collected in the previous year (sprayed methidathion and vamidathion) did not contain any urinary metabolites, pre-exposure urine samples of this study were not analysed. Serum ChE activity was assayed using the method of Ellman *et al* (1961). Serum paraoxonase and arylesterase activities were measured spectrophotometrically, using paraoxon and phenylacetate respectively as substrates. Dialkylphosphorus metabolites of dimethylphosphate (DMP), dimethylthiophosphate (DMTP), thiono-DMTP, thiolo-DMTP and dimethyldithiophosphate (DMDTP) [and a few diethylphosphorus metabolites (DEPs) because compounds like chlorpyrifos and diazinon had been used in the orchard previously] in urine were determined using a capillary GC method. Dimethyl phosphate (DMP) was not evaluated in this study due to limitations in detection procedure. No further details on experimental methods were provided.

Findings: No clinical signs related to azinphos-methyl exposure were reported. The data on serum ChE activity and total dialkylphosphorous metabolites in urine are given in the following Table.

Comparison between serum ChE activity and total dialkylphosphorus metabolites in the urine

Number of samples	Percent ChE activity*	Total metabolite range (nmol/mg of creatinine)
18	above 90	0.14-25.1
9	89-70	1.21-8.17
3	69-66	1.67-55.9

*Expressed as percentages of pre-exposure values. Pre-exposure serum ChE activity with acetylcholine as the substrate (mean ± SD, expressed as µmol/min/mL serum) was 1.95 ± 0.53. With the substrate propionylcholine, it was 3.44 ± 0.95.

According to the data, about 31-34% inhibition in serum ChE activity compared to the pre-exposure values was seen in 3 workers, with an indeterminate number of workers exhibiting about 20%-30% depression in serum ChE activity. No correlation was observed between serum ChE inhibition and the total concentrations of dialkylphosphorus metabolites in the urine. Paraoxonase and arylesterase activities also showed no correlation with either serum ChE inhibition or urinary dialkylphosphorus metabolite concentrations (r = -0.2-0.3).

Concentrations of different metabolites in urine are presented in the following Table. Median concentration of DMDTP appeared to be slightly higher in the urine than that of DMTP. It was stated that mixers were the most exposed group of workers with the median concentration of total metabolites of 19.9 nmol/mg creatinine (range: 3.32-55.9), followed by sprayers who had a median concentration of total metabolites of 2.19 nmol/mg of creatinine. According to the study authors, the predominant urinary metabolite in mixers was DMTP, while DMDTP being the major metabolite in other workers. But no further supporting data for these study parameters in different worker groups were provided.

Conclusions: The data presented in this study demonstrate that azinphos-methyl metabolites in urine could be considered as indicators in exposure assessments. No correlation was observed between serum ChE activity inhibition and the total urinary dialkylphosphorous metabolite concentrations. Paraoxonase and arylesterase activities also showed no correlation with either serum ChE activity inhibition or urinary dialkylphosphorous metabolite levels. Lack of correlation between serum ChE activity inhibition and urinary metabolite concentrations suggests that both these parameters need to be monitored in exposure assessments.

Concentrations of different urinary metabolites (including DEPs) in workers exposed to azinphos-methyl

Metabolite	Concentration (nmol/mg creatinine)		
	N	Median	Range
DMTP	34	0.78	0.07-53.7
DMDTP	35	0.92	0.03-14.3
DEP	0	0.00	-
DETP	22	0.04	0.01-1.37
DEDTP	25	0.11	0.03-2.23
Total	35	2.35	0.14-55.9

Hernandez BZ, Sanborn JR, Schneider FA, Spencer JR & Krieger RI (1992) Dermal and biological monitoring of tree fruit harvesters exposed to foliar azinphos-methyl residues. American Chemical Society Abstracts, 203rd National Meeting, (1-3): AGRO Abstract no: 62.

[Data for this study were provided in abstract form.]

Study and observations: This report presented the data collected from an occupational exposure assessment study conducted by the California Department of Pesticide Regulation (CDPR) as part of its exposure method validation process. Peach and apple harvesters were monitored for exposure to azinphos-methyl for over 2 years by measuring dermal residue (in t-shirts and hand washes) and dialkyl phosphate levels in urine. Cholinesterase (type unspecified) monitoring was performed as an additional monitoring tool.

Findings and conclusions: Based on the urinary dialkyl phosphate data, the mean percent dermal absorption of azinphos-methyl in peach and apple harvesters was 19% and 55% respectively. No significant change in ChE activity was observed. Further, no correlation between dermal dosimetry and urinary metabolites was seen.

Stokes L, Stark A, Marshall E & Narang A (1995) Neurotoxicity among pesticide applicators exposed to organophosphates. Occup Environ Med 52: 648-653.

[Although this report also presented epidemiological data in the applicators, only the data pertaining to urinary excretion of azinphos-methyl metabolite, DMTP were considered in this evaluation.]

Study and observations: This epidemiological study of licensed pesticide applicators (90 males) in New York, USA was conducted to investigate the effects of exposure to organophosphate pesticides on the peripheral nervous system. Azinphos-methyl had been sprayed for a mean of 14 years (range 1-39 years) by the applicators, and was also the predominant organophosphate sprayed (other major pesticides were phosphamidon, chlorpyrifos, phosmet and diazinon) during the growing season. During the growing season azinphos-methyl was sprayed by 61% of the applicators five times/season or more. As a part of the study, short-term exposure effects in workers were validated by monitoring clinical signs and measuring the concentration of dimethylthiophosphate (DMTP) in urine during the spraying season of April-August 1989 (purity, source, application method and rate unspecified). There were 68 population based and age-matched (± 1 year) persons in the control group. Urinary DMTP levels in 53/90 applicators (age, body weight range unspecified) were determined (sampling time and method unspecified) according to the

established methods. Ninety three percent of the applicators used some type of head covering more than half the time; at least 60% of them used overalls and rubber gloves more than half the time. About 35% of the applicators used respirators or rubber boots or both and a similar number of workers used tractor cabs for spraying. No further details on experimental methods were provided.

Findings: It was stated that the applicators reported headaches more frequently on than off-season, but no further details were provided. Urinary DMTP levels based on the extent of work involved and the time spent for spraying are presented in the following Table. According to the data, an apparent exposure-related increase in urinary DMTP concentration was seen in the applicators. Urinary DMTP level, however, did not increase with increasing number of exposure symptoms reported. The study authors hypothesised that the exposure levels may not have been high enough to produce acute symptoms in the workers.

Conclusions: A clear exposure-related increase in urinary DMTP level was seen in workers, who sprayed azinphos-methyl. Urinary DMTP level, however, did not increase with the increasing number of exposure symptoms reported.

Urinary DMTP levels in pesticide applicators

Group	Number of applicators (%)	DMTP recovered Mean (range) ppm
Total tanks loaded (1-4 days)		
0.5-5	16 (30.1)	40.5 (3.7-143.0)
6-12	21 (39.6)	75.4 (2.0-396.1)
13-53	16 (30.1)	96.8 (6.3-298.3)
Total acres sprayed (1-4 days)		
0.5-20	17 (32)	56.2 (2.0-396.2)
21-85	22 (41.5)	62.2 (3.7-274.3)
86-265	14 (26.4)	103.9 (6.3-298.3)
Total hours sprayed (1-4 days)		
0.5-8	21 (39.6)	39.0 (2.0-143.0)
9-18	19 (35.8)	72.5 (3.7-274.3)
19-61	13 (24.5)	121.8 (7.5-396.1)

Kehrig B (1999) Occupational medical experience with azinphos-methyl. In company communication to Dr KG Heimann dated July 21, 1999. WD-DO medical Services, Bayer AG. Report no: not stated.

This short, in-company communication reported that annual medical monitoring examinations of employees who have been working in a azinphos-methyl production (20 persons) or a formulation (20 persons) plant under the prevailing industrial hygiene conditions and plant and personal safety precautions did not reveal any clinically relevant sensitisation (term unspecified in the report) to this chemical. The conclusions in this communication were based on the findings of annual occupational medical examinations, experience gained by the physicians during their regular visits and information from the management of the relevant production or formulation plant. No further details on the chemical, workers involved, the types of medical tests conducted or the work environment were provided.

Heimann KG (2000) Azinphos-methyl: Determination of cholinesterase inhibition during spraying in orchards (operator exposure study). Study no: not stated. Labs: Azienda USL di Ravenna (Italy), Presidio Ospedaliero di Lugo, Laboratorio Analisi Chimico-Cliniche e

Microbiologiche; LCL 78 av de Verdum BP 10, F-94208 Ivry/Seine, and Laboratoire Marcel Merieux, Pasteur et de la Fondation Marcel Merieux, BP 7322-69357 Lyon Cedex 07, Avenue Tony Garnier, F-69007, Lyon, France. Sponsor: Bayer AG, Business Group Crop Protection, D 51368 Leverkusen, Germany. Study duration: September to October, 1998. Report no. not stated. Report date: January 10, 2000.

This study was not conducted in compliance with the OECD or FIFRA (40 CFR Part 160) GLP Principles. No quality assurance statement was provided.

Study and observations: According to the study author, this operator exposure study involving 8 male operators in Italy and France (25-36 yrs old), was conducted to determine the "real exposure under practical conditions". Plasma ChE activity in 5 workers in Italy, 2 workers in France, and erythrocyte (RBC) ChE activity in 1 operator in France were determined prior to and after exposure (exposure conditions unspecified) according to internal laboratory standards (method unspecified). No further details on experimental methods were provided.

Findings and conclusions: No relevant differences between pre- or post-exposure plasma or RBC ChE activities were noticed in any of the workers compared to the reference values of the laboratory (data provided). However, the validity of the findings of this study is reduced due to lack of details on the test substance, exposure conditions and experimental methods.

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[Figures in square brackets are an Australian identification code and indicate the location of the submitted data. MCW = Makhteshim Chemical Works; KI = Koor Inter-trade; BA = Bayer Australia Limited;]

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